

Book of Abstracts

**of the 76th Annual Meeting
of the European Federation of Animal Science**



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25 - 29 August, 2025

**Book of Abstracts of the 76th Annual Meeting
of The European Federation of Animal Science**



EAAP

European Federation of Animal Science

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Innsbruck, Austria, 25st – 29th August , 2025



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Welcome to the EAAP 2025 in Innsbruck

Ladies and Gentlemen,

on behalf of the organizing committee and the entire community of the Austrian Association for Animal Science and Production I am happy and honored to welcome you to the 76th annual meeting of the European Association for Animal Production, which will be held in Innsbruck on August 25th -29th, 2025. The overarching theme of the congress is "Future-proof livestock farming". Innsbruck, the capital of the Tyrol, is nestled in a breathtaking Alpine panorama. The city combines nature, culture, science, and business, blending traditional customs with modern innovations. Innsbruck promotes diversity and has a high quality of life, with clear air, pure water, and state-of-the-art infrastructure. The city's economy is strong, and its universities are renowned and rich in tradition, keeping the city vibrant with over 35,000 students.

Austria is renowned for their diverse and high-quality agricultural products. The region around Innsbruck is particularly famous for its dairy products, with about 35 to 40% of the agricultural production value coming from the milk industry. This sector includes around 4,200 farms that produce approximately 350,000 tons of milk annually. The region also boasts 44,300 dairy cows, 2,376 sheep, and 6,075 goats, all contributing to the rich dairy heritage of the Tyrol.

Tiroler Bergkäse is a protected designation of origin for a traditionally produced, matured hard cheese made from the milk of cows that graze in the Tyrolean mountains. Similarly renowned are the Tyrolean bacon specialties, which acquire a distinctive aroma through the unique climate and traditional smoking process. Additionally, the Tyrol is known for its direct marketing of agricultural products, with over 4,000 farms selling their produce directly to consumers through various channels such as farmers' markets, farm shops, and home delivery services.

In addition, Austria is renowned for the quality of agricultural products, like beef, grains, and wines. The country's beef industry is an important part of its agricultural landscape. In addition to livestock, Austria also produces a variety of high-quality grains such as wheat, barley, and corn that are crucial to the nation's food production.

Beyond meat and grains, Austria's winemaking tradition dates back centuries, with renowned wine regions like Lower Austria, Burgenland, and Styria producing world-class vintages like the famous Grüner Veltliner. These diverse agricultural products highlight the breadth and excellence of Austria's food and beverage industries. Some of these production and marketing systems will be subject of technical visits organized at the end of the technical session.

Welcome to Innsbruck!
President
Josef Wiesböck

Welcome to Austria!

We are honored to host the EAAP annual congress once again — more than two decades since it was last held in Austria. Welcoming guests from around the world is always a joy, especially when we get to showcase our unique landscape in combination with local agriculture and food production in the alpine setting of Innsbruck.

“Future-Proof Livestock Farming,” resonates with our national focus on circular economy and site-adapted agriculture in Austria. Favourable locations for fruit and vegetable cultivation, such as arable farming and fattening, alpine mountain regions skilfully used by ruminants for landscape maintenance and high-quality dairy products, as well as careful growth of beef cattle and animal-friendly husbandry in mother-bound calf rearing. Austrian agricultural systems combine both tradition and innovation.

As a member of the EAAP, the Federal Ministry of Agriculture is keen to share the latest research findings with the scientific community, particularly with regard to the transfer into practice, feasibility and profitability for production agriculture.

With a shared commitment to sustainable livestock farming, we are especially pleased to have the ‘Sustainable Animal Husbandry Austria’ association at our side as a competent and industry-recognised partner in the preparations leading up to the EAAP Annual Meeting here in Innsbruck as well as the Tyrolean Chamber of Agriculture – we thank our partners for their support!

We look forward to offering you enjoyable moments, interesting encounters and space for informative dialogue throughout the event.

**Warm regards,
The Local Organizing Committee**

Local Organizing Committee

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- **Julia Unseld** - BMLUK
- **Johanna Prodinger** – NTÖ
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**Federal Ministry
Agriculture and Forestry, Climate
and Environmental Protection,
Regions and Water Management
Republic of Austria**

**Federal Ministry of Agriculture
BMLUK**

As a member of the EAAP we are grateful for the great international exchange regarding science and research, further development and innovation, as well as learning from each other and talking to each other.

While agriculture, forestry and water management remain at the forefront, our agency has newly incorporated the agendas of climate, environment, and nature conservation. Especially with the takeover of climate and environmental agendas, it is a concern of our house to present agriculture - and especially animal husbandry - as an important factor with regard to circular economy, food safety, and food security.

But that's not all. As a sector that is heavily affected by climate change, we also contribute to mitigating the consequences proactively through our actions, behavior, and consumption, and support climate change adaptation measures in various ways, strengthen their resilience, work in a future-oriented manner, and take into account new requirements for agriculture without jeopardizing their competitiveness.

Agriculture is part of our identification and culture, creates jobs, shapes the rural landscape, contributes to the economy, and provides security. One of our main concerns is to create trust in the quality of our products, administration, and transparency.



Sustainable Animal Husbandry Austria
NTÖ – Nachhaltige Tierhaltung Österreich

As an umbrella organisation, Sustainable Animal Husbandry Austria (NTÖ) represents all producer associations and industry representatives of livestock farming in Austria. As well as acting as a communication platform, we are also a contact point for farmers' concerns and offer support in implementing various agricultural guidelines, drafting them and representing agricultural interests in various committees. We also provide education to farmers, and our close relationship with research partners helps us to shape the course programme and the interests of farmers.

It is essential to us that new scientific information is made accessible to farmers and linked to practice. Only in this way can ongoing developments be implemented directly on farms. NTÖ has a strong focus on public relations work and the creation of numerous teaching materials, information brochures, appealing short videos, and informative social media content to increase transparency and improve the public image of modern, innovative agriculture. Our vision is to strengthen the link between animal husbandry and consumers once again.



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- Sam de Campeneere (Chair)

 Federal Ministry
Agriculture and Forestry, Climate
and Environmental Protection,
Regions and Water Management
Republic of Austria



The European Federation of Animal Science (EAAP)

The main aims of the EAAP are to promote, by means of active co-operation between its members and other relevant international and national organisations, the advancement of scientific research, sustainable development and production systems; experimentation, application and extension; to improve the technical and economic conditions of the livestock sector; to promote the welfare of farm animals and the conservation of the rural environment; to control and optimise the use of natural resources in general and animal genetic resources in particular; to encourage the involvement of young scientists and technicians. More information on the organisation and its activities can be found at www.eaap.org.

Former Presidents

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1961-1967	R. Trehane (United Kingdom)
1967-1972	J.M. Rijssenbeek (The Netherlands)
1972-1978	J.H. Weniger (Germany)
1978-1984	E.P. Cunningham (Ireland)
1984-1990	A. Roos (Sweden)
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1996-2000	P. Solms-Lich (Germany)
2000-2004	A. Aumaitre (France)
2004-2008	J. Flanagan (Ireland)
2008-2012	K. Sejrsen (Denmark)
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The European Federation of Animal Science (EAAP) has close established links with its sister organizations of American Society of Animal Science (ASAS), American Dairy Science Association (ADSA), Canadian Society of Animal Science (CSAS) and Asociación Latinoamericana de Producción Animal (ALPA) and is also member of the World Association for Animal Production (WAAP).



Industry Members Club



EAAP started in 2023 a new initiative to create closer connections between European livestock industries and the animal science network. Therefore, the “EAAP Industry Club” was shaped with the specific aim of bringing together the important industries of the livestock sector with our European Federation of Animal Sciences. All companies dealing with animal production (nutrition, genetic, applied technologies, etc.) are invited to join the “EAAP Industry Club” because industries will have opportunity to increase their visibility, to be actively involved in European animal science activities, and to receive news and services necessary to industries. In addition, through the Club, industries will enlarge their scientific network and will receive specific discounts on sponsoring activities.

The Industries that already joined the “EAAP Industry Club” are:



The Club gives:

Visibility • Company name and logo at EAAP website and all relevant documents • Slides with name and logo at Official Events • Priority links with EAAP Socials • Invite, through EAAP dissemination tools and socials, people to events organized by your company • Information disseminated through a brand new Industry Newsletter • Networking • Joining the Study Commissions and Working Groups • Suggest topics to be considered for Annual Meetings Scientific Sessions • Organize Professional Panel through the EAAP platforms • Economic Benefits • One free registration to each Annual Meeting and at every meeting organized by EAAP • Five individual memberships at no cost • Many possible discounts (-30%) to increase company visibility through: EAAP Newsletter, EAAP website, EAAP Annual Meetings and workshops • Support young scientist by sponsoring scholarships named by the company • Co-Organize and sponsor webinars

Make yourself more visible within the livestock industry via the animal science network!

For more information please contact eaap@eaap.org

YoungEAAP



What is the YoungEAAP?

YoungEAAP is a group of young scientists organized under the EAAP umbrella. It aims to create a platform where scientists during their early career get the opportunity to meet and share their experiences, expectations and aspirations. This is done through activities at the Annual EAAP Meetings and social media. The large constituency and diversity of the EAAP member countries, commissions and delegates create a very important platform to stay up-to-date, close the gap between our training and the future employer expectations, while fine-tuning our skills and providing young scientists applied and industry-relevant research ideas.

Committee Members at a glance

- Ines Adriaens (President)
- Jana Obsteter (Vice President)
- Giulia Gislon (Secretary)

YoungEAAP promotes Young and Early Career Scientists to:

- Stay up-to-date (i.e. EAAP activities, social media);
- Close the gap between our training and future employer expectations;
- Fine-tune our skills through EAAP meetings, expand the special young scientists' sessions, and/ or start online webinars/training with industry and academic leaders;
- Meet to network and share our graduate school or early employment experiences;
- Develop research ideas, projects and proposals.

Who can be a Member of YoungEAAP?

All individual members of EAAP can join the YoungEAAP if they meet one of the following criteria: Researchers under 38 years of age OR within 10 years after PhD-graduation.

Just request your membership form (info@eaap-youngclub.org) and become member of this network!!!

EAAP Study Commissions



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Scientific programme

EXPLANATORY NOTES ON SESSIONS

Abbreviations after the title are (Organising Commission; Session Type)

Commissions are:

Cattle (C), Genetics (G), Health and Welfare (H+W), Horse (H), Insect (I), Livestock Farming Systems (LFS), Nutrition (N), Pig (P), Physiology (Ph), Precision Livestock Farming (PLF), Sheep and Goat (S+G), Animal Task Force (ATF)

Session types are:

Theme Sessions (T) are on key topics in animal science and consist of invited and offered papers.

Industry sessions (I) are led and supported by Industry and comprise invited and offered papers.

Free communications/Bottom-Up (B): these sessions will be created from submitted abstracts and titles will be announced in early April.

Discovery Sessions (D) are invited single/multiple presentations on hot/emerging topics.

Challenge Sessions (C) are free format (workshop, open committee, round table) to debate important issues, not theatre paper presentation.

Early-career (EC) scientist's Session.

Project session (P) are sessions based around a project which wants to share its results, often combined with submitted abstracts.

Working Group sessions (WG) are the results of an EAAP working group.



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Scientific programme

Session 1. How to mitigate livestock emissions through breeding

Date: Monday 25 August 2025; 8:30 - 12:30

Chair: Pszczola / Kargo

Theatre Session 1

Global Methane Genetics Initiative: Acceleration of genetic progress for reduced methane emission in ruminants	132
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Chair: Pinotti / Mele

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Chair: Berg / Camarinha Silva / Rahmatalla

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Chair: Verschuren / Chalvon-Demersay / De Grande

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Chair: Chalvon-Demersay / Lawlor

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Chair: Holinger / Trocino

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Chair: Camarinha Silva / Kreuzer-Redmer

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Chair: Morgan-Davies / Emsen

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Chair: Cesarani / Kuipers

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Chair: Maselyne / Odintsov Vaintrub

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Chair: Cesarani / Kuipers

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Chair: Schiavo / Maroto Molina

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Date: Monday 25 August 2025; 13:30 - 17:15

Chair: Giagnoni / Cieslak

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Chair: Das / Gierus / Trocino

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Chair: Kenny / Stella

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Date: Tuesday 26 August 2025; 14:30 - 18:00

Chair: Millet / Stadnicka / Bruggeman / De Grande

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Chair: Podstatzky / Almeida

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Chair: Sölkner / Berg

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Chair: Kreuzer-Redmer / Ranilla García

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Chair: Pocrnic / Lourenco

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Chair: Druyan / Gautier

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Chair: Karatzia / Lebreton

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Chair: Kiefer / Macciotta

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Chair: Dadousis / Wurzinger

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Chair: Kargo / Schiavo

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Chair: Bruggeman / Alvarez-Rodriguez

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Session 62. European research infrastructures to support livestock science: whom and how?

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Chair: Boichard / Cartick

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Chair: Adriaens / Gislon / Obsteter

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Chair: Egger-Danner / Sell-Kubiak

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Chair: Deruytter / Bellezza Oddon

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Chair: Vestergaard / Devant

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Chair: Foy / Cartick / Franchi

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Chair: Foy / Cartick / da Silva

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Chair: Klopčič / Hocquette

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Chair: Egger-Danner / Malchiodi

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Chair: Manaig / Alvarez-Rodriguez

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Global Methane Genetics Initiative: Acceleration of genetic progress for reduced methane emission in ruminants

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The Global Methane Genetics (GMG) initiative aims to reduce methane (CH₄) emissions from the single largest emitter in the agricultural sector: ruminant livestock. Genetic differences in CH₄ emission between animals can be identified and included in selection decisions to breed animals that emit less CH₄, whilst staying healthy and producing nutrient dense food. Several countries have started recording individual CH₄ emission in cattle and small ruminants on commercial farms allowing for estimation of breeding values for lower CH₄ emissions. However, for routine implementation globally, the number of phenotypes is still too small and limited to only a few populations around the world. The GMG initiative is a multi-million dollar investment by the Global Methane Hub's Enteric Fermentation R&D Accelerator and The Bezos Earth Fund, coordinated by WUR. The overarching aim is to accelerate genetic progress towards low-CH₄ emitting ruminants. The primary goals are to (1) significantly expand recording of CH₄ emissions in cattle and small ruminants, together with genotypes, pedigree and rumen microbiome, (2) share and disseminate knowledge via global working groups, and (3) develop standard operating procedures in partnership with the ICAR Feed & Gas working group. There is a portfolio of programs starting early 2025 and involving around 50 partners from academia, research organisations, cattle and small ruminant breeding organisations and other related stakeholders. This large-scale phenotyping and genotyping effort aims to result in more than 100,000 animals phenotyped and genotyped for CH₄ emissions, and around 20,000 animals with rumen microbiome information in more than 25 countries in the Global North and South. A global database hosting phenotypes, genotypes, pedigree and microbiome information will be developed within GMG, enhancing data sharing across the world. In this presentation we will show the potential of genetic selection as a CH₄ mitigation tool, as will be implemented in the GMG-program.

Innovative approach to data management of methane emissions in dairy cows

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Methane (CH₄) is a potent greenhouse gas linked to climate change, with ruminant livestock being a significant source of emissions. Accurate phenotyping of methane emissions in dairy cows is essential for developing mitigation strategies, such as selective breeding for lower emissions. In this study, we developed a data processing pipeline for methane measurements collected using sniffer devices installed in automated milking systems. The dataset included methane (CH₄) and carbon dioxide (CO₂) concentration measurements recorded every 7 seconds during milking sessions in Polish Holstein-Friesian cows at two commercial farms in western Poland. Data were collected over approximately 20 months on Farm 1 and around 6 weeks on Farm 2. The proposed pipeline consists of two key steps: (1) precise identification of milking start and end times using peak detection methods and (2) background correction based on stable methane plateau regions to account for ambient CH₄ variability. Visual inspection confirmed improved accuracy in detecting milking start and end times compared to previous methods, as well as a more reliable identification of non-milking periods. This approach enhances the precision of methane emission phenotyping in dairy cows. This work was funded by the European Union's Horizon Europe research and innovation program under grant agreement No. 1059609 (Re-Livestock project).

Converting Sniffer Data into Methane Yield Phenotypes – Experiences from the Swiss Project CH4COW

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Dairy cattle contribute to greenhouse gas emissions, particularly through enteric methane (CH₄) production. Breeding dairy cows with lower CH₄ emissions offers a promising approach to reducing agriculture's environmental footprint. However, direct measurement of CH₄ emissions is expensive and laborious. Nevertheless, sniffers installed in automatic milking systems (AMS) or in automatic feeding stations (AFS) open new perspectives for phenotyping many cows with a high throughput at a reasonable price. The sniffers measure gas concentrations that can be converted into yield per unit of time. These phenotypes are often required for education and consulting purposes and are particularly relevant for farmers to rank their cows. This study focuses on the development of methane yield phenotypes (g CH₄/day) from sniffer data, based on different methods, using the example of Switzerland, to identify potential pitfalls. In our study we have 2,400 Holstein (HOL) and Brown Swiss (BSW) dairy cows on 60 commercial farms with sniffer (MooLogger Plus, Tecnosens), AMS (n=50), AFS (n=10), type traits and milk recording data. Sniffer and milk yield (mil) data are available per cow per day from farms with AMS. This is only monthly the case for farms with AFS. Conformation data is mostly available once per cow in 1st lactation. Individual fat (fat) and protein (prot) contents are recorded monthly. To derive expected daily mil, fat and prot, cow specific lactation curves were fitted. Body weight (BW) was not directly available for cows with measured CH₄ concentration but was predicted using conformation data from 115 Swiss cows from two research stations for HOL and 40 cows from an agricultural college herd for BSW. Next to calving age and days in milk, type traits rump and dairy strength were kept as effect for the BW prediction. It can be concluded that the pitfalls for the prediction of CH₄ traits, using the example of Switzerland, are due to farm differences in recording of yield and type traits. In addition, BW measurements with associated conformation traits are essential, the more the better, to derive an accurate BW prediction.

Expectations of the genetic correlation between methane concentration and methane production

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Genetic selection is a promising method to reduce enteric methane (CH₄) emission in cattle. To breed cattle with lower CH₄ emissions, it is essential to collect CH₄ phenotypes from thousands of individual animals. Sniffer sensors, which measure CH₄ and carbon dioxide concentrations in the feed bins of automated milking systems (AMS), provide an efficient and cost-effective method, which enables large-scale CH₄ recording. However, sniffers measure concentrations in the air (in ppm) rather than the CH₄ production from breath (in grams of CH₄). Our objective was to demonstrate the expected relationship and genetic correlation between CH₄ concentration as measured by sniffers and CH₄ production measurements as measured by GreenFeed units. For this, we used quantitative genetics theory to estimate the expected genetic correlation between CH₄ concentration and CH₄ production based on the ratio between the coefficient of variation and the trait heritabilities and variances. In addition, a simplified breeding program was simulated using the R-package MoBPS to examine changes in genetic correlations over time between the two traits, with changes in the mean when selecting for lower CH₄ concentration. Our results showed that based on quantitative genetic theory the genetic correlation between CH₄ concentration and CH₄ production is expected to be close to unity. In simulations we showed how environmental factors and changes in the genetic variance can affect the genetic correlation between CH₄ concentration and CH₄ production. Our results provide a better understanding of the use of the CH₄ concentration phenotype in selection indices.

From sniffer measurement to phenotype for genetic selection

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Genetic selection is promising to reduce enteric methane (CH₄) emission of cattle. To breed cattle with low CH₄ emissions, it is essential to collect CH₄ phenotypes from thousands of individual animals. Sniffer sensors, which measure CH₄ and carbon dioxide concentrations in the feed bins of automated milking systems (AMS), provide an efficient and cost-effective method which enables large-scale CH₄ recording. Standardizing data processing steps is crucial to ensure accurate phenotype definitions and maximize genetic selection responses. Therefore, our aim was to describe the effects of various sniffer data processing steps on CH₄ phenotypes. The data processing steps focused on the detection and filtering of data from defective sensors and other recording errors, matching records to cow identification numbers, and averaging records per visit and per week. The filtering steps used for initial genetic parameter estimations included: an interquartile range larger than 200 ppm within an hour, an hourly mean above 30 ppm, excluding hours with at least 30% of data points within 10 ppm of the first two modes, no measurements above 3500 ppm within an hour, and individual measurements outside of the upper to lower 0.001st quantile were discarded. After filtering, background concentrations were estimated using the 0.001st lowest quantile of each day and subtracted from each measurement. Without applying any filtering steps, the dataset included CH₄ measurements recorded during 4,383,561 AMS visits from 12,296 cows across 76 farms. After filtering, the dataset included measurements recorded during 2,847,110 AMS visits from 12,009 cows across 75 farms. The data filtering improved the normality of phenotypic distributions. Our work underlines the importance of quality checks for sniffer-recorded data, which are essential to optimize genetic parameter estimations for breeding programs that aim to reduce CH₄ emissions using phenotypes from sniffers.

Session 1

Theatre 6

Evaluation of Genetic Correlations of Methane Emissions in Dairy Cattle: A Comparative Study of Estimation Methods Across Countries

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There is an increasing interest in reducing enteric methane emissions from cattle, with various estimation methods available, each with its own advantages and disadvantages. Sniffers devices are becoming increasingly popular for breeding purposes due to their high correlation with other devices and higher throughput. However, they showed high variability and moderate reliability. The aim of this study was to compare genetic estimates of methane emissions obtained with sniffers in Spain with other estimations obtained with different methodologies implemented in other countries. The dataset was obtained from the Efficient Dairy Genome Project and the Resilient Dairy Genome Project where collaborate Spain (ESP), Canada (CAN), Switzerland (CHE), Australia (AUS) and Denmark (DNK). In total, 28,755 weekly averages of methane estimates (g/d) from 4,731 dairy cows were analyzed, including measurements from sniffers (SN; ESP and DNK), GreenFeed (GF; CAN and CHE), respiration chamber (CR; CHE) and SF6 tracer (SF6; AUS). The genetic correlations between Spain and other countries were positive and moderate to high with CAN (0.29±0.23), CHE (0.71±0.16) and AUS (0.60±0.16); while it was negative with DNK (-0.22±0.34). The analysis of methane traits from different methodologies and regions is a useful tool for evaluating the reliability of methane estimates obtained from sniffers, but it is important to consider other factors that could also affect the methane emissions as particular conditions on each region.

Multi-country genomic prediction of methane emissions in dairy cattle

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Genetic selection for lower methane-emitting cows is a promising strategy to reduce methane (CH₄) emissions. Achieving high accuracy in the prediction of genomic estimated breeding values (GEBV) requires a large number of animals with CH₄ records. Previous studies have shown that a reference population of at least 50,000 cows is needed to achieve a reliability of 0.6 for a trait with a heritability of 0.2. Despite numerous efforts, the number of CH₄ records within countries remains limited. However, merging datasets from different countries can enhance the accuracy of prediction of GEBV for CH₄ emissions. This study examined whether incorporating data from other countries enhances the accuracy of prediction of GEBV for CH₄ emissions. Phenotypes for CH₄ methane concentration from sniffers from the Netherlands, Spain, and Poland were merged and standardized for editing. The CH₄ dataset comprised 132,961 weekly records from 7,669 cows in the Netherlands, 12,456 records from 3,426 cows in Spain, and 7,104 records from 483 cows in Poland. Genetic correlations between countries ranged from 0.53 to 0.67. Three scenarios were proposed: (1) base scenario using only within country data (2) treating each country as a different phenotype and (3) using the collated dataset, assuming the trait is the same across countries. Validation will be done applying a forward-cross-validation approach, where the dataset is divided into two subsets—older data and newer data—with the newest subset serving as the validation population and the oldest subset as the reference population. This work has received funding from the European Union's Horizon Europe research and innovation programme, grant agreement No 01059609 (Re-Livestock project).

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Theatre 8

Methane emission in dairy cattle breeding programs: sensitivity of genetic parameters, economic weights and phenotyping strategies

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Reducing enteric methane (CH₄) emissions by selective breeding is key to mitigating greenhouse gas emissions from livestock farming. Designing sustainable breeding programs requires understanding the impact of genetic parameter accuracy, economic weights, and phenotyping strategies. When considering CH₄ production as the main trait and CH₄ concentration (from sniffers) as an auxiliary trait, it is more important to estimate the heritability for CH₄ production accurately, than the heritability for CH₄ concentration. When 100 progeny per bull are phenotyped with sniffers, a one s.e. lower heritability for CH₄ production than the actual estimate results in 9.8% lower genetic progress (so directed to a higher CH₄ production), whereas a one s.e. lower heritability for CH₄ concentration results only in 0.5% lower genetic progress. Sensitivity analysis showed that under ceteris paribus assumption the phenotypic variance of both CH₄ traits in the population and the genetic correlation between CH₄ production and CH₄ concentration measured by sniffers strongly influence the genetic progress for lower CH₄ emissions. A genetic correlation between CH₄ production and CH₄ concentration one s.e. lower than the actual estimated correlation results in a 23.0% higher CH₄ production. The economic value of CH₄ considered in the selection index, e.g. determined by a carbon tax applied to livestock, also strongly influences genetic progress. In the selection theory-based calculation, the height of the Danish carbon tax on dairy cattle planned for the year 2035 leads to a situation that 29.7% of the total economic response to selection results from CH₄ production. In addition, we evaluate the impact on genetic progress of different CH₄ national phenotyping strategies and the integration of CH₄ phenotypes from other countries when forming genomic across-country reference populations as tested within the Re-Livestock project (Horizon Europe, GA No. 01059609). Phenotyping strategies include testing fixed numbers of phenotyped cows with sniffers, GreenFeed and sniffers, and different genotyping proportions.

Towards methane-efficient cattle: breeding solutions for the Netherlands and Flanders

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Dairy cows contribute to the emission of methane (CH₄), a strong greenhouse gas, into the atmosphere. Reducing CH₄ emissions from dairy cows will lower the impact of livestock on global warming. Breeding could be an effective reduction method, and estimating breeding values was the objective of this work. The CH₄ emission of 11,595 dairy cows on 89 Dutch herds was measured with sniffers in parts per million (ppm) of exhaled air. The CH₄ emission of 397 dairy cows from 1 Dutch herd was measured in grams per day (gpd) of exhaled air. CH₄ measurements took place from 2019 to 2025 (sniffer) and from 2022 to 2025 (GreenFeed). All observations during a week on a cow were averaged into week observations. There were 226,449 week observations for ppm and 11,824 week observations for gpd. Genetic parameters were estimated with ASReml 4.2 using an animal multi-trait repeatability model. Heritabilities (h²) were 0.14, 0.14 and 0.19 for ppm and 0.34, 0.37 and 0.37 for gpd, for respectively lactation 1, lactation 2 and lactation 3 and later (3+). Genetic correlations between different parities for both ppm and gpd were 0.75 between 1 and 2, 0.55 between 1 and 3+ and 0.80 between 2 and 3+. An overall breeding value was calculated for gpd based on traits in lactation 1, 2 and 3+. By using a selection index, extra information was added to the overall breeding value in gpd. Traits in the selection index were kg milk production, kg fat production, feed intake and body weight with genetic correlations of respectively 0.39, 0.19, 0.20 and 0.09. The average CH₄ emission of a dairy cow was 435 gram per day with a genetic standard deviation of 36 grams per day. The heritability of the trait, the size of the genetic standard deviation, and the fact that genetic correlations with health traits were estimated to be small, makes breeding an effective and powerful tool to mitigate CH₄ emissions from dairy cattle in the Netherlands and Flanders. The overall breeding value for CH₄ in grams per day is published in the Netherlands and Flanders from April 2025.

Session 1

Theatre 10

MicroHUB: An international initiative to modulate the rumen microbiome for methane mitigation

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Genomic selection is an efficient tool to reduce methane emissions in cattle. However, measuring methane from individual animal is not feasible in all production systems due to technical limitations, lack of labor and adequate infrastructure, or high costs. This may slow down genetic progress for mitigation of methane emissions. The microbiome has been proposed as an indicator of enteric methane. Furthermore, selection pressure on feed efficiency and reduced methane emissions can inflict a correlated response on the rumen microbiota composition, due to moderate (0.20) to strong (0.70) genetic correlations. Although sampling rumen microbiome is a labor-intensive and costly process, this information can be obtained for part of the population as a reference population. The microbiota composition shows larger heritability (~0.30) than methane traits measured at large scale (~0.15). The Global Methane Genetics initiative aims to reduce methane emissions from the largest single emitter in the agricultural and livestock sector: ruminants. Denmark, The Netherlands, Australia, New Zealand and Spain have joined forces in a project called Micro-HUB to accelerate this process by establishing a global reference population with metagenomic and genotype data, including dairy and beef cattle, and sheep. Our calculations showed that a reference population of 20 000 cows with microbiome phenotype can achieve GEBV reliabilities ~0.50, whereas reliabilities above 0.80 can be obtained for genotyped sires with at least 5 equivalent daughters with microbiome information. A composite microbiome phenotype can be defined, and a genomic evaluation system developed. Microbiome composition needs to be appropriately balanced in the breeding goal, to obtain a desired genetic response on methane while maintaining proper rumen health.

Exploring flexible microbiome-driven breeding strategies to mitigate methane emissions in beef cattle

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Microbiome-driven breeding (MDB), which utilises the abundances of the most informative ruminal microbial genes that are host-genetically correlated with methane emissions, has been proposed as a cost-effective strategy to breed cattle for reduced methane yield. In this study, we developed an MDB approach for methane production (CH4P) and analysed different strategies for integrating this methodology into the genetic evaluation of production traits. A Bayesian analysis using host genomics and whole metagenomic data was applied to a deeply phenotyped beef population. The genomic analysis of the data revealed that CH4P was strongly and unfavourably correlated with daily feed intake, average daily gain (ADG), and age-adjusted carcass weight. To mitigate these unfavourable correlations, we developed an MDB strategy that considered only microbial genes favourably correlated with both reduced CH4P and improved ADG (MDB41, where the number denotes the most informative microbial genes identified) and compared it to a scenario that included microbial genes targeting only CH4P (MDB43). Selection of the 10% best animals based on genomic estimated breeding values (GEBVs) using MDB43 resulted in a reduction in CH4P by 18% of its mean (GEBV accuracy = 0.72, HPD95%: -22% to -13%) and a 7% decrease in ADG (0.65, -3% to -10%). In contrast, equivalent intense selection using MDB41 led to a lower CH4P reduction of 14% (0.61, -19% to -10%) but a slight increase in ADG of 1% (0.58, -3% to 6%). We also integrated MDB into a multi-trait model alongside production traits and analysed the impact of selection on each of these traits on the correlated responses of the others. Based on these results, we developed a new selection criterion of residual methane emissions that did not negatively affect production traits. Our findings demonstrate that MDB can be flexibly incorporated into existing genetic evaluation systems to reduce methane emissions while simultaneously avoiding reduction in production traits.

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Theatre 12

Validating genomic evaluation for methane efficiency in dairy cattle using national and international data

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Genetic strategies can play a crucial role in reducing greenhouse gas (GHG) emissions in dairy cattle, contributing to sustainable breeding programs worldwide. Canada released a genomic evaluation for Methane Efficiency in April 2023 and successfully validated it within the country. Further validation using international data is warranted to ensure the applicability of estimated breeding values across diverse populations and production systems. The objective of this study was to validate the official genomic evaluation of Methane Efficiency using national and international data. First, the genomic evaluations from April 2023 were used to classify 141 genotyped cows from the Ontario Dairy Research Centre that had no available phenotype at the time of the evaluation but had methane measurements recorded in 2024 using the GreenFeed system. Relative Breeding Value (RBV) groups exhibited the desired decreasing trend in mean methane emissions as RBV increased, confirming that selecting cows with higher RBVs for Methane Efficiency (ME) effectively reduces methane emissions. In a second analysis, the study leveraged data from 272 cows with methane measurements collected through the GreenFeed system across multiple European countries to assess the transferability and effectiveness of methane efficiency traits. The results demonstrated significant genetic variation, with notable differences in methane production among animals with breeding values exceeding one standard deviation. Results across the two analyses confirmed the robustness of the genetic evaluation beyond Canadian datasets. These findings highlight the global relevance of genetic selection for reducing emissions and emphasize the importance of incorporating internationally validated data into breeding strategies. By demonstrating the applicability of genomic evaluations beyond national borders, this study supports the broader adoption of sustainable breeding practices in the dairy industry worldwide.

About the genetic relationship between methane emission traits and ketone bodies in Danish Holstein and Jersey cattle

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A key focus in current animal breeding is the mitigation of enteric methane (CH₄) emissions by introducing a selection index. This requires a thorough understanding of the new traits' genetic relationship with other traits related to e.g., fertility and health, to avoid compromising these traits. Of great importance in dairy cattle is thereby the metabolic disorder ketosis, characterized by increased level of ketone bodies (β -hydroxybutyrate (BHB) and acetone (AC)) in milk and blood, as it makes the cows susceptible to other health problems and reduces their (re-) production. Hence, this study aimed at assessing the genetic relationship between enteric CH₄ emission traits, i.e., the CH₄ (MeC) concentration and CH₄:CO₂ ratio, obtained using sniffers that were installed on 21 Danish dairy farms, and test-day levels of BHB and AC in the milk, obtained from the routine milk recording scheme. The applied dataset comprised of 4,120 Holstein (HOL) and 2,782 Jersey (JER) cattle. Mean levels of BHB and AC were slightly higher in HOL (0.06 mmol/l for BHB, 0.03 mmol/l for AC), than in JER (0.05 mmol/l for BHB, 0.01 mmol/l for AC). In contrast, JER had a higher mean MeC (450.52 ppm) than HOL (386.86 ppm). The estimated heritabilities were low to moderate and ranged from 0.08 (se = 0.01) for the CH₄:CO₂ ratio (HOL) to 0.19 (se = 0.02) for the MeC (JER). Most genetic correlations were low and not significant, only the correlation between AC and the CH₄:CO₂ ratio was significantly negative for HOL (-0.23, se = 0.07), indicating an unfavorable association, i.e., a lower CH₄:CO₂ ratio with increasing AC in the milk. In general, the results reveal heritable enteric CH₄ emissions and differences between breeds that might be caused by distinguishing metabolisms. Furthermore, the observed undesired correlation between the CH₄:CO₂ ratio and AC raises concerns regarding the use of this trait for genetic evaluations. However, further studies to illuminate the genetic relationship between CH₄ emissions and dairy health in more detail by applying direct health traits are required.

Session 1

Theatre 14

Genetic variation in the cow's response to methane-mitigating feed additives

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Feed additives such as 3-NOP can reduce the dairy cow's methane (CH₄) emissions. The individual response to methane-mitigating feed additives may vary among cows. Our study aimed to examine whether there is genetic variation in the cow's response in CH₄ emission after receiving 3-NOP. Data was available from a project run by MetanHUB (www.metanhub.no) where the CH₄-mitigating effect of 3-NOP (Bovaer®) was tested for dairy cows from June 2023 to June 2024. The trial took place in a commercial dairy herd and included 79 Norwegian Red cows. Methane emissions were measured by GreenFeed. A total of 14,166 daily CH₄ measures were analyzed and the overall mean (standard deviation) was 387 (96) grams CH₄ per cow per day. The response trait was defined as the cow's change in CH₄ after introducing 3-NOP in the diet compared to the cow's "base level CH₄" in periods without 3-NOP. Cows were in 2 groups, where 54 cows received between 1.2 and 1.5 grams 3-NOP per day, and 25 cows were in a control group without any feed additives. The base level period consisted of 3 weeks in September and 4 weeks in January/February. The trait daily CH₄ (gram per day) was analyzed with a linear animal repeatability model with fixed effects of 3-NOP group, parity, lactation week, and test-day, and random animal genetic and permanent environment effects. Fixed effect solutions from this model were used to compute "Yield Deviations" for CH₄ (YD_CH₄), a corrected phenotype, i.e. daily CH₄ corrected for effects of parity, lactation stage, and test-day. For cows fed 3-NOP, we computed their base level CH₄ as the average YD_CH₄ from the periods without 3-NOP. The trait of interest, CH₄-response, was then calculated as YD_CH₄ minus base level CH₄. We had a total of 7,293 records of daily CH₄-response from 42 cows. The trait CH₄-response was analyzed with a linear animal repeatability model with fixed effect of test-day and random additive genetic effect of cow. Although this is a small dataset and results should be interpreted with caution, the estimated heritability of 0.15, with standard error 0.03, suggests that genetic variation for response in CH₄ emission after receiving 3-NOP feed additives exists.

Impact of introducing methane emission traits in the breeding goal for Polish Holstein-Friesian cattle

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Livestock, especially ruminants, produce methane and other greenhouse gases, contributing to climate changes. One potential solution to reduce methane production is selective breeding. Yet, the impact of including methane traits into breeding goals is largely unknown. In Polish Holstein-Friesian cows the selection index consists of six sub-indices: production traits and somatic cell count, conformation traits, fertility traits, calving ease-calf survival, longevity, milkability traits. The aim of this study was to evaluate the effect of incorporating methane emission traits into selection index for Polish Holstein-Friesian cattle on the genetic gain of traits currently included in the breeding goal and the potential of reducing greenhouse gas production in the next generation. The simulation of various breeding scenarios was done using the Modular Breeding Program Simulation (MoBPS) package in R. The following strategies were used: selection based on Polish-Holstein breeding program and compare to additional selection that included methane emission. Genetic parameters were obtained from existing breeding program. Genetic correlation between methane emission and these traits were also taken into account, and various scenarios for the impact of selection intensity and the weighting of traits were evaluated. The finding from this study, can be used to evaluate the effect of inclusions methane emission into cattle breeding program. The European Union's Horizon Europe research and innovation program provided funds for this work under grant agreement no. 1059609 (Re-Livestock project).

Session 1

Poster 16

Differential gene expression analysis of blood transcriptome and enrichment of potential cell types involved in beef methane production

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Introduction Climate change has emerged as an increasingly pressing issue, particularly regarding the need for reducing greenhouse gas emissions, which are a primary contributor to global warming. Among the various sources of greenhouse gases, ruminal fermentation stands out as a significant contributor to methane (CH₄) emissions. Objective This study aimed to investigate the blood transcriptome and methane production, measured by the GreenFeed system, in beef cattle that originated from a dairy herd. The objective was to identify gene expression patterns and cell types associated to methane emissions in dairy beef. Materials and Methods Total 12 blood samples from dairy beef were exposed to high-throughput RNA sequencing. The raw data were underwent quality control, trimming, genome alignment and read count by using FastQC, V0.11.9, Trimmomatic, 0.39, STAR, 2.7.11b and FeatureCounts, 2.0.2 tools, respectively. Differential gene expression analysis using DESeq2 was performed on the produced count matrix to explore the differentially expressed genes (DEGs). To enhance the understanding of the involved cell types, PanglaoDB was utilized to identify potential cell types involved in methane production. Results In the present survey several cell types were enriched, including Enterochromaffin Cells, Crypt Cells, Enteric Glia Cells, Parietal Cells, Chondrocytes, Oligodendrocytes, Dendritic Cells, Germ Cells, and Olfactory Epithelial Cells. Conclusion The findings indicated that the identified cell types predominantly reside within neural, immune, and endocrine tissues, suggesting that these three systems actively participate in the ruminal methane production of beef cattle. Keywords: Ruminal methane, Beef, Transcriptomics, Cell type enrichment

Characterization of methane emissions in autochthonous Spanish dairy sheep breeds

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Small ruminants are responsible for 7% of greenhouse gas emissions from livestock farming, which in dairy sheep is mainly attributed to enteric methane (CH₄). Genetic selection of low-methane emitter animals is a promising mitigation strategy because it is cumulative and permanent over time. For this, it is essential to collect and accumulate phenotypic data to estimate the variability of the trait, as well as characterize it at individual level in the population under selection. Thus, the objective of this work is to make a first characterization of methane emissions by analysing different phenotypes collected in experimental herds of Manchega and Latxa Cara Negra autochthonous dairy sheep breeds. Data on CH₄ emission in exhaled air were taken three times per month during the same week, throughout the milking period (5 months), on 100 Manchega and 120 Latxa ewes. A total of 1375 Manchega and 2136 Latxa records were collected using a portable non-dispersive infrared methane detector. Based on this, the following phenotypes were calculated on a daily and monthly basis: CH₄ concentration (ppm), CH₄ production (g/day) and CH₄ intensity (g/milk yield). Variance components were estimated together with genomic information (50K SNP), using the AIREMLf90 program. Phenotypic means were 1090.36 ppm, 56.62 g/day, and 29.42 g/milk yield, with a low to moderate correlation between phenotypes (0.35-0.60). Estimated heritability was low, ranging from 0.004 to 0.10, being higher for methane intensity (on a daily or monthly basis). Repeatability was estimated between 0.10 and 0.19, which might indicate the necessity to record more phenotypes on these animals to have more consistent results. Moreover, the exploration of other phenotypes of emission would be advisable to define the most appropriate one to be consider for implementation in Manchega and Latxa breeding programs.

Session 2

Theatre 1

Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants—Approaches and recommendations

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Antimethanogenic feed additives (AMFA) can considerably mitigate enteric methane (CH₄) emissions from ruminants. Mathematical models are essential for understanding and predicting the quantitative impact of AMFA across diverse diets and production systems. This study provides a comprehensive overview of modelling methodologies, concluding with recommendations for quantifying AMFA's impact in support of farm-level, national, regional, and global inventories. Key considerations encompass the type of models employed (i.e., empirical, machine learning, and mechanistic models), alignment of modeling objectives, data availability and quality, modeling synergies and trade-offs associated with using AMFA, and model applications for enhanced understanding, prediction, and integration into higher levels of aggregation. Careful evaluation of additive dosage, delivery methods, and transient effects is essential, the chosen modeling approach should align with specified objectives, and synergies of diverse modelling methodologies should be used to improve understanding and accurately predict AMFA's impact on CH₄ emissions.

Recommendations for identification and selection of bioactive compounds to develop anti-methanogenic feed additives

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This work provides technical recommendations and guidelines for research to identify and screen different compounds to reduce enteric methane (CH₄) emissions before they can be further assessed in vivo. For the initial identification of anti-methanogenic feed additives (AMFA) candidates, two approaches can be used: mechanistic (combining computational tools -in silico- and docking studies, using a modelling technique to predict how target enzymes interact with candidate molecules, and then synthesizing the candidate compounds), and empirical (finding published evidence of specific bioactive effects, by searching different repositories, followed by obtaining bioactive material from the identified source). While both approaches offer potential to identify a large number of compounds, sophisticated equipment and specific expertise are required for computational/modelling work and extracting bioactive compounds from a biological matrix, respectively, in mechanistic and empirical studies. Once candidates have been identified, the next step is to evaluate experimentally the activity and there is an array of in vitro methodologies that can be used for this purpose (enzymatic assays, pure cultures of methanogens batch cultures and continuous or semi-continuous cultures). We will present and discuss the main aspects to consider when using different approaches, including identifying the target enzymes to study, developing the enzyme material for running inhibition kinetics, culturing conditions and selection of archaeal species to study and the processing of the rumen inoculum used for experiments conducted using batch cultures and fermenters. Critical overarching methodological considerations for all in vitro approaches are: i) appropriate experimental design and statistical tests, ii) selection of doses depending on the nature and mode of action of the AMFA, iii) careful preparation of solid or liquid formats for appropriate delivery of the additives and iv) when and how to measure CH₄ and units to express its production.

Session 2

Theatre 3

Recommendations for testing enteric methane-mitigating feed additives in ruminant studies

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There is a need for rigorous and scientifically-based testing standards for enteric methane mitigation technologies, including anti-methanogenic feed additives (AMFA). Recommendations include study design and statistical analysis of the data, interactions with diet composition, associative effect of AMFA with other mitigation strategies, appropriate methods for measuring methane emissions, production and physiological responses to AMFA, and effects on animal health and product quality. Animal experiments should be planned based on clear hypotheses and experimental designs must be chosen to best answer scientific questions, with pre-experimental power analysis and robust post-experimental statistical analyses being important requisites. Experimental conditions should be representative of the production system of interest, to ensure that results and conclusions are applicable and practical. To explore additivity and synergism, as well as trade-offs, including relevant manure emissions, AMFA may be combined with other methane mitigation strategies and studied in appropriately designed experiments. Methane emissions can be successfully measured, and efficacy of AMFA determined, using respiration chambers, the sulfur hexafluoride method, and the GreenFeed system. For proper assessment of an AMFA, it is critically important that representative animal production data are collected and reported. In addition, evaluating the effects of AMFA on nutrient digestibility, animal physiology, animal health and reproduction, product quality, and how AMFA interact with nutrient composition of the diet is necessary and should be conducted at various stages of the evaluation process. The authors emphasize that enteric methane mitigation claims should not be made until efficacy of AMFA is confirmed in animal studies designed and conducted considering the guidelines provided

Feed additives for methane mitigation: How to account for the mitigating potential of antimethanogenic feed additives – Approaches and recommendations

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Here we examine the approaches used for accounting of enteric CH₄ abatement through the use of AMFA in livestock systems across scales. This accounting process starts with the ruminant animal, with estimates obtained from simple empirical emission factors or equations, to complex process-based models. The accounting needs to consider the potential synergies and trade-offs in GHG emissions at levels before and after being offered to the animal. The choice of methodology and level of complexity must be tailored to the targeted scale of analysis, the availability of input data and the accounting objectives. Assumptions on the additivity of AMFA abatement should follow insights from experimental work, and in the future, from models as these continue to improve the simulation of combined AMFA efficacy and effectiveness. Collectively, the accounting of enteric CH₄ abatement by feed additives remains to be fully assessed beyond experimental results to address pragmatism, potential for adoption and societal acceptance.

Session 2

Theatre 5

A guideline to uncover the mode of action of anti-methanogenic feed additives for ruminants

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We discuss guidelines of research required for understanding the mode of action of anti-methanogenic feed additives (AMFA). According to their mode of action, AMFA can be classified into 4 categories: 1) lowering H₂ production; 2) inhibiting methanogens; 3) promoting alternative H₂-incorporating pathways; and 4) oxidizing methane. In-depth research should be conducted only after the AMFA effectiveness and safety have been established. Research questions guiding this investigation should cover 5 perspectives: 1) microbiology, identifying the targeted microbes and the potential side effects on non-targeted microbes; 2) cell and molecular biochemistry; identifying the active compounds, elucidate mechanisms of action on targeted molecules, and investigating microbial resistance and adaptation; 3) microbial ecology, analyzing the effects of on microbial communities, shift in metabolic pathways, accumulation of fermentation products and AMFA degradation; 4) animal metabolism; studying effects on DMI, digestibility, absorption, metabolism, excretion, and safety; and 5) holistic approach; describing the modulatory effects of the diet, type of animal and management on the mode of action and effectiveness. In conclusion, complementary approaches are required to fully understand the mode of action of AMFA at various levels, moreover addressing critical knowledge gaps is essential for the successful adoption of AMFA.

Regulations and evidence requirements for authorization of enteric methane-mitigating feed additives

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A review of the regulatory and evidence requirements for authorizing anti-methanogenic feed additives (AMFA) for ruminants was conducted for seven jurisdictions. The objectives were to provide an overview to help applicants and scientists understand evidence requirements to meet regulations, identify differences and similarities between jurisdictions, and provide recommendations. Regulations protect animal health, promote food safety, and prevent unsafe practices and misleading claims. These mandatory rules cover ingredient safety, manufacturing practices, product labeling, and permissible limits for substances. The intended use is the specific purpose for which AMFA are authorized and determines the evaluation and approval process. This includes practical aspects of administration like active ingredients, dose, mixing, and feeding frequency. Each jurisdiction has unique criteria for legally classifying AMFA, making it challenging to meet all classifications with the same scientific evidence. However, all jurisdictions need robust evidence on efficacy, safety, and product quality, despite different study requirements. The applicant must provide the scientific evidence. Applicants and scientists must consult with authorities before designing studies. Scientists conduct efficacy and risk assessments following good scientific practices that inform regulatory decisions. Collaboration with regulatory agencies may refine legal classifications to improve harmonization. Educating stakeholders about AMFA's advantages and proper use encourage correct usage increasing understanding and transparency.

Session 2

Theatre 7

Long-term effects of 3-nitrooxypropanol on methane production, fecal bacteria and blood immune cells in dairy cows

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The compound 3-nitrooxypropanol (3-NOP) is known to effectively reduce methane emissions, but its long-term effects and possible effects on immunity are not well studied yet. Therefore, we investigated effects of 3-NOP on performance, methane production, fecal bacteria and blood immune-related cell profiles in a one-year feeding trial with 28 Holstein cows. Half of the cows were offered 80 mg/kg DM 3-NOP via concentrate feed, administered up to 5 times daily, alongside a season-dependent basal diet composed of silage, hay, and, from May to August, night pasture. The other half served as controls (CON), receiving concentrate feed without 3-NOP and the same basal diet as 3-NOP cows. Cows started the trial in early lactation and ended in the dry period or early lactation of the subsequent calving. Feed intake, milk yield, and methane production (via GreenFeed®) were recorded continuously. Fecal and blood samples were taken every 3 months and analyzed by flow cytometry. Data were analyzed using linear mixed models, with treatment, experimental month and their interaction as fixed, and animal as random effect. Feed intake in the barn and milk yield were not affected by treatment, mean 3-NOP intake was 1426 mg/d. Methane was reduced on average for 10% by 3-NOP, varying by experimental month. In months +1 and +12, feces of 3-NOP cows contained less archaea, but a higher percentage of IgA-coated archaea, compared to those of CON cows. Total fecal IgA abundance was not altered by 3-NOP supplementation. Throughout the experiment, immune cell subsets did not differ between 3-NOP and CON cows, neither in number nor percentage. However, 3-NOP cows had less polymorphonuclear cells than CON cows in month +3. In conclusion, the relatively low methane reduction is likely explained by the high dietary NDF content and the interval-fed 3-NOP. A possible IgA-mediated mechanism in the reduction of archaea after 3-NOP treatment requires further investigation.

Effect of a novel calcium peroxide based dietary additive on performance and methane emissions

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This study aims to evaluate the effect of dietary inclusion levels of a novel calcium peroxide-based feed additive on beef cattle's production performance and methane emissions. Seventy-two spring-born Charolais cattle (466kg; 15 months of age) were randomly assigned to one of the four dietary inclusion levels of CaO₂, based on total dry matter intake. The control diet with 0% CaO₂ inclusion (CON), a low inclusion diet with 0.4% CaO₂ (LID), a medium inclusion diet with 1.2% CaO₂ (MID), and a high inclusion diet with 2.0% CaO₂ (HID) (n=18 per group). All animals were fed a basal diet containing 60% grass silage and 40% concentrate. Animals received their respective treatments over a 12-week period, during which dry matter intake (DMI), average body weight (ABW), average daily gain (ADG), feed efficiency (FCE) and gaseous emissions (C-Lock Inc., Rapid City, SD) were measured. The average weekly individual DMI, growth, and gaseous emissions data were analysed using a linear mixed model in R studio. The results were reported as least square means with significant differences at $P \leq 0.05$. Total DMI varies across the treatment groups with animals offered MID having the lowest (8.91 kg/day) compared to CON (9.10 kg/day) and HID (9.11 kg/day) ($P < 0.001$). Growth performance improved with CaO₂ inclusion, as HID had the highest ABW (507 kg), ADG (1.16 kg/day), and FCE (0.13 kg ADG/kg DMI) ($P < 0.001$). Absolute CH₄ emissions were lower in MID and HID (211 and 209 g/day respectively) compared to CON (233 g/day) and LID (228 g/day) ($P < 0.001$). Animals offered HID had the lowest CH₄ yield with a reduction of 2.6, 2.3 and 0.6 g/kg DMI compared to the CON, LID, and MID treatment groups, respectively ($P < 0.001$). Similarly, animals offered HID had lower CH₄ intensity up to 43, 52 and 13 g/kg of ADG compared with CON, LID, and MID groups, respectively ($P < 0.001$). Supplementing a forage-based diet with 2.0% CaO₂ improved growth rate and feed efficiency in beef cattle while reducing methane emissions and intensity up to 10% and 19%, respectively.

Session 2

Theatre 9

Dose Dependent Response of L-Carvone on Methane Production and Digestibility in Ruminants: An In Vitro Study

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There is growing interest in natural feed additives to reduce ruminal methane emissions. This study evaluated L-Carvone (LC), an essential oil compound, for its dose-dependent effects on fermentation, in vitro dry matter digestibility (IVDMD), and methane production. Three treatments were assessed: control (0 µL/L), LC250 (250 µL/L), and LC500 (500 µL/L), using a 55:45 forage: concentrate substrate (14.2% CP, 32.2% NDF). Fermentation parameters were analyzed using the Gas Endeavour system, and data were evaluated via linear mixed models with treatment as a fixed factor and experimental runs as a random factor. Pairwise comparisons were performed using Bonferroni correction. LC500 significantly reduced ($P < 0.001$) cumulative gas production at 24 h (157.1 vs. 170.5 vs. 185.6 mL/g substrate for LC500, LC250, and Control, respectively). IVDMD was highest in the Control, decreasing by 2.10% and 17.85% in LC250 and LC500 ($P < 0.005$). Total volatile fatty acids and the acetate-to-propionate ratio declined with LC500 ($P < 0.005$), accompanied by a substantial reduction in methane yield (28.24% vs. Control, $p < 0.001$). LC250 lowered methane by 5.41% while maintaining digestibility, but its methane-to-gas ratio was slightly higher than the Control ($p = 0.015$). In conclusion, LC250 effectively reduced methane production while maintaining digestibility, whereas LC500 had a stronger methane reducing effect but compromised digestibility. Further studies on intermediate doses are needed to optimize LC's role in rumen fermentation.

Medium-Chain Fatty Acid Products Derived from Agriculture and Food Production Sidestreams Decrease Cattle Greenhouse Gas Emissions in Vitro

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Eructation of greenhouse gases from cattle represents an environmental concern and can significantly impact cattle's energetic metabolism. This study was designed to assess the effectiveness of medium-chain fatty acids (MCFA) on ruminal greenhouse gas production (CH₄ and CO₂) and diet digestibility (IVDMD). The MCFA were produced by a novel bioprocess of microbial chain elongation using agriculture and food production sidestreams, resulting in the production of caproic acid (C6) and caprylic acid (C8). Seven different treatments were tested in in vitro fermentations that lasted 24 or 48 hours: 1) Basal diet with no inclusion of MCFA (CTRL); 2) C6 with maltodextrin (C6_P1); 3) C6 with maltodextrin and gum arabic (C6_P2); 4) C8 with maltodextrin (C8_P3); 5) C8 with maltodextrin and gum arabic (C8_P4); 6) C6 salt (C6_Salt); and 7) C8 salt (C8_Salt). The MCFA were added to the CTRL diet at 1% of dry matter to create the aforementioned treatments 2 to 7. As expected, IVDMD was greater for 48 than 24 hours (57.3 vs. 42.6%; $P<0.001$). No treatment-by-time interaction was observed for IVDMD ($P=0.56$), but an overall effect of treatment was observed ($P=0.03$) with C6_P2 having the highest overall IVDMD (51.21%), which differed from CTRL (48.98%). There was no difference in the total volume of gas produced by any treatment ($P=0.58$). However, the percentage of CH₄ was lowest ($P<0.001$) in C6_P1, followed by C8_P3, and both differed from CTRL. The percentage of CO₂ was lowest ($P<0.001$) in C8_Salt, and all the MCFA treatments had lower CO₂% than CTRL. Furthermore, CH₄ yield was lowest ($P=0.001$) in C6_P1, which differed from CTRL (73.39 vs. 78.52 mL/gDMD). The CO₂ yield was lowest ($P<0.001$) in C8_Salt, and all the MCFA treatments had lower CO₂ yield than CTRL. Although additional studies are currently being performed with the MCFA, these initial results reveal that these novel products can significantly reduce in vitro production of greenhouse gases from ruminal fermentation.

Session 2

Theatre 11

Lysozyme supplementation reduces methane emissions in Italian Holstein Heifers

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The objective of the study was to assess the effect of lysozyme supplementation at various concentrations on enteric methane emissions, performance, rumen metabolism, and overall health of Italian Holstein heifers. The trial was performed at the Research center (CZDS) in Lodi on 36 heifers housed in a free stall barn. The trial lasted 42 days and the animals were divided into 4 homogenous (age, weight) experimental groups ($n=9$). Each group was fed with an experimental diet: a control diet (C), a diet supplemented with 2,000 U of lysozyme (T2), a diet supplemented with 4,000 U of lysozyme (T4), a diet supplemented with 10,000 U of lysozyme (T10). Heifers were fed with a total mixed ration (TMR) diet ad libitum once daily (12 am). Feed intake and dry matter intake were measured daily and individually thanks to the Roughage Intake Control (RIC) system. Live body weight (LBW), body condition score (BCS), withers height, and heart girth were measured weekly. At day 0, 21 and 42 of the trial, samples of urine and blood were collected. Ruminal fluid samples were collected at day 0 and 42 of the trial. Concurrently, methane emissions were recorded using the Laser Detector Methane System. Data were analyzed by mixed procedure of SAS for repeated measurements. Significance was set up for $p<0.05$. No significant differences were observed among the experimental groups for feed and water intake, LBW, BCS, withers height, heart girth, and feed conversion ratio. The administration of lysozyme did not influence urinary and ruminal pH, nor did it affect the parameters of blood analysis, maintaining the ruminal, metabolic and overall health of the animals. Regarding the enteric methane emissions, instead, our study demonstrated that the administration of 10,000 U and 4,000 U of lysozyme significantly reduced ($p<0.05$) the enteric methane emission peaks at day 42, compared to C. We can conclude that under our study conditions, the inclusion of lysozyme in heifers' diet significantly reduced methane emissions after 42 days of treatment, without negatively impacting performance and health status of the animals. Keywords: methane production, lysozyme, heifers

Optimizing Essential Oil Mixtures: Synergistic Effects on Rumen Fermentation and Methane Emissions

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The integration of essential oil (EO) combinations in ruminant nutrition presents a sustainable approach to enhancing fermentation efficiency while mitigating methane (CH₄) emissions. This study employed two 24-hour in vitro experiments to evaluate the synergic effects of different blends of EOs on rumen microbial fermentation. In Exp.1, five EOs were assessed into two different combining strategies: Strategy 1 with 10 different combinations of thyme oil (THY), peppermint (PPM), and cinnamon leaf oil (CIN), and Strategy 2 with 10 different combinations of anise oil (ANI), clove leaf oil (CLO), and peppermint (PPM). Each blend of EO was tested at a consistent single dose of 400 mg/L, using an in vitro batch culture method determining: pH, ammonia-N, and volatile fatty acids (VFAs). Based on results from Exp.1, the two most effective EO blends, designated as T1 and T2, were selected for further evaluation in Exp.2 assessing their effects on total gas and CH₄ production. All treatments were added to a rumen fluid-buffer mixture, with a substrate of 50:50 forage: concentrate diet at an initial pH of 6.6. In Exp.1, data were analyzed according to the Simplex Centroid Design using R-Studio. In Exp.2, analysis was conducted using the MIXED procedure in SAS. Results from Exp.1 identified a blend of CIN+PPM (80:20) (T1), and ANI+CLO (80:20) (T2) as synergistic. They demonstrated promising results by increasing total VFAs, reducing the Acetate: Propionate ratio, and decreasing ammonia-N concentration. In Exp. 2, both T1 and T2 demonstrated significant reductions in total gas and CH₄ production compared to the negative control. Between treatments, T1 demonstrated the highest reductions, resulting in a lower CH₄/total gas ratio when compared to T2 (4 vs. 7 %). These findings suggest specific EO blends can enhance rumen fermentation and reduce CH₄ emissions, supporting sustainable livestock production. However, in vivo studies are needed to optimize dosing and assess long-term effects.

Session 2

Poster 13

Short-term behavioural effects of 3-Nitrooxypropanol on dairy cows

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The feed additive 3-nitrooxypropanol (3-NOP), which is marketed as Bovaer® and EFSA-approved in the EU for use in dairy cows in doses between 60-100 mg/kg DM, is a tool to reduce enteric methane emissions. However, earlier studies reported that cows supplemented with doses of 60-80 mg of 3-NOP per kg DM reduced feed intake, which may indicate discomfort. Although the mechanisms underlying this behavioural change are currently unknown, the behavioural and welfare effects of 3-NOP on cows must be studied to ensure an environmentally viable and animal welfare oriented milk production. Hence, we examined the short-term behavioural effects of 60 mg of 3-NOP per kg DM on 46 healthy, loose-housed, primiparous, lactating Danish Holstein cows. This study was part of a larger nutritional study following a 3×3 Latin Square design with 3 21-d periods (14-d adaptation; 7-d sampling), in which cows started treatments 14-21 d after calving. Cows were grouped in 2 pens with 24 cows/pen. Groups were dynamic as cows were continuously housed upon calving and moved out at the end of the third period. Treatments – 3-NOP, nitrate (not examined), and control – were included in maize-based partially mixed rations (roughage:concentrate=55:45) offered in individual automatic feed bins with ad libitum access. Based on footage and using 1-min instantaneous sampling, individual cow behaviour was labelled twice daily (10.00-13.00h; 20.00-23.00h) on d18 and d20 relative to period start. Data (% undisturbed scans of each behaviour) was analysed with GLMM in R. When provided 3-NOP, cows reduced the % of time spent in upright posture (3-NOP:52.7% vs. Control:56.8%; SEM=2.8%; P<0.05) and tended to be more vigilant (Odds Ratio:4.6; SE=2.7; P=0.059). No effects of 3-NOP (P>0.1) on % of time spent lying, feeding, perching, self-grooming, and in social contact were detected. Knowing that 3-NOP also reduced feed intake (N=186 cows; 3.7% reduction; P<0.001), the increased standing and vigilance warrant further examination of the behaviour and welfare effects of 3-NOP on dairy cattle.

Effect of combining 3-nitrooxypropanol and direct-fed microbials on enteric methane emission, performance and rumen microbiome of lactating dairy cows

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We hypothesized that a combination of 3-nitrooxypropanol (3-NOP) with direct-fed microbials (DFM= *Propionibacterium acidipropionici* strain P169 (2.0×10^{11} CFU/g) + *Bacillus subtilis*, and *B. licheniformis* (3.2×10^9 CFU/g) would mitigate enteric methane (CH₄) and improve milk production in dairy cows by re-directing hydrogen into non-methanogenesis pathways. Forty-eight lactating Danish Holstein dairy cows were enrolled in the study. The experiment consisted of a 2-wk covariate period followed by a 12-wk experimental period. Four experimental diets including: (1) Control; (2) Control with 3-NOP (60 mg/kg dry matter); (3) Control with DFM; and (4) Control with 3-NOP and DFM. Individual feed intake was recorded using automatic feed bins. Milk yield was recorded automatically at every milking. Individual CH₄ emissions were measured using GreenFeed systems. On days 14, 56 and 91 of the experiment, buccal swabs were collected for rumen microbiome characterization. 3-NOP supplementation reduced CH₄ production and intensity (g/kg energy-corrected milk) by 11 and 14%, respectively ($P < 0.05$). Neither 3-NOP alone nor in combination with DFM had a significant effect on dry matter intake or milk production ($P > 0.05$). α - and β -diversity remained unchanged between treatments and control, yet differences were observed in microbial composition across the experimental time-points (d7 vs. d56 and d91). 3-NOP supplementation increased *Megasphaera elsdenii* abundance compared with control at d91 ($P < 0.05$). 3-NOP alone decreased CH₄ emissions whereas combination of 3-NOP and DFM did not provide additional CH₄ mitigation or improved production parameters. 3-NOP supplementation resulted in higher abundance of certain lactate utilizing bacteria such as *Megasphaera*.

Session 2

Poster 15

Effects of Dietary Palm Oil on Rumen Fermentation, Growth Performance, and Enteric Methane Emissions in Hanwoo Steers

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This study evaluated the effects of palm oil feed on rumen fermentation, growth performance, and enteric methane emissions in Hanwoo steers. Twelve steers with an average body weight of 698.42 ± 61.86 kg at approximately 25 months old, were assigned in a replicated 3×3 Latin square design with 3 treatments and 3 periods of 21 days. Steers were randomly assigned to three diet groups: CON (orchard grass + control concentrate), T1 (orchard grass + 2.5% palm oil concentrate), and T2 (orchard grass + 3.0% palm oil concentrate). Rumen fluid was sampled using an oral stomach tube to analyze rumen fermentation parameters. Steers were weighed at the start and end of each period to assess weight gain, average daily gain (ADG), and feed efficiency (FE). Enteric methane (CH₄) emissions were measured using the GreenFeed system. Data were analyzed with ANOVA using the GLM procedure of SAS and means were compared using Tukey's HSD test, with statistical significance set at $P < 0.05$. Results showed that palm oil significantly altered rumen fermentation parameters, influencing rumen pH, ammonia-nitrogen concentration, and levels of acetate, propionate, total volatile fatty acids (VFA), and the acetate to propionate ratio ($P < 0.05$). Steers fed palm oil feed had significantly lower weight gain, ADG, and FE than the control group ($P < 0.0001$). Moreover, steers fed palm oil demonstrated a significantly reduced dry matter intake, with increased forage and reduced concentrate intake ($P < 0.0001$). Methane production (21.26% and 22.19%) and intensity (19.16% and 20.96%) were significantly reduced in steers fed 2.5% and 3.0% palm oil diets, respectively ($P < 0.05$). However, CH₄ yield increased by 5.25% and 21.21% in steers fed 2.5% and 3.0% palm oil diets, respectively. Overall, the inclusion of palm oil feed in Hanwoo steer diets altered rumen fermentation parameters, and negatively affected growth performance by lowering weight gain, ADG, and feed efficiency, along with changes in feed intake patterns. While palm oil feed reduced total CH₄ production and intensity, it increased CH₄ yield, indicating higher methane production per unit of feed intake.

The effect of oregano oil on in vitro methane emissions

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Methane production of dairy cows is not only disadvantageous for the animal due to the loss of energy, but also an environmental issue. The objective of this study was to investigate the effect of oregano essential oil (OEO) (DOSTO® Liquid, Dostofarm GmbH, Westerstede, Germany) on ruminal in vitro total gas (TG) and methane (MET) production. Rumen fluid samples were collected from two non-lactating rumen-cannulated Dexter cows in the morning, immediately before the start of each run. Substrate samples (1 g ± 5 mg) consisting of 60 % ground lucerne hay, 10 % ground oat hay, 25 % maize meal, and 5 % soybean oil cake meal, were weighed out into 200 mL glass digesters. Volumes of 40 mL of rumen fluid inoculant and 160 mL of buffered incubation medium were added to each digester. The Gas Endeavour System (BPC Instruments) was used and during the 48-hours incubation period, gas measurements were recorded every 5 minutes. Doses of 0, 50, 100 and 200 µL of OEO per 100 mL of incubation medium was examined in six in vitro runs. Results were analyzed with ANOVA, using Statistica 14. Multiple comparisons (Fisher's LSD) were made post hoc to discern differences among treatments with a significance of $P < 0.05$. Measured against the negative control, TG production was increased in the 100 and 200 µL treatments, and MET production was decreased at all the OEO dilution levels (Table 1). When both TG and MET production are considered, it was concluded that rumen fermentation appeared to be improved at a dosage of 100 µL of OEO / 100 mL incubation medium. Further research is needed to identify the mechanisms by which oregano oil inhibits methane production in the rumen, e.g. studies involving dose-response trials on shifts in the rumen microbiome, and volatile fatty acid production and ratios. Table 1: Effect of different OEO dosages on ruminal in vitro total gas and methane production after 48 h of incubation

Parameter	OEO (µL/100 mL incubation medium)	P-value
TG (mL)	185a, 195ad, 212c, 200bd	< 0.01
MET (mL)	108a, 97b, 88c, 91bc	< 0.01
MET (% of TG)	58.5a, 49.5b, 41.5c, 45.5bc	< 0.001

Session 2

Poster 17

Evaluating the efficacy of combined additives to inhibit methanogenesis and enhance hydrogen utilisation in vitro

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The study assessed the efficacy of combining methanogen inhibitors, such as iodoform (IOD), bromoform (BRO), and 3-nitrooxypropanol (3NOP), with hydrogen-utilizing additives enhancing ruminal VFA synthesis (fumaric acid [FA], encapsulated fumaric acid [EFA], and phloroglucinol [PHG]). Each methane inhibitor was tested in combination with FA, EFA and PHG, resulting in nine treatments. The dosages, expressed as % of DM incubate, were: 0.001% for BRO, 0.01% for IOD, 0.04% for 3NOP, 3% for both FA and EFA, and 5% for PHG. Treatments were evaluated on a 1 g of TMR diet (60:40 F:C ratio) in three in vitro incubations, alongside a control diet (CON). Gas production was continuously monitored for 48 h while methane (CH₄) and hydrogen (H₂) concentrations were measured at 2, 4, 8, 24 and 48 h. At the end of the incubation, pH was recorded, and samples were collected for dry matter digestibility (DMD), ammonia nitrogen (NH₃-N), VFA and microbiota evaluation. The pH, gas production and composition were affected by treatments ($P < 0.01$) and differences between treatments were assigned using Tukey's HSD test. BRO and IOD showed similar pH to CON, while 3NOP reduced it. Gas production (mL/g DM) at 48 h was 5% higher in BRO combinations and 7% lower in IOD combinations compared to CON. At 48 h, CH₄ production (mL/g DM) was zero in IOD treatments, while BRO and 3NOP decreased CH₄ production, respectively by 70 and 90% compared to CON. At each measured time points, H₂ proportion of BRO combinations were similar to CON, whereas 3NOP combinations had higher H₂ production. IOD combinations accumulated H₂ in the first 8 h, although to a lesser extent than 3NOP; however, H₂ production was similar to CON at 48 h. All treatments reduced CH₄ production without negative effects on DMD. The changes in gas composition during the incubations, especially for H₂, highlight the importance of evaluating the effect of additives over time. The NH₃-N, VFA, and microbial data will provide valuable insights into ruminal fermentation responses and microbial adaptations.

Evaluation of the efficacy of three different additives for reducing enteric methane emissions in vitro
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Livestock farming is responsible for 14.5% of anthropogenic greenhouse gases (GHGs) emissions, with 46% of these represented by enteric methane (CH₄) released by ruminants, mainly through belching. Enteric methanogenesis not only impacts the environment but also causes animal energy loss of 2 to 12%, depending on diet. Several feed additives were tested to reduce enteric CH₄ emissions, but with contrasting results. This trial aimed to determine the in vitro efficacy of three additives (biochar, tannins and an essential oil blend) in reducing CH₄ emissions. The procedure involved the incubation of 3 g of a concentrate for beef cattle using the Ankom RF Gas Production system with three concentrations of each additive: 0%, 1.9%, and 3.8% (DM basis), corresponding to 0, 333, and 667 mg/L, respectively. High concentrations were used to increase additive–microorganism interactions in the fermentation substrate, consisting of ruminal fluid sampled at the slaughterhouse from beef cattle and buffer solution. The efficacy of the additives was investigated after 24 hours of incubation at 39°C by measuring total gas production (GP24) and CH₄ concentration, the latter analysed by gas chromatography. Data were analysed using the GLM procedure in SAS 9.4, considering additive type and concentration as the main effects. The results showed a statistically significant reduction in GP24 when the tannin additive was used. It reduced GP24 by 11.0% and 14.1%, at the lowest and highest doses, respectively. No statistical differences were observed with the other two additives. The absence of CH₄ reduction, even at high doses, may be attributed to factors such as the low fibre content of the concentrate, the limited methanogenic population in the ruminal fluid, and experimental setup constraints from the in vitro trial. Further tests with ruminal liquids collected from animals fed different diets will be necessary to determine the relevance of the above variables.

Session 2

Poster 19

Evaluation of alternative additives on in vitro ruminal fermentation, gas production kinetic, and enteric greenhouse gas emissions

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The objective of the present study was to evaluate alternative additives on in vitro ruminal fermentation, gas production (GP) kinetic, and enteric greenhouse gas emissions in finishing diets for feedlot cattle. Three treatments were arranged: 1) Basal diet (Control; no additives inclusion); 2) Basal diet + Kingbrown (0.5 g/ kg of DM); and 3) Basal diet + Multienzyme (0.25 g/ kg of DM). Kingbrown® (MixScience Animal Health and Nutrition, Bruz, France) is composed by chestnut tannin extract (hydrolysable polyphenols). Multienzyme® (Beijing Smile Feed, Beijing, China) is composed by beta glucanase (*Trichoderma longibrachiatum* – CGMCC 7290), cellulase (*Trichoderma longibrachiatum* – CGMCC 7289), xylanase (*Aspergillus oryzae* – CGMCC 7.70). An in vitro GP system with 12 bottles (AnkomRF) was used in four consecutive fermentation batches to evaluate total GP (TGP), volatile fatty acids (VFA), enteric methane (CH₄) and carbon dioxide (CO₂). The basal diet consisted of a typical finishing beef cattle's diet (10:90 roughage: concentrate ratio). The dosages were calculated proportionally considering a rumen of 80 L according to manufacturing recommendations. The diet was formulated to meet the nutrient requirements of a beef non-castrated bull with an ADG of 1.8 kg/d (NRC, 2016). Data was analyzed using the software SAS 9.4 considering batch as experimental unit. No differences were detected in kinetic variables among treatments. Kingbrown had decreased GP (24h) compared to other treatments; but, after 48h, similar GP were detected among treatments. Multienzyme reduced propionate proportion compared to other treatments; however, it did not differ from control. Finally, Kingbrown and Multienzyme reduced CH₄ production, by □ 29% in terms of mmol, mmol/g dry matter, mmol/g organic matter, and mmol/g organic matter digestibility compared to control treatment.

Evaluation of neem cake on in vitro ruminal fermentation, gas production kinetic, and enteric greenhouse gas emissions

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The objective of the present study was to evaluate increasing doses of Neem cake (*Azadirachta indica*) as well as a unique monensin (positive control) dose on in vitro ruminal fermentation, gas production kinetic, and enteric greenhouse gas emissions in finishing diets for feedlot cattle. Four different doses were tested independently, resulting in 6 treatments (negative control + 4 doses of neem cake + positive control MON). The basal diet consisted of a typical finishing beef cattle's diet (15:85 roughage: concentrate ratio) and was composed by the following treatments: negative control (CON; basal diet with no additive); Neem cake at levels of 240, 480, 720, and 960 mg/kg DM; and monensin as positive control at 30 mg/kg DM. The dosages were calculated proportionally considering a rumen of 80 L according to manufacturing recommendations. The diet was formulated to meet the nutrient requirements of a beef non-castrated bull with a ADG of 1.8 kg/d (NRC, 2016). An in vitro gas production (GP) system with 12 bottles (AnkomRF) was used in four consecutive fermentation batches to evaluate total GP (TGP), volatile fatty acids (VFA), enteric methane (CH₄) and carbon dioxide (CO₂). Supplementation levels of each experiment were analyzed for linear and quadratic responses using the software SAS 9.4. Slight effects were detected for Neem cake supplementation on gas production kinetics and VFA profile. At highest dose (960 mg) neem cake had similar VFA production to monensin supplementation. In addition, Neem cake increased BCVFA linearly compared to monensin. Further, Neem cake was effective in increasing pH of ruminal fluid compared to monensin and quadratically increased ammonia-N; however, it did not affect greenhouse gas emissions.

Session 3

Theatre 1

ROH: Reflecting On Homozygosity - from inbreeding to adaptation

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Genetic diversity is the foundation of evolution and breeding. In breeding programs, maintaining population-level diversity and minimizing inbreeding is key. With the rise of studies on runs of homozygosity (ROH) in livestock and wildlife, ROHs have become a standard tool in genomic population assessments. The genomic inbreeding coefficient (F_{roh}) now complements the pedigree-based F_{ped}, providing an alternative measure of inbreeding. With increasing functional genomic understanding and routine whole-genome sequencing, a shift in perspective may be warranted. We can predict harmful mutations from sequence data with increasing accuracy. Rather than broadly minimizing homozygosity, a more targeted approach—focusing on the avoidance of deleterious alleles—could be more effective. Meanwhile, genomic selection has driven localized allele frequency changes, creating instances where homozygosity is not only tolerated but actively selected for. Now what has F_{roh} revealed about individual and population viability? ROHs play a dual role in genome studies, offering insights into both inbreeding and selection. In this talk, I reflect on over a decade of ROH research in livestock, exploring emerging general patterns and the key insights gained.

Mining genomic data in Reggiana cattle genetic resource to identify and manage potential deleterious alleles

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The Reggiana cattle breed, native to the Emilia-Romagna region in the North of Italy, holds cultural and economic value due to its exclusive production of mono-branded PDO Parmigiano-Reggiano cheese. The conservation of this genetic resource is crucial for the sustainability of the value chain linked to this breed. The selection of animals in breeding management is a key factor in preventing inbreeding depression and the emerging disadvantageous alleles. High-throughput genotyping technologies offer a cost-effective solution for analysing livestock populations at a genome-wide level. This study aimed to identify potentially disadvantageous alleles or haplotypes in the Reggiana breed population combining different genomic information. A total of 4,000 Reggiana cattle, representing nearly the entire breed, and including 280 sires, was genotyped using the GeneSeek GGP Bovine 150k SNP chip and the genome of approximately 100 cattle was sequenced at 20X. Through the use of PLINK 1.9, SHAPEIT and ad-hoc scripts, several low-frequency alleles or haplotypes, which were never identified in homozygous state and located in genomic regions known to harbour reproduction relevant genes, were detected to be potentially associated with reduced fertility, stillbirths, and postnatal mortality. The study revealed up to 143 carriers of undesirable alleles, underscoring the importance for careful breeding strategies. These findings highlight the need of targeted breeding practices to mitigate the risks posed by these alleles while preserving diversity. The results could play a key role ensuring the long-term sustainability of the Reggiana breed and serve as a model for other local livestock conservation efforts. Funded within the AgriTech National Research Center (Spoke 1) and the European Union Next-GenerationEU under the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.4, Call D. D.3138 published on 12/16/2021 amended D.D. 3175 on 18/12/2021, CN00000022 – CUP J33C22001150008

Session 3

Theatre 3

Genetic and Historical Diversity of Horses: Local Breeds in France

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Working equines (horses and donkeys) historically played a significant role in France's social and economic development. However, technological advancements limited their traditional uses, leading to the demographic decline and a global reduction in the genetic diversity of these species. While in-situ conservation and dedicated breeding programs exist, they rely on incomplete or shallow pedigree information. To overcome these limitations, we investigated the usefulness of molecular markers to improve the management of these genetic resources. We sampled 609 animals from 17 local horse breeds (~35 per breed) using the IMAGE 10K SNP array. First, we conducted a genomic analysis aimed at retracing the breeds' demographic history. Second, we aimed at evaluating the impact of breed management on their genetic diversity using various pedigree and genomic-based metrics. For this, we benchmarked various estimators of kinship, inbreeding and effective population sizes, to propose the most suitable set of estimators to monitor their genetic diversity. The genetic structure of the breeds clearly distinguished light "territorial" breeds from heavier draft horses, and also indicated potential gene flow. Genetic diversity analysis with multiple estimators revealed potential consequences of different management practices, for instance: the Boulonnais breed had the highest inbreeding (0.201) and a moderate Ne/N ratio (0.038), while the Breton and Ardennais breeds exhibited lower inbreeding (0.081 and 0.100, respectively) and Ne/N ratios (0.010 and 0.018, respectively). This suggests a greater improvement potential for the Boulonnais despite its high inbreeding. These results led us to propose a new metric that combines kinship (expected inbreeding) and realised inbreeding estimates which can be applied to breed management. Our study demonstrates the usefulness of genomic markers, even at low density, in monitoring genetic diversity and evaluating the effectiveness of management programs. We also emphasize the need for cautious interpretation of molecular metrics in conservation programs.

Genomic index of diversity as a tool for management of genetic diversity in livestock populations

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Breeding programmes for livestock aim to improve several traits while maintaining long-term genetic variation. Although genotyping technologies provide tools for monitoring genetic diversity, integrating genomic estimates of diversity into breeding programmes remains challenging. This study addresses this gap by developing a genomic diversity index (GDI) composed of the inbreeding coefficient (FROH>16Mb), the average relatedness to the reference population, and a new indicator assessing the impact of individuals on allele frequencies at risk of disappearance. The founder population for the simulation was based on real genomic data from Holstein cattle, comprising 2,441 individuals with 31,978 markers on 29 chromosomes. Two scenarios were tested: one based only on breeding values and another considering both GDI and breeding values. The use of GDI showed a significant positive effect on genetic diversity and led to slower inbreeding accumulation (0.023 with GDI vs. 0.039 without GDI). Our simulations also showed a decrease in monomorphic alleles from 631 to 262 and higher observed heterozygosity (0.351 with GDI vs. 0.319 without GDI). However, considering genetic diversity as a selection criterion resulted in lower short-term genetic gain for all quantitative traits, with decreases of 19.55% for milk yield, 23.75% for fat yield, 20.22% for protein yield, and 27.61% for longevity after 25 generations. This pilot study emphasises the importance of balancing genetic gain and preserving genetic diversity to ensure the long-term adaptability and sustainability of livestock populations. This research was supported by grants APVV-17-0060 and APVV-20-0161, while the work of Ino Curik was funded by NextGenerationEU, project “NEXT GENERATION ANIMAL PRODUCTION” (NPOO.C3.2.R3-II.04.0141).

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Using Low-Coverage Nanopore Data to Assess Population Structure in an Endangered Pig Breed

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Genotyping arrays are the default technology for routine genomic characterization in breeding and conservation. They are highly efficient in terms of precision, speed and cost, but also afflicted with ascertainment bias and limited to a preselected SNP set. Cost efficient use often requires a minimum number of samples, which makes them unattractive for scenarios where small breeds or just small batches must be characterized. Long-read sequencing promises to overcome some of these issues, as it can be done even under field conditions and for very low numbers of samples. Additionally, it holds the potential for enhanced detection of InDels, structural variation and methylation. We use barcoded Nanopore sequencing to generate low coverage sequences of an endangered pig breed. Six animals were sequenced per flowcell, leading to a depth of coverage per sample from 1 to 9 X. Currently, 48 animals are finished. Samples are processed with a Dorado/ Minimap based pipeline. Stitch is used as a reference free imputation tool to generate genotypes for the positions of a commercially available 80 k Illumina array. All samples were also genotyped with the respective array. Covariance matrices are generated for both datasets. Both methods can consistently identify second degree relationships. The covariance matrices show a correlation of about 0.8, with line-by-line comparisons ranging from 0.62 to 0.9. This makes the current approach suitable for prioritization of animals in, e.g. a gene-banking context, but would require further improvement to precisely resolve looser relationships. As accuracy of an individual's estimates seems to be dependent on the product of relationship with other animals in the set times their depth of coverage, an ever increasing number of samples is expected to lead to further improvements.

Estimation of Demographic Parameters of Major Aquaculture Species Using PSMC and GONE

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Over the years, selective breeding has revolutionized aquaculture, with the implementation of genomics further accelerating the recorded increases in the genetic gain of production traits such as growth, disease resistance, and feed efficiency in some major economic species. To continuously improve the next generations for better welfare, environmental resilience, dietary adaptability, resistance to new strains of pathogens, and other complex traits through selective breeding, it is imperative to maintain high genetic diversity in the population to reduce inbreeding and the effects of selection pressure. The knowledge of how the current population genetic structure of major species has evolved is crucial for maintaining genetic variation for future selection. As a result, inferring demographic parameters such as the change in effective population size (N_e) over time is critical to understand how random ecological and evolutionary changes such as genetic drift, mutation, natural selection, and recombination in the population have affected genetic variability in our Atlantic salmon population. In this study, we used pairwise sequentially Markovian coalescent (PSMC) and GONE software to infer the ancient and more recent historical changes in N_e , respectively. For the PSMC analysis, whole genome sequence data for four individuals was aligned and mapped onto the reference genome using the BWA tools. For the diploid consensus sequences conversion and variant calling, we used samtools and bcftools, respectively. GONE leverages estimates of linkage disequilibrium between molecular markers in single nucleotide polymorphism (SNP) data. We expected to see a very recent decline in the effective population size because domestication and artificial selection of aquaculture species are relatively recent compared to terrestrial livestock. This ongoing study aims to generate an Atlantic salmon demographic model to expand StdpopSim, an open-source catalog containing simulation parameters of plants and animals.

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Demographic history of conserved Iberian pig populations inferred from genomic estimates of historical effective population size

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The Iberian pig is the source of some of the most prestigious pork products, and plays a crucial role in sustaining the ecologically rich dehesa landscapes of the southwestern Iberian Peninsula. The experimental herd at ‘Dehesón del Encinar’ consisting of the Guadyerbas and Torbiscal strains, serves as a key resource for preserving the genetic diversity of Iberian pigs. Guadyerbas is the only representative of the black hairless genetic type while Torbiscal originated from the mix of four ancestral populations, including Guadyerbas. Genomic estimates of the historical effective population size based on linkage disequilibrium have been previously obtained for both strains. However, the method used implicitly assumes that demographic events occurring in a given generation do not affect subsequent generations, making it reliable only for detecting linear population size changes. Recently, a novel method has been developed to identify sudden and drastic shifts in population size. This study aims to estimate the historical effective size of the Torbiscal and Guadyerbas strains using this novel method. A total of 217 Torbiscal and 219 Guadyerbas pigs were genotyped using the Illumina Porcine SNP60 BeadChip. In both strains, a sharp decline in effective population size was observed, about 21 and 16 generations ago for Guadyerbas and Torbiscal, respectively. This decline coincides with the establishment of the herd (Guadyerbas) and the creation of Torbiscal. Also, an increase in effective size was observed for both strains around 8 generations ago, aligning with the implementation of more effective management practices. These findings highlight the effectiveness of the new method in capturing the demographic history of populations. This work was funded by MCIN/AEI/10.13039/501100011033, Spain (grants FPI PRE2021-096997 and PID2020-114426GB-C2).

On the use of ROH length distribution to estimate inbreeding age and past effective population size

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In recent years, it has become common practice to date runs of homozygosity (ROH) classes based on their length, and also to use the frequencies of these classes to estimate past Ne. ROH reflect homozygosity-by-descent (HBD), which occurs when an individual inherits twice the same segments identical-by-descent from an ancestor through two distinct paths. Their length is related to the time to the most recent common ancestor (TMRCA), and their distribution has therefore proved useful in characterizing inbreeding and past demographic history of populations. Using simplified demographic scenarios, we have previously shown that estimating TMRCA from ROH classes using simple rules is inaccurate and that model-based approaches perform better. However, it remains unknown which generations of ancestors are captured by the different HBD classes. We have therefore updated our model to define HBD classes as intervals of past generations. This new feature facilitates the interpretation of the HBD partitioning by allowing to assign HBD classes to past generations of ancestors and by directly estimating inbreeding rates in the HBD classes. We then used SLiM to simulate a range of complex demographic histories. These realistic simulations were used to evaluate the usefulness of ROH and HBD classes for estimating TMRCA and the inbreeding levels, both of which are required to estimate past Ne. We first assessed whether the partitioning of inbreeding into different classes was consistent with the simulated values. Next, we measured the true TMRCA of segments assigned to different classes. In general, age estimation was relatively inaccurate. The ROH-based approach tended to underestimate TMRCA, while our model shifted the estimated TMRCA towards the main HBD class. Although the ZooRoH model outperformed ROH in most scenarios, it was less efficient in scenarios without a dominant demographic event. ROH-based approaches captured less inbreeding and overestimated Ne, especially at medium marker densities. True age in a ROH class depended not only on ROH length, but also on demographic history and parameter settings. In conclusion, more work is required to better understand the distribution of ROH or HBD segments in different classes under different demographic scenarios.

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Characterization of the genetic diversity of two main breeds used in Canada using genomic metrics

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By 2027, the Canadian ovine selection program will be upgraded to include genomics in estimation of breeding values. Over 8,500 sheep from which 3,000 Romanov and 3,000 Rideau Arcott, the two main breeds used in Canada, will be genotyped. Since selection programs are known to impact genetic diversity, its characterization before and during selection is crucial. The objective of this project is to characterize the initial genetic diversity of these breeds using genomic metrics. To date, data from 900 animals from each two breeds have been obtained using a custom 65K Illumina genotyping panel. Mean run of homozygosity-based inbreeding coefficients of 14.6% and 9.3% have been calculated for Romanov and Rideau Arcott sheep respectively. The intensive selection for scrapie resistance genotypes in Romanov, which is visible by a ROH around PRNP gene in over 50% of individuals, and the recent creation (1988) of Rideau Arcott from a composite of more than ten breeds, could explain this ranking. Estimated linkage disequilibrium-based effective population sizes of 108 and 179 individuals for Romanov and Rideau Arcott also supported that the former breed is slightly less diversified. These results, along with diversity trends calculated along the project and comparison with other Canadian breeds, will be a key referential to monitor genetic diversity once the implantation of the genomic assisted selection program. This project is supported by a grant from the Genomic Applications Partnership Program (GAPP) from Genome Canada and Genome Québec and a scholarship from the Natural Sciences and Engineering Research Council of Canada.

Utilizing Genome-Wide SNP Arrays to Predict Heterosis Effects for Body Weight, Body Temperature, and Blood Parameter Traits in a Heat-Stressed Advanced Inter-Cross Chicken Population

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The aim of this study was to estimate breed differences as well as dominance and epistatic components of heterosis using genome-wide local genetic ancestry for body weight, body temperature and blood parameters in the 18th and 19th generations of an advanced intercross line (AIL) of chickens derived from two highly divergent chicken lines (Fayoumi and commercial broiler). A total of 839 chickens with phenotypes and 431,399 SNPs (Affymetrix 600K) genotyped in 462 chickens were analysed. The local ancestry of the AIL population was derived from the genomic data of the AIL founders (six female Fayoumi chickens and one male broiler) using ELAI software. Genome-wide locus-specific breed ancestry was then used to estimate breed differences, dominance and epistatic loss components of heterosis. A linear model was used to predict heterosis, with sex and hatching period as fixed effects and additive, dominance and epistatic loss components as covariates. A genome-wide association study (GWAS) was also performed. Significant breed differences were found for most traits, while significant dominance was observed only for partial pressure of carbon dioxide (PCO₂), ionised calcium (iCa²⁺) and bicarbonate (HCO₃). Negative epistatic losses were significant for hatching weight, body weight at 28 days, HCO₃, PCO₂ and total carbon dioxide (TCO₂), while positive epistatic was observed for oxygen saturation and partial pressure. No significant GWAS signals were detected after Bonferroni multiple comparison corrections. The non-significant estimates of dominance indicate intermediate performance of AIL chicken compared to the mid value of ancestral pure breeds.

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Validating runs of homozygosity in pig populations

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Mating strategies and higher selection intensities can accelerate genetic progress, but they may also increase inbreeding and genetic diversity. Inbreeding is more accurately estimated through runs of homozygosity (ROH) than based on pedigree, as ROH refers to contiguous chromosomal segments with homozygous marker genotypes. Long ROH segments indicate recent inbreeding, while shorter segments reflect older inbreeding events. The aim of this study was to evaluate the accuracy of ROH detection at two different SNP densities (50K and 660K) in a total of 622,447 animals from seven different pig lines. Prior to ROH identification, 10% of the SNPs from the 50K panel and 1% from the 660K panel were masked. The validation of ROH was performed by estimating the IBD (Identity-By-Descent) probability of each masked SNP within an identified ROH region to be indeed homozygous. The results showed an IBD probability of 92.7% within ROHs for the 50K panel and 94.5% for the 660K panel. Longer ROH segments (greater than 16 Mb) exhibited higher IBD probabilities compared to shorter segments (less than 2 Mb). Specifically, for the 50K panel, the IBD probability ranged from 82.5% for segments shorter than 2 Mb to 97.6% for segments longer than 16 Mb. For the 660K panel, IBD probabilities ranged from 89.4% for the shorter segments to 98.6% for the longer segments. Minimal variability was observed between lineages, with short segments showing IBD probabilities from 81% to 86% (both panels) and long segments from 97% to 98% (50K) and 97% to 99% (660K). Thus, the comparison across both panels revealed similar validation values for all seven lines, indicating no line with less accurate ROH. The higher IBD probability obtained with the 660k panel than with the 50k panel highlights the importance of utilizing a higher-density SNP panel for inbreeding assessments. The next steps will include comparing inbreeding estimates and applying the findings to breeding programs for better genetic diversity management.

Optimum contribution selection applied to bull selection in a genomic cattle breeding program

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Optimum contribution selection (OCS) is a selection procedure that minimizes increase in coancestry, while maximizing genetic gain. Simulations and real data have shown the benefits of OCS over truncation selection. Nonetheless, OCS has to our knowledge not been widely adopted in dairy cattle breeding programs. Icelandic Dairy Cattle is a dairy breed local to Iceland with a genomic breeding program. In 2024, ca. 200 bull calves were genotyped. The bull calves with highest genomic predicted total merit are selected by modified truncation selection such that AI bulls are not too related. The current selection is ad hoc by limiting the size of the largest AI-sire offspring groups. This reduces the increase in coancestry but also reduces the rate of genetic gain. Selection accuracy affects genetic gain. We hypothesize that bulls selected based on OCS will have smaller changes of TMI with new information than bulls selected based on truncation. This would be explained by the OCS selected bulls being more diverse. The accuracy of selection of AI bulls can be approximated by the correlation of the bull calves predicted total merit at selection and the predicted total merit as proven bulls. The aim of this study is to compare the genetic gain and increase in coancestry observed when modified truncation selection and OCS is applied to a cohort of bull calves with a genomic TMI. We will use real data from AI sires in the Icelandic Dairy Cattle breeding program. Both genomic and pedigree OCS will be compared. We will compare the number of bulls selected, their genomic and pedigree relationship, and changes of TMI.

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Robust optimal contribution selection, accounting for uncertainty

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Managing the conversion of genetic variation into genetic gain is crucial for the long-term sustainability of breeding programs. Standard optimal contribution selection (OCS) balances short-term genetic gain with long-term genetic variation within the population by balancing the expected response to selection against the expected group coancestry weighted by the parameter λ . However, OCS assumes that the selection criterion is known without error, which is not the case when using phenotypic values or estimated breeding values (EBV). The aim of this work is to evaluate how to take into account the uncertainty and correlation of EBV in OCS. To address this issue, we used robust optimization when solving for OCS. The approach extends the standard OCS into the robust OCS by including the square root of the conditional variance of the EBV weighted by the optimised contributions, giving the standard deviation of the estimated genetic gain ('group uncertainty'), and κ parameter that controls emphasis on the 'group uncertainty'. To demonstrate the difference between standard and robust OCS, we simulated a selective breeding program in which we obtained the true and estimated breeding value alongside their conditional covariance and genetic variation. We compared how genetic gain, group coancestry and group uncertainty, and corresponding optimised contributions changed as we varied the parameter κ while λ was fixed. The preliminary results suggest that there is a non-zero correlations between the group uncertainty and group coancestry. We will demonstrate how this correlation changes as we vary the κ parameter and how it affects the optimised contributions, genetic gain, and group coancestry in short- and long-term

Unveiling Gene Regulatory Mechanisms Behind Variability in Average Daily Live Weight Gain of Lambs through RNA-Seq Analysis

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Average daily live weight gain (ADG) is a crucial trait that affects productivity and economic benefits in sheep production. Understanding genetic factors underlying ADG is essential for uncovering the molecular mechanisms regulating this trait and provide information to target and support genetic improvement strategies. Therefore, RNA sequencing analysis was conducted on blood samples from lambs (n=36, male, Texel crossed, average weight 30 kg), randomly allocated to four dietary groups (n=9 per group) and fed a total mixed ration basal diet composed of 50:50 grass silage and concentrate for 14 weeks. The diets were supplemented with four varying levels of micro algae oil (*Schizochytrium* sp.): 0 g, 5.4 g, 10.8 g, and 16.2 g per kg of dry matter, as part of a study aiming to affect methane emissions. While ADG, measured over the 14-week period, exhibited a decreasing trend (from 0.19 to 0.15 kg/day) with increasing micro algae oil, this reduction was not statistically significant (P-value = 0.41). Bioinformatic analyses identified 40 differentially expressed genes significantly associated with ADG variation. Upregulated MFSD5, SCN3B, and SNAP25 genes were enriched in functions like salt transmembrane transporter activity, voltage-gated channel activity, and passive transmembrane transporter activity, indicating roles in cellular processes affecting lamb growth and metabolism. Pathway analysis revealed ILK, ACOX2, and LOC101115115 genes were enriched in peroxisome proliferator-activated receptor (PPAR) signalling pathway, which regulates lipid metabolism, energy homeostasis, anti-inflammatory responses, and cellular differentiation, highlighting their potential role in metabolic processes influencing ADG. Collectively, these findings suggest that lambs with higher ADG may benefit from enhanced metabolic and immune function, leading to better growth performance. The key genes identified in this study provide potential targets for genetic selection to improve growth rates in sheep.

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Poster 15

Development and application of 50K ovine liquid gene chip

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China possesses rich sheep breed germplasm resources, which are characterized by valuable traits, such as high prolificacy and adaptation of harsh environments. As the commercialized ovine genotyping chips lacked Chinese local sheep breed genotypes during their development, detection efficiency of genetic variation is reduced. Therefore, to improve the capture rate of novel variant sites, an ovine 50K liquid chip was designed based on whole-genome data from approximate 500 sheep individuals. In addition, the custom-designed chip was used to perform genetic diversity of native Chinese sheep populations and genome-wide association analysis for growth traits of Chinese breeds. Genetic diversity and genetic structure of Chinese native sheep breeds were revealed. Body weight and body size are crucial economic traits for sheep, significantly influencing growth and development. Given the critical role of body weight and body size in determining the meat production of livestock, screening for candidate genes related to growth traits is crucial for the genetic improvement of meat sheep populations. Seven phenotypes of growth traits were measured in 1074 Dumeng sheep, which is a Chinese local developed breed characterized by fast growth rate and harsh environment adaptability. After quality control, 35,824 high-quality Single Nucleotide Polymorphisms (SNPs) were retained. SUPER(Settlement of MLM Under Progressively Exclusive Relationship) method was applied to perform the GWAS analysis using GAPIT software package. 805 SNPs significantly associated with growth traits were identified. Among them, 106 SNPs were closely associated with body weight. The significantly associated SNPs were annotated to a total of 290 candidate genes. These genes were enriched in growth trait-related signaling pathways. Key genes identified include MEF2C, CAMK2D, PLCB4, PDE4D, PIK3C3, ITGA11, PRKN, DGKB, ENPP2, FGF10, PDGFD, GHR, SOAT1, MINPP1, HSD17B12, and PLPP3. This study provides an insight to the molecular genetic markers of growth traits in sheep, which may be helpful for small ruminant breeding and genetic improvement for growth traits.

Runs of homozygosity and inbreeding in Swiss pigs

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The study aimed to analyze Runs of homozygosity (ROH) in four Swiss pig breeds – Duroc, Swiss Landrace, Swiss Large White dam line and Swiss large White sire line – using SNP genotype data to assess genomic inbreeding levels and identify ROH islands indicative of genomic regions with high frequency of homozygosity (selection signatures). Data sets received from SUISAG comprised 2744 Duroc, 4743 Swiss Landrace, 11383 Swiss Large White dam line and 15464 Swiss Large White sire line pigs, with approximately 70000 imputed SNP per breed. As genotypes were imputed, call rate filtering was not necessary and filtering for minor allele frequencies was not applied according to suggestions from other studies. The genomic inbreeding coefficient (FROH) varied among breeds, with Duroc showing highest (25.1%) and Large White dam line the lowest (19.8%). Long ROH segments (>16 Mb), reflective of more recent inbreeding, contributed significantly to overall inbreeding, ranging from 4.8% in Large White dam line to 6.9% in Large White sire line. The observed heterozygosity ranged from 0.29 (Duroc) to 0.33 (Large White sire line). ROH island analysis revealed genomic regions with high SNP incidence within ROH. More than 80% of Duroc animals had an ROH on SSC2, SSC3, SSC7, SSC9, SSC10 and SSC15. About 80% of Swiss Landrace animals had an ROH on SSC6. Around 80% of Large White dam animals had an ROH on SSC6. In Large White sire animals, no SNP had an incidence within ROH of 80% or more. Nevertheless, it should be noted that in all examined breeds, further indications of interesting regions were found, albeit with a frequency of less than 80%. This study highlights the extent of genomic inbreeding across Swiss pig breeds and identifies candidate regions under selection, providing insights into breeding strategies and genetic diversity conservation.

Comparative Analysis of Demographic Inference Tools in Livestock Population Decline Mode

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Demography plays a crucial role in understanding evolution, domestication, and hence much of the work with genomes within modern breeding programs. As a core aspect of demography, population dynamics measured with effective population size (N_e) provide a quantitative summary into how the genetic landscape of a population has been shaped. Although numerous tools exist for inferring temporal changes in N_e , comparative evaluations under declining N_e scenarios, common in livestock and endangered species, remain scarce. Additionally, different parameters in theoretical frameworks and input formats among these methods pose barriers to their application. We compared the performance of eight major demographic inference tools under six simulated scenarios, with the sixth scenario closely resembling the demography of pigs. Publicly available whole-genome sequencing data for pigs were also used. We have built a pipeline of widely used coalescent-based methods (PSMC, MSMC2, and SMC++), recent tools optimised for larger-scale datasets (pHlash, tskit, Relate), and approaches leveraging SNP frequency spectra and linkage disequilibrium (Stairway Plot, GONE). Our findings show that tskit and SMC++ closely track the true N_e across generations, while PSMC significantly overestimates N_e between 1000 and 10,000 generations ago. GONE demonstrates superior short-term estimation performance, despite showing an artificial dip around 100 generations ago under a declining population model. This analysis can enhance understanding of domestication and breed formation, and the pipeline offers a practical framework for inferring livestock demography. It can potentially support scientists and breeders in simulating data tailored to breeding strategies and testing novel genomic prediction techniques.

Runs of homozygosity in a population of SRC Iberian pigs selected for birth weight uniformity

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In the last decades, there has been an increase of interest in the search for homogeneity by reducing the variability in certain traits. The meat industry incentivizes farmers who provide homogenous batches of animals. Moreover, selection for homogeneity has been related to greater robustness and resilience in other populations. An experiment for selection for birth weight uniformity in a Sánchez Romero Carvajal Iberian pig population has been developed since 2022 with discrete generations. This study aimed at analyzing runs of homozygosity (ROH) patterns in the generations 0 and 1 of this population. A total of 95 individuals were genotyped using the Axiom PigHDv1 Array. After quality control, non-mapped SNPs, the SNPs mapped in sexual chromosomes and SNPs with Mendelian errors were removed. Finally, 550,896 SNPs were kept for analysis. The pedigree included 6,075 individuals with a mean of 4.61 equivalent generations depth. Pedigree inbreeding coefficient (FPED), ROH inbreeding coefficient (FROH), FROH obtained from six ROH length classes (FROH1-2Mb, FROH2-4Mb, FROH4-8Mb, FROH8-16Mb, FROH16-20Mb and FROH20-26Mb) and in six FROH accumulative categories (FROH $\geq$ 2Mb, FROH $\geq$ 4Mb, FROH $\geq$ 8Mb, FROH $\geq$ 16Mb, FROH $\geq$ 20Mb and FROH $\geq$ 26Mb) were calculated. Pearson's correlations were computed between the inbreeding coefficients. The software PLINK v1.9 and ENDOG v4.9 were used. A total of 13,516 ROHs were detected. Of these, 54.47% were segments of 1-2 Mb. The FPED-FROH correlation was 0.50. The correlation of FPED-FROH1-2Mb was -0.32. Nevertheless, the correlation of FPED-FROH $\geq$ 16Mb was 0.57. FPED and FROH had a negative correlation when calculated from older (shorter) ROH whereas that correlation was positive when longer (more recent) segments were used. This suggests that FPED captured better the recent inbreeding than the older one.

Session 3

Poster 19

Synchrotron FTIR microspectroscopy investigation on the sperm cryopreservation of Anglo-Nubian goat

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Cryopreservation and artificial insemination improve livestock reproduction, but sperm still experiences cryo-damage during freezing and thawing processes, despite efforts to enhance efficiency across ruminant species. This study aimed to investigate the effects of various cryoprotectant combinations on post-thaw sperm quality and biomolecular changes in Anglo-Nubian goat semen. Synchrotron-Based Fourier-Transform Infrared Spectroscopy (SR-FTIR) was used to assess biomolecular changes. A tris-based extender supplemented with 5% glycerol was tested in combination with different concentrations of cryoprotectants, including 1% and 3% soybean lecithin and 10% and 18% egg yolk, with Andromed® serving as the control. SR-FTIR analysis revealed that the combination of 5% glycerol and 3% soybean lecithin (T2) resulted in significantly higher levels of lipids, ester lipids, and nucleic acids compared to other treatments ($p < 0.05$). The percentage of curve fitting of amide I and secondary protein structures (α -helix) was significantly higher at 52.65% compared to other treatments ($p < 0.05$). Analysis of the PCA scores plot and correlation loadings revealed a positive association between the cryoprotectant combination of T2 and increased levels of lipids and ester lipids, as well as enhanced sperm motility, progressive motility, and viability. The results suggest that specific cryoprotectant formulations, particularly 5% glycerol + 3% soybean lecithin (T2), enhance the biomolecular and functional integrity of frozen-thawed Anglo-Nubian goat semen.

Genomic insights into congenital disorders in pigs: a GWAS approach

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Porcine congenital splay leg syndrome (PCS) and scrotal hernia (SH) are among the most common congenital disorders in pigs, posing significant challenges to both animal welfare and economic efficiency of pig farming. PCS is characterized by hindlimb weakness and an inability to stand or walk properly, compromising the survival of affected piglets. SH occurs in male piglets when the small intestine protrudes into the scrotum, forming a visible 'bulge'. Despite their considerable impact on the pig industry, the genetic background and pathophysiology of these conditions remain largely unknown. In this study, the genetic background of both disorders was investigated using a genome-wide association study (GWAS) on 1413 Piétrain boars genotyped with the GGP Porcine 50K medium-density SNP chip (43845 SNPs after quality control). For these boars, data was collected on 14743 crossbred litters (Piétrain sire, hybrid dam) which were scored for the number of PCS/SH piglets per litter. Multiple GWAS approaches were performed using GCTA software. In the qualitative analysis, boars were categorized as 'cases' or 'controls' based on their minimal number of offspring and the prevalence of PCS or SH in their litters. In the quantitative approach, the prevalence was used as a continuous phenotype. Additionally, a haplotype-based GWAS was performed. To account for population stratification, corrections were applied across all analyses using the genomic relationship matrix and principal component analysis. These analyses revealed several associations for both disorders, with varying levels of significance. For PCS, associations ($p < 5 \times 10^{-8}$) were detected in genomic regions on SSC1, SSC4, and SSC-Y. For SH, a signal was found on SSC1. Fine-mapping of these regions and candidate gene detection with imputed sequences is currently ongoing. This study provides a promising foundation to find candidate genes linked to congenital defects in pigs, which will enhance genetic selection against these disorders.

Session 4

Theatre 2

Mineral calibration and analysis of swine feces via portable X-ray Fluorescence device

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AniGun®, a portable X-ray Fluorescence (XRF) device (Animine, France), has been calibrated for rapid, cost-effective mineral analysis of forages for precision ruminant nutrition. This study evaluated the feasibility of using portable XRF for calibrating and analyzing the mineral composition of swine feces. It offers a potentially cheaper and simpler alternative to the widely accepted inductively coupled plasma-optical emission spectroscopy (ICP-OES) method for measuring trace mineral concentration in digestibility studies. Fecal samples from a previous trial, where 70 pigs were fed varying levels of Cu and Zn, were lyophilized and analyzed for minerals (Cu, Zn, Mn, Ca, K, S, P) using ICP-OES. Thirteen fecal samples representing the highest, lowest, and median values for each mineral were selected to build a multi-elemental calibration, covering ranges: 30-210 mg/kg for Cu; 144-849 mg/kg for Zn; 305-523 mg/kg for Mn; 9.2-16.9 g/kg for Ca; 3.1-7.8 g/kg for K; 1.1-1.9 g/kg for S; and 4.6-8.8 g/kg for P. Using these data, linearity, limits of detection, and quantification were established for each element. To validate the calibration, accuracy and precision of the XRF measurements were evaluated using random samples from the same pool. Agreement between portable XRF and ICP-OES was assessed through mean percentage bias and recovery, with results indicating mean percentage recoveries of 97.4% for Cu, 93.9% for Zn, 96.5% for Mn, 99.2% for Ca, 95.8% for K, 94% for S, and 95.6% for P, with a mean bias of ~4%. In summary, these findings demonstrate the potential of this portable XRF as a valuable tool for advancing the understanding of trace mineral digestion and excretion in swine. With the device's efficiency, it offers an effective alternative in analyzing mineral concentration for digestibility and performance trials. Future research should explore expanded calibrations and applications to tissues, feed, and feed ingredients, contributing to digestibility studies.

Serum FITC-d as biomarker to investigate intestinal integrity in piglets

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Oral inoculation of fluorescein isothiocyanate-labelled dextran (FITC-d) and subsequent determination of systemic levels has been used in broilers as model for gut integrity. The objective of the current trial was to evaluate this application in weaned piglets. Diets were formulated to a low acid-binding capacity of 215 meq/kg (i.e. LOW) or to a high acid-binding capacity of 450 meq/kg (i.e. HIGH) and diets were fed from weaning (29.6 ± 0.03 days old; 8.5 ± 0.19 kg). Each treatment consisted of 32 pens with 16 non-sample pens (6 piglets/pen) and 16 sample pens (7 piglets/pen). At D5 and D12 post-weaning, 16 piglets/treatment (one per sample pen, 1:1 sex ratio) were fasted for 3 hours, orally inoculated with 4.16 mg/kg body weight of FITC-d dissolved in water, followed by blood sampling after an interval of 2h to study FITC-d levels and dissection after 3.5h to collect tissue of duodenum, jejunum and ileum for morphometric measures. Pig performance and faecal consistency were reported on pen level. Serum FITC-d levels were detected by fluorescence spectroscopy. Data were analysed with ANOVA and Pearson correlation analyses. LOW-piglets tended to have a better faecal score than HIGH-piglets (5.2 vs. 5.0; $P=0.06$) with a numerical better average daily feed intake (306 vs. 292; $P=0.14$), average daily gain (222 vs. 207; $P=0.11$) and feed conversion (1.40 vs. 1.47; $P=0.14$) between D0-14 post-weaning. At D7 post-weaning the prevalence of diarrhoeic pens was higher in HIGH than in LOW (75% vs. 50%; $P=0.02$), which effect was not significant any longer at D14 post-weaning (50 vs. 38%; $P=0.15$). A very strong positive correlation ($R^2=0.95$) was found among systemic FITC-d levels at D5 and D12 post-weaning ($P<0.001$). A significant day effect was found with higher FITC-d levels at D5 versus D12 post-weaning (2.10 vs. 1.17 ± 0.060 , $P<0.001$). LOW-piglets tended to have lower FITC-d levels than HIGH-piglets (1.95 vs. 2.24 ± 0.124 , $P=0.13$ at D5 and 1.09 vs. 1.23 ± 0.055 , $P=0.09$ at D12). Histology of the intestinal sections are being performed and the effect of diet and correlations among FITC-d and morphometric measures will be presented. FITC-d is suggested as a promising biomarker to determine effects of (dietary) interventions on in vivo intestinal integrity in weaned piglets.

Session 4

Theatre 4

Histological and Microbiota Responses to *Arthrospira platensis* in Pig FeedingA. Toscano¹, D. Giannuzzi¹, Y. Ramayo-Caldas², R. Verdighione¹, S. Pegolo¹, G. Chillemi³, G. Don¹, S. Schiavon¹, L. Gallo¹¹ University of Padova, DAFNAE, viale dell'Università, 35020 Legnaro, Italy, ² IRTA, Animal Breeding and Genetics Program, Torre Marimon, 08140 Torre Marimon, Spain, ³ University of Rome, Department of Experimental Medicine, Via Montpellier, 00133 Tor Vergata, Italy

Protein is the most significant macronutrient in pig nutrition for economic and ecological reasons. Previous research evidenced that *Arthrospira platensis* (AP) may replace soybean meal (SBM) without affecting pigs' growth and carcass traits. This study evaluated the impact of a dietary replacement on gut health and microbiota in 60 growing pigs. Animals were randomly allocated to one of four isoenergetic and iso-amino acid feeds, a control diet (CTR), based on cereals and SBM, or an experimental diets (AP33, AP66, or AP100) replacing 33%, 66%, and 100% of SBM, respectively, with a nucleus composed of AP, sugar beet pulp, wheat meal and amino acids (640, 308, 40, and 12 g/kg, respectively). Ileum and colon samples were collected at the slaughterhouse (238 d of age) for histological and microbiota analyses. Histological evaluation concerned the height and width of the villi, and crypt depth (ileum), crypt depth (colon), and inflammatory status (ileum and colon). For microbiota analysis, DNA was extracted using the QIAamp PowerFecal Pro DNA (Qiagen), while libraries were prepared using the QIAseq 16S Region Panels kit, amplifying V3-V4 regions. Sequencing was performed with an Illumina MiSeq. A linear model was used to analyse the histological data, including diet and sex as fixed effects; however, no significant effect was found for either diet or sex. Conversely, diets had an effect on gut microbiota. Then, the histological data were associated with the gut microbiota. In ileum, villus height and crypt depth were negatively correlated, with villus height positively influencing microbiota structure and diversity, while crypt depth had a negative effect on both. The discriminant analysis identified members of the Cellulosilyticum and Intestinibacter genera as key indicators of gut health in the ileum. Acknowledgements: Agritech National Research Center funded by the European Union Next Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA – PNRR – MISSIONE 4 COMPONENTE 2, CUP C93C22002790001)

Influence of sex type on bone characteristics in pigs fed a phosphorus depletion-repletion strategy

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Bone characteristics of pigs depend on diet and sexual type. However, most studies are focused on gilts, entire males and barrows and do not evaluate individual bone traits. For this reason, the objective of the present study was to evaluate the influence of sexual type (entire male EM, entire female EF, surgically castrated male CM, immunocastrated male IM and immunocastrated female IF) on metacarpal and rib bone characteristics of pigs fed the same P depletion-repletion strategy. In this study, 70 pigs of 5 sexual types (14 each) were used. The pigs were fed in 2 phases, the first with a restricted diet (60% of the digestive requirements of P and Ca) and the second with a control diet (all nutritional requirements). The pigs were slaughtered at 25 weeks of age. At 24 h post mortem, bones (metacarpal III and IV, 1st rib and 4th last rib) were removed from the left carcasses. The bones were scanned using the Philips Brilliance 16 computed tomography device and the proportion of low, medium and high-density bones was computed from the total bone volume according to the Hounsfield values (+141 to +499, +500 to +999 and +1000 to +1499, respectively). The average density of the whole bone was also calculated. The statistical analysis was carried out with SAS software, considering sexual type as fixed effect and pen as experimental unit and applying Tukey test. The results showed that ribs' density characteristics were not significantly different between sexual types ($P > 0.05$). Regarding metacarpal III, the proportion of low-density bone was higher in the EM than in the EF pigs; while the proportion of medium-density bone was higher in the EF than in the EM pigs. Metacarpal IV of EM pigs showed higher proportion of low-density bone than EF and IF pigs; while the proportion of medium-density bone was higher in the EF and IF animals than in the EM ($P < 0.05$). The average density of the metacarpals of the EF pigs was higher than those of the EM pigs ($P < 0.10$). It can be concluded that the sexual type of the animals had an effect on bone characteristics, especially in the metacarpals. The research is within the project SUSPORK PID2021-124423OR-100 financed by MICIU/AIE/10.13039/501100011033 and by FEDER, UE.

Session 4

Theatre 6

Nitrogen and Phosphorus reduced feeding strategies and its influence on feeding behavior, efficiency and carcass traits in pigs

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Agriculture and especially animal husbandry leads to different environmental pollutions. The biggest focus in this is on nitrogen (N) and phosphorus (P) excretion. This requires changes in the amount of nutrients in animal diets and its efficiency to utilize these nutrients. The genotype of an animal contributes to the variation in adaptability to dietary changes. As animals with a different genetic background adapt better to certain diets, a genotype $\times$ feed-interaction ($G \times F$) can be assumed. Two trials were conducted to assess the effects of lowered dietary N and P concentrations on feeding behavior, efficiency and carcass composition traits. In total 1,118 purebred landrace boars (LR) and 883 female and male crossbred Pietrian $\times$ F1 pigs (PiF1) were investigated. One group received a control feeding and the other group a N/P-reduced feeding based on recommendations of DLG. In total there were 6 (0) feeding behavior, 4 (4) efficiency and 10 (9) carcass composition traits for LR (PiF1). Variance component estimation was performed with ASReml and heritabilities (h^2) and correlations (rg) were determined using uni-, bi-, and multivariate analysis. For feeding behavior traits h^2 ranged from 0.40 to 0.67, for efficiency traits h^2 from 0.33 to 0.49 and for carcass composition traits h^2 from 0.40 to 0.85 were detected. The bivariate analyses in DL revealed a $G \times F$ in average feeding rate per visit, residual feed intake, residual growth and 6 out of 7 carcass traits, whereas for PiF1 $G \times F$ was detected in residual growth and pH1. Multivariate analysis showed high negative genetic correlation (rg) -0.77 between feed conversion ratio and meat content in DL. In PiF1 this correlation was even higher with rg -0.93. This study indicates that the N-/P-reduction could lead to significant $G \times F$, which might be considered in pig breeding programs.

Effects of feed processing methods on nutrient digestibility and performance in early broiler

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The first two weeks of life are critical for broiler development, as early nutrition influences organ growth, gut health, and performance. Feed processing methods, such as pelleting and expanding, can increase nutrient digestibility by modifying feed structure. However, excessive energy input during processing may negatively impact nutrient digestion. This study hypothesized that reducing the specific energy (SME, kWh/t) of the expander (exp) would improve feed digestibility and broiler performance without adverse effects during early nutrition. A total of 792-day-old male Ross 308 chicks (average BW: 41.5g) were randomly placed among four diets with six repetitions each. The diets were isonitrogenous (22% CP) and isoenergetic (AMEN 11.72 MJ/kg), but different processing methods (mash, pellet, exp 6 kWh/t, and exp 13 kWh/t). Performance parameters were measured at 7 and 14d. Afterwards 10 broiler per pen were randomly sampled for organs weights and ileal viscosity for both time points. Data were analyzed (ANOVA) and means compared via Tukey's test ($P \leq 0.05$). At 7 and 14d broiler fed mash diets presented poorer BWG, FI, and FCR compared to the other diets. No differences in BWG were observed among pellet, exp 6, and exp 13 diets. However, pellet diets led to higher FI and worse FCR. Exp 6 improved FCR due to lower FI compared to pellet diets, but no differences were observed between exp 6 and exp 13. Mash diets resulted in higher liver, proventriculus, and gizzard weights at 7d, with persistent results for proventriculus and gizzard weights at 14d, except liver weight. Mash diets exhibited lower ileal viscosity at both ages. At 14d, exp 13 diets had higher ileal viscosity compared to exp 6 and pellet diets. In conclusion, feed processing methods such as pelleting and expanding improved early broiler performance compared to mash diets. Exp 6 diets offered the best balance between FI and FCR, supporting the hypothesis that reduced SME in the exp could optimize nutrient utilization during early broiler nutrition. However, the increased ileal viscosity observed with exp 13 should be further investigated due to its potential impact on fat digestibility and gut health.

Session 4

Poster 8

Purebred-crossbred correlation of resilience indicators in Swiss Large White pigs

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The genetic correlation between purebred and crossbred performance (RPC) is an important parameter in pig breeding, as phenotypes are often recorded in purebred pigs, while the goal is to improve crossbreds. The RPC is often below one due to genotype by environment effects (nucleus vs commercial), genotype by genotype interactions (GxG; epistasis) or measurement differences (e.g. live vs carcass measurements). Values for RPC below 0.8 suggest that crossbred data should be used in breeding value estimation. Recently, automated feeding stations enabled resilience indicator estimation by quantifying deviations in observed vs expected patterns of feed intake. However, these costly machines are usually used to phenotype purebreds, raising questions about the utility of these measures to predict resilience in crossbreds. This study used data from Swiss Large White pigs raised at the experimental farm of SUISAG. Daily records of feed intake, feeding duration and frequency between 90-160 days of age were available for 2921 purebred Large White pigs and 3090 crossbreds (Large White sire line x hybrid dam (Large White dam line x Landrace)). Pedigree data was available for all pigs and 461 purebreds were genotyped. Resilience was estimated as the ln-transformed variance (lnvar) of expected vs observed feed intake, duration and frequency via linear regression. The RPC was estimated using bivariate Bayesian animal models (gibbsf90+) with a purebred and a crossbred trait. Heritability estimates were moderate and similar for purebreds and crossbreds of lnvar of feed intake ($h^2=15.5$ and 19.4%), duration ($h^2=35.0$ and 28.7%) and frequency ($h^2=36.6$ and 35.0%). The estimated purebred-crossbred correlations were high for lnvar of feed intake (RPC=0.93), duration (RPC=0.81) and frequency (RPC=0.99). Since all pigs were kept in a similar environment with the same measurement method, deviations from one mainly reflect GxG interactions. The high correlations show these GxG interactions are limited in Swiss Large White pigs. This finding is highly relevant for the pig breeding sector as it indicates that selection for resilience in purebred pigs will improve their crossbred offsprings' resilience, at least in a similar environment.

Refining the kinetics of salivary oxytocin in response to farrowing, lactation and exogenous oxytocin injection in pigs

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Oxytocin is a neuropeptide involved in essential physiological processes, such as uterine contractions during parturition and milk let-down during lactation. Recent studies suggest that salivary oxytocin may serve as a non-invasive biomarker for monitoring animal welfare, particularly in response to physiological events. However, the kinetics of oxytocin release into saliva, and its validity as a welfare indicator, remain poorly understood. This study aims to refine the kinetics of salivary oxytocin by assessing its levels during three biologically relevant events: farrowing, nursing, and the injection of exogenous oxytocin. We collected saliva samples from ten sows at an experimental farm at multiple time points before (n=1-2), and during/after (n=7-14) each event to capture oxytocin secretion dynamics in saliva. The primary objective is to better understand the temporal diffusion pattern of oxytocin in saliva during these physiological events as a basis to determine its viability as a welfare indicator in pigs. Although data analysis is ongoing, preliminary observations suggest that oxytocin levels vary in response to each event. We anticipate that farrowing and nursing will result in a marked increase in salivary oxytocin, reflecting the physiological processes during these stages, while the injection of exogenous oxytocin may result in more immediate but transient increases in oxytocin concentrations. Data analysis will help to further establish whether the observed patterns of oxytocin release align with the known functions of oxytocin in physiological contexts. This research contributes to the growing field of salivary oxytocin studies, highlighting the importance of understanding its temporal profile, and supporting the development of standardized protocols for oxytocin measurement. These findings may help assessment of salivary oxytocin as a quantifiable biomarker in farm animals.

Session 4

Poster 10

Effect of an antioxidative stress cocktail in weaned pigs after E. coli challenge

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Alternative solutions that increase the resilience of pigs postweaning are crucial to reduce the use of antibiotics in pig production, prevent further antimicrobial resistance, and ensure sustained efficacy of antibiotics. This study investigated the effect of an antioxidative stress cocktail on oxidative stress as an innovative solution for enhancing oxidative status and intestinal health, thereby potentially reducing postweaning diarrhea. Seventy-two newly weaned pigs were allocated to two different diets: Anti-Low (Se 0.20 and vit C 0 mg/kg feed) and Anti-High (mix of different antioxidants including Se 0.35 and vit C 500 mg/kg feed). Half of the pigs were challenged with E. coli F18 at 8 and 9 days postweaning (dpw) providing four experimental groups: Anti-Low diet and non-challenged; Anti-Low diet and challenged with E. coli; Anti-High diet and non-challenged; Anti-High diet and challenged with E. coli. Blood samples were collected at 1, 8, 11, 14 and 17 dpw. Fecal samples were collected at 1 and 8 dpw before E. coli inoculation and at 9, 10, 11, 12, 13, 14, 16 and 17 dpw. Fecal DM and plasma vit C and acute phase proteins were analyzed. At 12 dpw, fecal DM dropped in challenged pigs compared to non-challenged pigs for both diets (16.1 vs 24.1%; $P < 0.01$) but the drop was more pronounced in pigs fed Anti-Low compared to Anti-High (13.8 vs 18.4%; $P = 0.5$). Haptoglobin and Pig-MAP increased in challenged pigs compared to non-challenged pigs fed Anti-Low diet (1.66 vs 0.95 mg/mL; $P = 0.09$ at 14 dpw and 0.40 vs 0.28 g/L; $P = 0.05$ at 17 dpw, respectively). At 17 dpw, vit C was higher in non-challenged pigs fed Anti-High compared to Anti-Low (39.3 vs 22.3 $\mu\text{mol/L}$; $P = 0.02$), and in non-challenged pigs compared to challenged pigs fed Anti-High (39.3 vs 25.9 $\mu\text{mol/L}$; $P = 0.09$). The results indicate that an antioxidative stress cocktail enhances oxidative status of the pigs and reduces severity of postweaning diarrhea, suggesting a promising alternative to antibiotics for promotion of enteric health in pigs.

The microbiota-gut-brain axis as a key regulator of physiology, brain function and behaviour: implications for human and animal health

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The microbiome-gut-brain axis comprises an extensive communication network between the brain and the gut. It is increasingly appreciated that the gut microbiome exerts a marked influence on host physiology with implications for brain function and behaviour. This includes key roles in regulating the stress response as well as brain functions such as myelination, microglia maturation, neuronal morphology, and blood-brain barrier integrity across the life span, from early life to adolescence to old age. Appropriate early-life assembly of the gut microbiota is also believed to play a role in subsequent emotional and cognitive development. Compositional and functional alterations in the gut microbiome have also repeatedly been associated with host metabolism and body composition. Microbial metabolites are key effectors within the gut-brain axis, both at baseline and following stress exposures, and include indoles produced from tryptophan and short chain fatty acids. Stress can exacerbate both gastrointestinal symptoms and CNS phenotypes and is an important common risk factor for disorders of gut-brain interaction where comorbid gut and brain symptoms are expressed. Therapeutic targeting of the gut microbiome may thus be useful in treating or preventing stress-related microbiome-gut-brain axis disorders and metabolic diseases. Much current research focuses on establishing causality and achieving a greater understanding of the molecular mechanisms underpinning signalling in these interconnected gut-brain pathways to influence neuroendocrine responses. The implications of these findings need to be considered in the context of human brain and gut health but also farm and domestic animal physiology where managing stressful situations can increase productivity.

Session 5

Theatre 2

Prolonged stress and its effects on the microbiota-gut-brain axis in the development of post-weaning diarrhea in piglets

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Weaning is one of the most stressful periods in pig production and can contribute to the onset of post-weaning diarrhea. Emerging studies have identified the microbiota-gut-brain axis as a promising therapeutic target for gastrointestinal disorders, including diarrhea. We aimed to evaluate the effect of prolonged stress on diarrhea aspects, intestinal barrier function, and gut microbiota and its metabolite profiles. A total of 64 piglets were assigned to a 2x2 factorial design with the following factors: (1) with/without adrenocorticotrophic hormone (ACTH) injection to induce a physiological stress response and (2) with/without *Escherichia coli* (ETEC) F18 challenge. Piglets received ACTH (20 µg/kg) or saline intramuscularly daily from d 2 to d 8 post-weaning (PW). In the challenged groups, piglets were orally inoculated with ETEC (1×10^{10} cfu) on d 3 and 4 PW. The ETEC challenge did not significantly affect plasma cortisol levels ($P > 0.05$). Plasma cortisol significantly increased 1h after ACTH injection ($P < 0.0001$), and the cortisol responsiveness at 1h after injection remained relatively stable on d 2, 4, and 7 PW. Cortisol levels in all groups returned to baseline 24h after the final ACTH injection ($P > 0.05$). Challenged piglets developed diarrhea starting on d 6 PW, with ACTH tending to exacerbate the condition ($P = 0.08$). In the distal small intestine, the IDO-1 gene was upregulated with a 1.49-fold change ($P = 0.02$) in the presence of ETEC. In the proximal colon, ACTH significantly downregulated TNF- α gene expression (0.80-fold, $P = 0.04$) and tended to upregulate Claudin-1 gene expression (1.27-fold, $P = 0.07$). Performance, salivary cortisol, and plasma acute phase proteins (APPs) were not affected by ACTH injection or ETEC challenge ($P > 0.05$). Taken together, prolonged stress induced by ACTH injection influenced gene expression and diarrhea severity but did not impact performance and APPs. ETEC faecal shedding and gut microbiome- and metabolite profiles including neurotransmitters and tryptophan-derived metabolites, driving signaling between gut and brain are currently being analyzed to add to these findings.

Brain-Immune-Gut (BIG) benefits Milk Fat Globule Membranes (MFGM) to ensure a smooth transition during weaning.

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The idea is rising that brain and nervous function is tied to the gut microbiome and involves epigenetic and immunoregulatory changes. Current evidence indicates that MFGM has the potential to benefit the BIG axis through better cognitive development, reduced infection risks (acute otitis media, respiratory tract infections, and diarrhea), and shaping the gut microbiome activity and function. MFGM is a natural bioactive component of cow's milk. Orally administrating MFGM during the first postnatal week stimulated short-chain-fatty-acid producing bacteria colonization, enhanced intestinal barrier functions, and consequently improved the growth performance of neonatal piglets on 21 d (Wu et al., 2020). The hypothesis is that supplementation of these natural bioactive milk molecules in piglet feed strengthens piglets to transition smoothly through weaning. Two piglet trials were performed to examine the effect of MFGM combined with selected dairy proteins and an emulsifier (MFGM+) in the postweaning phase. In trial 1, 256 piglets (Danbred x Pietrain) were used in two treatments, Control and MFGM+ in weaner and starter diets, respectively. For each treatment, 8 replicates x 16 piglets per pen were enrolled for 49 days. In trial 2, 288 piglets (TN70 x Pietrain) were used in two treatments, Control and MFGM+ in the weaner diet. Eight and 16 pens with 12 piglets each in Control and MFGM+ treatments, respectively were followed for 37 days. In both trials, weaner and starter diets were barley, wheat, and soya-based, without medication nor pharmaceutical levels of ZnO. Measurements were body weight at the start, 14 days, and end of the trial and feed intake per pen. No differences were reported in mortality or morbidity in both trials. In both trials, the final weight was improved ($P < 0.05$) with 1.5 and 0.8 kg in the MFGM+ treatment, respectively. Furthermore, in both trials feed intake was increased with 4 and 3% in the MFGM+ treatment, respectively. Moreover, feed conversion rate was improved in both trials with 4 ($P < 0.05$) and 1% in the MFGM+ treatment, respectively. In conclusion, MFGM+ supplementation in piglet diets improves growth performance and feed efficiency, thereby ensuring a smooth transition at weaning.

Session 5

Theatre 4

Exploring microbiome-gut-brain axis connections in a porcine model of social stress

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Multi-omics signatures of social stress along the microbiome-gut-brain axis (MGBA) in pigs remain largely unexplored. Herein, we investigate the effects of social stress on MGBA by detecting fecal metabolites that discriminate control ($n=30$) from stressed ($n=30$) pigs, employing deep neural networks to infer microbiome-metabolite interactions, and comparing brain stress-related proteomic biomarkers between the experimental groups. Social stress was induced during the fattening period by reducing available space and mixing pigs from different pens of the stress group. Brain tissues, hair, and fecal samples were collected at the end of the experiment for protein, cortisol, metagenomic, and metabolomic analyses. Compared to control, stressed pigs exhibited decreased body weight and higher hair cortisol levels. The microbiome of this group showed an increase in opportunistic genera, followed by a reduction in butyrate and propionate-producing species. We tentatively identified 11 metabolites that differentiated stressed from control pigs with an accuracy of 80%. Among them, stressed pigs showed higher levels of xanthosine and pyrimidine bases, while serotonin and 2-acetamidophenol were indicative of the control group. Additionally, we detected 64 microbial genes that increased classification accuracy to 91.06%, improving the prediction of serotonin and xanthosine. Notably, stressed pigs exhibited lower levels of Brain-derived neurotrophic factor in the hippocampus and higher levels of astrocytes and microglia markers, indicating glial reactivity. In summary, our findings highlight the value of the porcine model in advancing our understanding of the MGBA, offering insights that could improve animal welfare and inform human health interventions.

The effect of the fermented component content with probiotic additives in the diet of pigs on metabolic processes including the regulation of gut-brain axis activity

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The study aimed to evaluate the effect of 8% dried fermented rapeseed meal (FRSM) and *Bacillus subtilis* 87Y on redox status and gut-brain axis in 384 Yorkshire x Danish Landrace piglets. Animals were divided into four groups: G0-KN (negative control), G1-KP (positive control with feed additives), G2-F (standard diet + 8% FRSM), and G3-FE (standard diet + 8% FRSM + 0.1% *B. subtilis* 87Y). The study began at weaning (day 28) and continued until the piglets reached ~ 30 kg, at which point biological material (blood, small intestine, liver, hippocampus) was collected for analysis. Antioxidant activity of superoxide dismutase (SOD) and catalase (CAT) increased in G2-F and G3-FE, while lipid peroxidation markers malondialdehyde (MDA), lipid hydroperoxides (LOOH) were higher in G0-KN and G1-KP. G2-F and G3-FE showed reduced mesophilic aerobic bacteria and *Escherichia coli*, with increased *Lactobacillus*. In the hippocampus, protein kinase B (Akt) was elevated in G2-F, while neurotrophic factors, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and tropomyosin receptor kinase B (TrkB) increased in G2-F and G3-FE, indicating improved neuroplasticity. Extracellular signal-regulated kinase (ERK) was highest in G2-F and G1-KP, glycogen synthase kinase-3 (GSK-3) increased in G3-FE and N-methyl-D-aspartate receptor (NMDA) expression was highest in G3-FE. In conclusion, FRSM and *B. subtilis* 87Y positively influenced redox status, gut microbiota and neuroplasticity-related proteins, highlighting their potential benefits for piglet health and production efficiency. Research conducted as part of the project titled "Support for Students in Enhancing Their Competencies and Skills" (Measure 01.05 Skills in Higher Education, European Funds for Social Development Program 2021-2027) initiated by the Ministry of Science and Higher Education.

Session 5

Theatre 6

Do piglets crave sweet and salty taste?

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In early-weaned piglets, adding sweeteners to their diet can increase feed intake during the first days post-weaning. However, the effects of salt (above requirements) or combinations of sweeteners and salt remain less studied. This study aimed to evaluate post-pellet coating with sugar beet molasses (sweet), mixer addition of salt above requirements (salty), and the combination of both on feed intake during the first 4 days post-weaning and weekly until 28d post-weaning. A total of 144 weaned piglets (Pietrain x (Large White x Landrace); 7.93 ± 0.97 kg BW) housed in 36 pens (4 piglets per pen; 2 males and 2 females), were used. Piglets were blocked by BW, and randomly assigned to four dietary treatments arranged according to a 2x2 factorial design with the reinforcement of sweet (0 or 1.5% molasses; pellet coating) and salty (0 or 0.13% salt; in mixer) tastes as main factors. Performance parameters were recorded daily during the first 4 days post-weaning and then weekly starting from d7 until d28. Data were analyzed as a randomized complete block design using the GLM procedure of SAS. The statistical model included the effects block, sweet, salty, and sweet x salty interaction. Statistical difference was considered at p<0.05. Sweet taste increased ADFI on d1 post-weaning (14 vs 30 g), whereas Salty taste reduced it on days 1 and 2 (33 and 140 vs 11 and 94 g, respectively). Over the first week, the Salty taste worsened ADG, ADFI and FCR (111, 164 and 1.60 vs 74, 137, and 2.11 g, respectively), while the Sweet taste improved ADFI during the 2nd week (278 vs 307 g). In general, there were no interactions between Sweet and Salty tastes, except for ADG during the second week and ADFI during the 4th week, in which Sweet taste had a positive effect on the diets that had additional salt but not in those without it. In conclusion, molasses coating enhanced feed intake during the first day and second week post-weaning, confirming the piglets' natural preference for sweetness. On the contrary the extra addition of salt is not well accepted by the piglets during the first week post weaning. The interactions between molasses and salt past the first week post-weaning deserves further investigation.

Identification of Key Commensal Microbes Enhancing the Production of Intestinal Secretory IgA in Early-Weaned Piglets

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Innate immune system plays a vital role in maintaining intestinal mucosal homeostasis, nutrients absorption and metabolism. Secretory IgA (SIgA) is the first line of defense against pathogenic bacteria in protecting the intestinal epithelium. Emerging evidences indicate that gut microbiota has a crosstalk with SIgA. Early-weaning strategy in the swine industry might disturb the level of SIgA in the intestine of piglets, thus influencing the immune response and causing early-weaning stress. Different pig breeds exhibit the different levels of resistance to early-weaning stress. However, whether SIgA level varies among breeds in the early-weaned pigs and which microbes could regulate SIgA production remain unclear. We firstly compared the levels of SIgA and the abundance of SIgA-coated bacteria among 5 pig breeds, and found that Huanjiang miniature (HM) early-weaned piglets have significantly higher levels of SIgA and SIgA-coated bacteria than Duroc×Landrace×Yorkshire (DLY) early-weaned piglets. Next, we sequenced the fecal metagenome of early-weaned HM and DLY piglets and analyzed 15 bacterial species that were significantly linked to the intestinal SIgA levels and the abundance of SIgA-coated bacteria. We then chose *Akkermansia muciniphila*, *Lactobacillus amylovorus*, *Lactobacillus acidophilus*, and *Lactobacillus crispatus* as four key candidate bacterial species for further validation in C57BL/6J mice. The results showed that *Akkermansia muciniphila* administration significantly increased levels of SIgA, SIgA-coated bacteria, and IgA+ plasma cells. Retinoic acid concentration was also increased after *Akkermansia muciniphila* administration, which lead to IgA class switching. Furthermore, we orally delivered *Akkermansia muciniphila* to Landrace×Yorkshire (LY) piglets from 10 to 20 d of age and found that *Akkermansia muciniphila* administration increased the intestine SIgA concentration. Our findings suggest that *Akkermansia muciniphila* may contribute to the intestinal health in the early-weaned piglets.

Session 5

Poster 8

Impact of gut microbiome composition on growth and feed efficiency in Duroc pigs

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Feed efficiency is an important trait in terms of the feeding costs in pig production. Recent studies have reported correlations between the composition and alpha diversity of the gut microbiota in pigs and nutrient digestibility, average daily gain, and feed efficiency. The purpose of this study is to investigate the impact of gut microbiome composition on growth and feed efficiency traits in Duroc pigs in Japan. A total of 102 Duroc boars were used in this study. Total feed intake (TFI), average daily feed intake (ADFI), body weight gain (BWG), average daily gain (ADG), and feed conversion ratio (FCR) were calculated using feed intake and body weight measured by Feed Intake Recording Equipment (FIRE) feeder during the growth trial period. DNA was extracted from rectal dry swabs along with feces from each animal at the end of the trial period. The fecal microbial communities were analyzed by sequencing the V3-V4 region of the 16S rRNA gene. A total of 298 genera were identified from the pig population, with *Ruminococcaceae* UCG-014 and *Lactobacillus* being the most dominant genera in the population. There was no significant correlation between alpha diversity (Simpson and Shannon) index and the five traits (TFI, ADFI, BWG, ADG, and FCR) for each animal. There were 21 genera with a correlation between the proportion of each genus and FCR stronger than ± 0.2 . In the 21 genera, the proportion of *Flavobacterium* had a negative (favorable) correlation with FCR, but had no correlation with BWG and ADG. In contrast, the proportion of *Eubacterium brachy* group and *Eubacterium xylanophilum* group were positively (unfavorably) correlated with FCR, but negatively correlated with BWG and ADG. Animals with better FCR tended to have higher proportions of *Ruminococcaceae* UCG-014 and *Lactobacillus*. These results suggest that several genera of the gut microbiota could be useful biomarkers for productively improving growth rate and feed efficiency in pigs.

Genetic analysis of feeding behavior and its relationship with key economic traits in a French male pig line
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Feed efficiency is key in pig production, influencing economic and environmental sustainability. While selection has traditionally focused on improving feed conversion ratio (FCR), feeding behavior traits (FBT) provide additional insights into how animals interact with their environment and utilize feed resources. Understanding the genetic basis of FBT and their relationships with production traits can help refine selection strategies. Consequently, the objective of this study is to estimate the heritability of FBT and their genetic correlations with traits of economic importance in a male pig line. Data were obtained from 74,727 Piétrain pigs over six years (2017-2023). Among them, feeding behavior data were recorded for 4,788 males over three years (2021-2023). The FBT that will be included in the study are: number of visits per day, time spent feeding per day, daily feed intake, time spent in feeding per visit, feed intake per visit, feed intake rate, number of meals per day, time spent in feeding per meal and feed intake per meal. Production traits to be analyzed will include age at 100 kg, backfat thickness at 100 kg, loin muscle thickness at 100 kg, FCR, lean meat percentage, pH of ham at 24 hours postmortem, drip loss, and boneless ham weight. All traits were measured in both sexes, except for FCR, which was recorded only in males. The pedigree file includes 84,714 individuals, among them, 1,557 animals were genotyped with the porcine SNP60 Illumina BeadChip. Variance components will be estimated using the single-step GBLUP employing programs of the BLUPF90+ family. Preliminary analyses on a subset of this population showed high heritability estimates (0.44-0.56) for FBT, highlighting the potential for incorporating these traits into genetic selection programs. Final results, including genetic correlations with production traits, will be presented and discussed during the conference.

Session 5

Poster 10

Voluntary feed intake of local Krškopolje pig breed from 30 to 100 kg: variability, sex differences and comparison to modern hybrids

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Key words: local breeds, Krškopolje pig, feed consumption Local pig breeds are early maturing, exhibit lower performances and higher adiposity compared to modern genotypes. However, their feed intake capacity is not sufficiently explored, and available data is limited and inconsistent, influenced by factors such as feed type, environment, housing conditions, and growth phase. Feeding stations with individual recording of feeding behavior enables to evaluate the feed intake capacity of Slovenian local breed Krškopolje pig (KKP). Thus we used 28 KKP pigs (15 barrows, 13 gilts) from three different litters and for comparison of feed intake capacity we used the data from 11 commonly used crosses (MP, Landrace × Pietrain; 5 barrows, 6 gilts). Monitoring was performed over three fattening phases (25–50 kg, 50–75 kg, and 75–100 kg) and weight gain and feed intake were recorded as g/day, kg feed/day, MJ metabolizable energy (ME)/day, respectively. KKP pigs consumed in average 1.93, 2.77 and 3.30 kg feed/day in first to third phase, respectively, and consumed consistently more than MP (1.62, 2.42, 2.82 kg feed/day, respectively). Within KKP breed, feed intake varied significantly between sexes and litters. Barrows consumed more feed than gilts (2.79 vs. 2.41 kg/day, 35.2 vs. 30.3 MJ ME/day, $P<0.05$) and exhibited higher growth rates (843 vs. 760 g/day, $P<0.05$), particularly in the first phase (798 vs. 674 g/day, $P<0.05$). We observed high individual variation and within litter variation in feed intake capacity, which was related to growth rate. Overall, our results indicate higher feed intake capacity of local pig breed compared with modern genotype. Acknowledgements: Funding by Funding by Slovenian Research and Innovation Agency (grants P4-0133, Z4-60178, J4-3094, V4-2201) and Slovenian Ministry of Agriculture, Forestry and Food (V4-2201) are acknowledged.

Animal Welfare Knowledge Platform: Benefits and Challenges

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Animal welfare (AW) is regarded as a ‘public good’ by European citizens and is recognised as a fundamental component of the sustainability of the food value chain. Standards of AW are ensured through legislation but also via stakeholder initiatives. To meet societal expectations, AW should be continuously monitored using valid and reliable AW indicators (or measures) and harmonized protocols. The European Partnership on Animal Health and Welfare is developing a knowledge platform with the objective to collect, analyse, share and use integrated AW data on farm, during transport, and at slaughterhouse. The platform’s development is structured around three parallel activities. The first is the creation of a network of stakeholders and the assessment of their expectations and concerns about participating in the platform and data sharing. Simultaneously, AW indicators, methods and protocols from existing databases are being collected and analysed for harmonisation. Thirdly the platform is being developed by: i) a mapping analysis of existing platforms in AW monitoring; ii) defining the requirements for a FAIR (Findability, Accessibility, Interoperability & Reuse) platform through a literature review on FAIR data in AW and a description of the data management practices in previous EU AW projects; iii) piloting of the platform by the collection of specific animal-based indicators. The Knowledge Platform will facilitate the integration, interpretation, and sharing of AW data and information from various stakeholders, the main objective of which is to facilitate the monitoring and potential surveillance of AW. One of the main challenges is to engage with potential data providers and meeting their expectations and requirements. This work is co-funded by the European Union’s Horizon Europe Project 101136346 EUPAHW.

Session 6

Theatre 2

Building a Knowledge Platform for Animal Welfare Data in the EU: Requirements and Challenges

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The welfare of food-producing animals is gaining increasing attention from EU policymakers, citizens, industries, and the scientific community. Animal welfare is regulated through EU and Member States legislation which mandates inspections on farm, during transport, and at slaughterhouses. Each Member State interprets the legislation and implements its own inspections and/or welfare assessment approach independently. This potentially results in variations in animal welfare inspections/assessments and in types of data collected. In response to these challenges, the European Partnership on Animal Health and Welfare (EUPAHW) aims to develop a centralized digital knowledge platform that will serve as a comprehensive repository for animal welfare data collected from key stakeholders, legislative updates, and harmonized welfare assessment protocols. This study first describes the obligations regarding the monitoring and surveillance of animal welfare as specified in EU animal welfare legislation, followed by an in-depth analysis of animal welfare projects at both the EU and Member state levels. This process identifies variations in animal welfare inspection/assessment and data collection methodologies as well as the absence of standardised data management practices. Finally, it explores the requirements for an integrated animal welfare data management framework. By promoting interoperability and data-driven decision-making, this platform will enhance transparency, improve compliance, and strengthen the enforcement of animal welfare standards across the EU. Keywords: Animal welfare data, key stakeholders, data collection, data management framework, Data driven, Knowledge platform, EU, Member States, EUPAHW This work was co-funded by European Union’s Horizon Europe Project 101136346 EUPAHW

A participatory approach to identify relevant indicators for the assessment of pig health

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Antimicrobials use (AMU) has largely decreased in pig farming in recent years, following sector's initiatives and regulations. To provide guidance in this minimal AMU while ensuring a preserved pig health and welfare, we are currently developing a multicriteria assessment protocol for monitoring these aspects. However, to our knowledge, if such protocols exist for welfare assessments, there are none that combines validated and operational indicators for the assessment of pig health in farms. Thus, there is a need to determine which health indicators are relevant. A participatory approach with veterinarians was developed and implemented, as they are considered experts of health assessment. During a first meeting, a group of 4-5 veterinarians were asked to select pig health indicators in a list coming from a literature search and rate them based on perceived sensitivity, specificity, reliability and feasibility. The experts had several weeks to complete the rating on their own. Then, during a second meeting, the results were discussed until reaching an agreement on the most relevant indicators for assessing pig health, that will be kept for the assessment protocol later on. Discussions were facilitated by the supervisor of the meeting. This process was repeated for every biological system, e.g. respiratory or digestive systems. Results show that our proposed framework allows identifying pig health indicators regarded as relevant through the elicitation of expert opinion. With the ratings, discussions and debates were facilitated, and indicators were also characterised (e.g. if they are perceived, or not, as feasible, reliable, sensitive and specific). In the same way, the most suitable measure method is identified. Our study provides a participative and iterative framework to identify and characterize pig health indicators through expert elicitation. Thereafter, selected indicators will be integrated and aggregated into a protocol for monitoring pig health, welfare and AMU on farms.

Session 6

Theatre 4

Experts' vs. farmers' opinion on "Best Practices" to improve animal welfare in pig farming

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Animal welfare problems such as tail biting pose a big challenge for livestock owners. Scientific literature offers various measures to use as remedies for these issues, however their effectiveness in practice is not always clear. This study, carried out in the scope of the EU project "aWISH", aims to identify so-called "Best Practices" in pig farming for certain animal welfare issues through surveys with scientific experts as well as pig farmers. The study commenced with a literature review to identify Best Practices on farm to reduce tail/ear lesions, skin lesions and bursitis in fattening pigs. The collected measures were included in two surveys: first, scientific experts were consulted to rate the Best Practices on a scale from 0% (unsuitable) to 100% (very suitable). The second survey was aimed at pig farmers who evaluated the same measures using the same scale (0% – 100%). Data analysis, including a mixed linear regression model, was performed with the software R. Overall, 127 completed questionnaires were collected (47 experts and 80 farmers). Experts gave a higher score than farmers to almost all proposed measures. For instance, only four out of 21 measures for tail and ear lesions received a score below 50% in the survey with experts, while more than half (11) of the Best Practices were rated below 50% by farmers. Big differences were seen in the measures "Organic manipulable material on farm except straw" (experts: $68 \pm 24.1\%$ vs. farmers: $45.5 \pm 28.1\%$) and "no storage of manure in the stable" ($55.5 \pm 28.8\%$ vs. $29.6 \pm 29.5\%$) (both $p < 0.001$). Similar findings were made for the indicator skin lesions. For bursitis, all six proposed Best Practices showed a significant difference between the two groups (all $p < 0.01$). In the comparison of experts' and farmers' views on Best Practices, a clear discrepancy was detected between the two groups. While experts regarded most measures as beneficial, the farmers' ratings provided important insights on practical challenges like feasibility. Further on-farm studies during the project are intended to identify effective and feasible Best Practices for improved animal welfare.

Farmer perception of fish welfare in rainbow trout farms

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This preliminary study aimed to assess farmer perceptions of on farm welfare in rainbow trout (*Oncorhynchus mykiss*) as for potential welfare indicators, risk factors, and corrective measures based on their experience and evaluation. A structured questionnaire covering five areas—farm data, management, on farm welfare, welfare at slaughtering, and perception at the wholesale level—was administered through interviews with 11 breeders managing a total of 23 farms in northeastern Italy, where 70% of Italian trout production occurs. The collected data underwent a descriptive analysis and were then categorized based on welfare criteria, risk factors, and corrective measures following the four welfare principles: Good Housing, Good Feeding, Good Health, and Appropriate Behaviour. Potential welfare indicators were abnormal behaviours (as for species-specific behaviour, swimming, social behaviours, human-fish relationships), performance, clinical signs of illness and mortality. The interviewed farmers identified trout welfare as influenced by both uncontrollable external factors (light, noise, piscivorous birds) and controllable environmental conditions (tank bottom, water flow, water quality) (Good Housing); as well as feed composition (proportion of vegetable ingredients), pellet type (sinking or floating), feeding frequency (Good Feeding); and fish health status (lesions and diseases) (Good Health). Risk factors for Appropriate Behaviour are related with those identified in the previous principles. In view of developing on farm welfare assessment protocols, the validity, repeatability and feasibility of the welfare indicators used by the farmers should be tested under field conditions.

Session 6

Theatre 6

Planning GIS projects in the aquatic animal health domain

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Implementing Geographic Information Systems (GIS) to manage aquatic animal health (AAH) calls for custom-made approaches to develop AAH-targeted GIS projects, which, however, appear to be lacking. Hence, the need for a framework providing a structured methodology for such project development and implementation. Ten international experts in both AAH management and GIS technologies were involved to design and validate the framework. A first version was drawn and then shared with the experts, who provided their revisions and further improvements that were then discussed and agreed on during a panel (Padua, February 2025). The resulting framework is a step-by-step guide divided into five development phases: i. conceptual design (development of the information product, including identification of project goals, stakeholders, and data requirements), ii. logical design (translation of goals into a detailed plan; i.e., data schemas, relevant technologies, reusable resources), iii. development plan (methods for data collection, system implementation, pilot testing), iv. maintenance plan, v. spatial communication. Two innovative concepts were introduced: strategic consultation of external expertise for more complex tasks and inclusion of a GIS data plan document to structurally manage and document geospatial data. The experts' interdisciplinary and collaborative work allowed producing a framework that was both technically robust and that also addressed real-world challenges and critical aspects of GIS planning for AAH, providing tailored, sustainable and adaptable solutions. The framework application is being carried out both in marine and freshwater environments so as to test its applicability and effectiveness.

Characterization of vocalisations associated with behaviours related to tail biting

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Raising pigs with long-tailed pigs requires increased surveillance on the part of farmers in order to intervene as soon as possible to put an end to tail biting. However, this monitoring remains ad hoc. The aim of the SOLBI project is to use vocalisations as a means of preventing bites. Audio and video recordings were made on two experimental farms and four commercial farms, for a total of 32 pens of long-tailed piglets housed in 8 different post-weaning rooms. Farmers recorded the condition of the animals' tails on a daily basis to determine when tail biting started. All animal interactions and associated vocalisations were recorded during the 24 hours prior to the onset of tail biting in one pen in each of the 8 environments. Ten categories of vocalizations were defined, with an eleventh category grouping indistinct vocalizations. The analysis focused on the nature and frequency of 7 types of interactions (biting or sniffing/manipulating the tail or another body part, aggression, pursuit, mounting) and the 10 categories of vocalizations, which corresponded to over 15000 behaviours and 5573 categorized vocalizations. Given the noisy environment, the relationship between behaviour and vocalization was not indisputable. Therefore, we established a quality index for behaviour/vocalization association, with scores ranging from 1 (best) to 3, taking into account the presence of one or more behaviours within 2 seconds before or after each vocalization. Only scores 1 and 2, representing 2399 associations, were retained for the chi-square analysis on R. The profiles of the eight environments did not highlight any typical behaviour or vocalizations in the 24 hours preceding the onset of tail biting, which may illustrate the variety of causes behind the emergence of this phenomenon. The relationship between vocalizations and behaviours showed preferential associations. Screams, regardless of their nature, were associated with tail-directed behaviour, while mounting was characterized by grunting. The database, along with the audio recordings, will help develop a machine learning algorithm to characterize the sounds and subsequently detect early signs of tail biting.

Session 6

Theatre 8

Skin lesions and lameness in group housed sows and gilts during mating and gestation in Swedish commercial piglet producing farms

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Moving towards the end of caging animals includes the expansion of group housing of sows and gilts beyond the gestation period. However, group housing of sows and gilts involves mixing of unfamiliar individuals which may result in injuries. In Sweden, sows and gilts have been housed in groups during both mating and gestation since the 1980s. This study maps occurrence of skin lesions and lameness in group housed gilts and sows in commercial Swedish pig farms. Data was gathered from 37 piglet producing farms during 2022 and 2023, covering about 10 % of the piglet producing farms in Sweden. On each farm, skin lesions and lameness were recorded on 10 randomly selected animals in the gilt rearing, gilt gestation, sow mating and sow gestation unit respectively. The scoring protocol was developed based on the Welfare Quality® protocol and assessments were conducted by one trained observer. Data analyses included in total information on 1446 animals of which 330, 352, 353 and 411 from the gilt rearing, gilt gestation, sow mating and sow gestation units respectively. Data was analysed with general linear models in SAS. Preliminary results show that only 1 % of the assessed animals had a lameness remark. The proportion of animals with a lameness remark was highest in the gilt gestation (2 %), compared to other units (<1 % for all). The occurrence of skin lesions was higher in the mating units compared to the other units ($p < 0.001$), with a majority of lesions on the front (indicating agonistic behavior) and middle (indicating riding mating behavior) parts of the body. There was a substantial variation in occurrence of skin lesions among farms ($p < 0.001$) with an average number of lesions per sow (entire body) ranging from 4.9 to 45.6. In all units and for all parts of the body (ears, front, middle, legs, tail and back) there were significant effects ($p < 0.05$) of days since mixing into the group, indicating that damaging behavior decreases over time after mixing. The results contribute with core information for future benchmarking of group housed gilt and sow welfare.

Overview of dairy calf housing in various production backgrounds in France

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Following a referral from the European Commission, EFSA recently published an opinion on the impact of group housing on calf welfare. Among other conclusions, experts recommended group housing for calves from the first week of life. In the vast majority of French dairy farms, individual housing remains the dominant practice during the first weeks of the calf's life. However, in order to assess the necessary adaptations to move towards collective housing for calves before weaning, an accurate snapshot of the current situation is essential. As part of a multi-annual national project led by Idele (COLOCAVO), a quantitative survey was conducted to provide a representative overview of calf housing practices before weaning. This survey was carried out with the support of the ELIANCE network of livestock advisory organizations. The sample of 230 surveys ensured the representativeness and diversity of the farms surveyed, considering factors such as lowland/mountain distribution, herd size, system types, barn construction date, and labor availability. The findings reveal that collective housing for pre-weaning calves is already widely practiced, mainly for replacement heifers and calves intended for rearing after their first three weeks of life. It is also mandatory from day 8 in organic farming systems. The main category of calves housed individually consists of those sold off the farm at an average age of three weeks, a timeframe that could be extended to five weeks by future regulations. This overview has helped identify the animal categories for which practical solutions, including technical guidelines and collective housing systems, need to be tested and proposed to dairy farmers in different production contexts. This work also made it possible to identify the main concerns of the surveyed farmers regarding the transition to group housing from the first week of life (increased health risks). The main positive aspects identified were equally the well-being of the calves (cognitive and social development) and that of the farmer (time savings in feeding and housing maintenance).

Session 6

Theatre 10

Relationships between individual daily water intake, rectal temperature, and treatment frequency in Limousin beef cattle during their first month of adaptation after arrival in Italy

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Advanced technologies that consistently monitor specific behaviours have rarely been used in intensive finished beef cattle. Consequently, there has been limited research into the individual daily water intake (DWI) of beef cattle and its relationship with their health status. This study aims to examine whether the individual DWI of beef cattle could serve as an early indicator of health issues during their first month of adaptation after being transported from France to the Italian fattening unit. It was carried out on a beef farm located in Padova province (Italy) and considered a total of 72 Limousin young bulls imported from France in 2 different batches at the end of 2024. Upon arrival, the bulls were distributed among 4 pens, each equipped with a prototype individual drinking station. The station identified each bull via RFID ear tags as they entered, while a flowmeter automatically measured their DWI. The farmer monitored the bulls' health, measuring their rectal temperatures (RT) twice weekly and recording all medical treatments administered. The DWI was categorized into 9 distinct levels, each representing a 10-liter increment. All medical treatments administered by the farmer targeted bovine respiratory disease (BRD) and were provided, on average, 17 days after the bulls' arrival. During the 30-day adaptation period, 52 out of 72 bulls (72%) were treated at least once for BRD. Of the 52 bulls that received treatment, 30 (58%) required multiple interventions. 52% of the treatments occurred on days when bulls exhibited DWI levels 1 and 2, corresponding to a DWI of less than 20 L. Concerning the relation between DWI and RT, nearly 60% of bulls with DWI less than 10 L had a critical RT higher than 39.8. As the individual DWI level increased, the prevalence of bulls with a critical RT higher than 39.8 decreased progressively. These preliminary findings confirm the high vulnerability of young beef bulls during their initial adaptation period after importation to Italy. Furthermore, the results suggest that DWI could potentially serve as an early indicator of health issues in these animals.

Pecking Blocks in the Pullet House Improved Pullet Distribution and Reduced Litter Moisture

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In aviary systems, laying hen pullets can move freely and perform natural behaviours. However, freedom of movement can result in uneven distribution in the barn, leading to local crowding and underutilized areas. This study investigated whether provision of pecking blocks (PB) affects pullet distribution and litter quality. Two flocks of Lohmann Brown pullets were housed in identical commercial rearing barns divided into 3 pens (~12,000pullets/pen), 5 rows/pen. At 12 weeks of age (woa), 3 pens were provided PB on the litter and 3 pens served as controls (C). PB were distributed evenly throughout the rows at a ratio of 1 PB/500 pullets. At 18woa, cameras mounted on the ceiling recorded behaviour, focusing on the PB in the treatment pens and on the litter of identical locations in C. Counts of pullets within 12 areas/pen (#pullets/0.53m²) were quantified through instantaneous scans every 15min from 0415-1715Hrs. Samples were collected in the same areas for litter moisture (%LM) determination. Data were analyzed using GLMM with fixed effects of PB and row for counts and, PB, row, season, and their interactions for LM, pen nested within flock was used as a random effect. Counts of pullets were higher ($p<0.0001$) in the two outside rows adjacent to exterior barn walls in PB (6.8 ± 0.19 and 5.6 ± 0.19) compared to C (3.9 ± 0.24 and 3.06 ± 0.15 , respectively). Counts were higher in inside rows with PB, but only significantly different in one of the rows (PB= 8.9 ± 0.19 vs. C= 5.5 ± 0.21). Overall, there were fewer pullets in outside rows ($p<0.0001$), but counts were higher when PB were provided ($p=0.0005$). LM% was higher ($p<0.0001$) in the winter ($32.4\pm0.64\%$) compared to summer ($19.8\pm0.37\%$), and PB lowered LM (PB= 22.8 ± 0.73 vs. C= $29.4\pm0.91\%$; $p=0.0002$). PB significantly affected pullet distribution and lowered LM in areas around PB. Providing PB may not only enhance foraging behaviour but may also improve bird distribution throughout the barn.

Session 6

Theatre 12

Effects of keel bone damage and foot health on the use of ramps to access elevated structures in older laying hens

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It is unknown whether painful conditions such as keel fractures or foot health problems affect a laying hen's ability to navigate her environment. We housed 32 Dekalb White (DW, 72 Weeks of Age (WOA), from aviaries with ramps), 32 Bovans Brown (BB, 72 WOA, from aviaries with ramps) and 32 White Leghorn hens (WL, 54 WOA, from aviaries without ramps) in 4 floor pens/strain (N=12). In each pen, there were 2 birds of each health status (n=8); healthy, keel bone damage (fractures and deviations, determined by palpation and radiographs), footpad problem (footpad lesions or toe damage), or both, with birds individually identified using numbered backpacks. Three roosting structures were offered 81cm above ground; round perch (diameter=2.54cm), flattened oval perch (2.54x5.08cm), wire platform. A ramp was placed 45° to each roosting structure. Videos, over 12 days following lights on (dawn) and preceding lights off (dusk), were used to observe if birds used ramp (R) or aerial (A, jumping/flying), to ascend to or descend from roosting structures. A total of 769 descents for dawn and 801 ascents for dusk were analysed using GLMM with fixed effects of condition, strain, method, structure and interactions. Interactions were used to test the hypotheses that condition, strain, and roosting structure would affect method of navigation. At dawn, A was the predominant method for descent (A:85.6% vs R:14.4%, $P<0.001$) with WL using R less than other strains ($P=0.003$). There were no effects of condition*method ($P=0.643$) or structure*method ($P=0.574$). At dusk, there were no effects of condition*method ($P=0.728$) or structure*method ($P=0.338$), but there was an interaction of strain*method ($P<0.001$). DW and BB used R for 72.6% and 61.7% of ascents, respectively, versus WL using 26.0%. Keel damage and foot health did not influence how older laying hens navigated elevated structures. Hens generally used different methods for ascending to and descending from roosts and the ramp was the preferred method for ascent by the majority of hens who had experience with ramps.

Behaviour of laying hens fed with live insect larvae (*Hermetia illucens*) as enrichment in a cage-free system
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The present study evaluated the effect of live insect larvae (*Hermetia illucens*, HI) supplementation as an environmental and nutritional enrichment on the behaviour of laying hens reared in a cage-free system. Along 5 weeks, 1800 laying hens of two genotypes (Novogen Brown and White) aged 36 to 40 weeks were randomly assigned to 8 pens of an experimental aviary. Live insect larvae were distributed (10% of the daily feed intake) in circular feeders once a day at 10 a.m. in four pens, whereas no enrichment was provided in the other 4 pens. Hen behaviour was assessed analyzing video recordings made 24 hours a day, one day a week. Data were submitted to ANOVA with live HI larvae supplementation, genotype, and hour of observation as main effects and the pen as a random effect by PROC GLIMMIX of SAS. The enrichment with HI larvae increased the number of hens observed on the floor (22.6 vs 24.2 hens; $P < 0.001$), which were found pecking insects (1.64% of observed hens) and feeders (5.19%). The HI enrichment also decreased the rate of hens pecking the litter (54.8% to 52.8%; $P < 0.01$), inactive (8.23% to 7.47%; $P < 0.001$) and moving (18.8% to 16.8%; $P < 0.001$) compared with the hens without enrichment. As for the effect of the genotype, more brown hens were found pecking the litter (56.2% vs. 51.5%; $P < 0.001$), inactive (10.1% vs. 5.64%; $P < 0.001$), and preening (14.0% vs. 9.93%; $P < 0.001$) compared with white hens, whereas the latter ones showed more piling (3.60% vs. 0.62%; $P < 0.001$) and locomotion (19.2% vs. 16.4%; $P < 0.01$) behaviours. The human confidence test revealed that the man-hen distance was lower in hens that received live larvae compared with the control hens (23.3 cm vs. 55.6 cm; $P < 0.001$), and lower in brown hens compared to white hens (12.6 cm vs. 66.5 cm; $P < 0.001$). In conclusion, the use of live HI larvae as enrichment in diets for laying hens increased their behavioural repertoire and confidence towards humans, which could likely have improved their affective status. On the other, the use of enrichment did not impact on hen aggression and piling behaviours.

Session 6

Poster 14

Building a Data-Driven Future for Animal Welfare: Stakeholder Insights for the EU Knowledge Platform

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The set of Actions n. 9 (SoA9) of the European Partnership on Animal Health and Welfare (EUPAHW) aims to establish the Knowledge Platform on Animal Welfare, which will harmonize data collection at farm, transport and slaughter. After a survey aimed to investigate the engagement, as potential data providers, of European stakeholders from several key sectors, an interactive workshop was organised to include needs and expectations. One-hundred-and-thirty-eight (138) stakeholders from 20 European countries were divided into 6 online breakout groups: i) animal production and inter-professional associations, ii) Industries iii) Research Performing Organisations iv) Animal health and welfare v) Competent Authorities vi) NGOs. Discussions in the groups were led by project partners, to identify sector-specific requirements before convening for a final plenary session. Findings indicate that 35% of participants view animal welfare monitoring primarily as a contribution to scientific research and welfare improvement, while the 17% focus on regulatory compliance, productivity, and market competitiveness. Ethical responsibility was also emphasized in 9% of cases. Expectations from the platform changed according to the type of stakeholders. Those directly involved in animal production and industry ask for access to reports (43%) and benchmark themselves with others (14%), while research institutions would like to access data for research (61%) and competent authorities mostly look for a new monitoring tool to refine policy strategies (50%). The results will be used to design the new platform, considering the need for a harmonised, research-driven framework to enhance animal welfare across sectors. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Advancing Animal Welfare in Europe: Survey to check stakeholder engagement in a knowledge platform for data collection

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A key project (SoA9) of the European Partnership on Animal Health and Welfare (EUPAHW) is the development of the Knowledge Platform on Animal Welfare, which will harmonize data collection, surveillance and welfare monitoring at farm, transport and slaughter, in different food-producing species. To engage, as potential data providers, different stakeholders including farmers, transporters, slaughterhouses, industries, NGOs, competent authorities and researchers, a survey was conducted. A questionnaire gathered insights from 200 stakeholders across 20 Countries and international bodies, checking the type of data collected and assessing their approach to animal welfare, data-sharing concerns, and expectations regarding potential platform functions such as trend analysis and repositories of best practices. First contact with stakeholders was made by email with a presentation document, describing the project. The questionnaire was completed via interview by researchers from 21 EUPAHW institutions, from 10 different Countries. The vast majority of stakeholders (95.5%) considered the use of animal welfare data as strategic for their organisation, with 81% of stakeholders interviewed regularly collecting data, 65% agreed to share their data with the platform, under certain conditions which might include case-by-case agreement, data anonymization and clarification of data ownership. The network born from this initiative was then consolidated through an online workshop, where 138 stakeholders attended. Following FAIR principles and ensuring GDPR-compliant data management, the platform will foster transparency, collaboration, and animal welfare-friendly livestock management. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Session 6

Poster 16

THIcare app: An innovative tool for assessing environmental welfare conditions in livestock farms

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The objective assessment of small ruminants' welfare needs of unified and easily interpretable standards for real-time monitoring and rapid decision-making actions, through early warning systems. As a part of the TechCare project (www.techcare-project.eu), a specialized tool was developed to assess the environmental welfare conditions (THIcare), initially for sheep and goats but with future adaptation to other species. THIcare® is a registered application operating on a multiplatform based on Microsoft Azure cloud. It collects environmental sensor data, integrating them into an Application Programming Interface (API). The API-based architecture of THIcare ensures scalability and compatibility with various sensors and data providers. Current implementation on commercial farms in the TechCare project can be seen in Elhadi et al. (2025). Data were structured as follows: 1) THI-outdoors, based on the Mader et al. (2006) equation, with temperature (T, °C) and relative humidity (RH, %) from an external weather station; 2) THI-indoors, similarly calculated from 8 T-RH indoor sensors placed at the animals' head height; 3) Aire Quality Index (AQI), derived from 2 internal sensors measuring particle matters <2.5 µm (PM/m³) and 1 supplementary for CO₂ (ppm), according to the US Environmental Protection Agency standards; and 3) Bedding Quality Index (BQI), based on water-soil capacitance (%) from 8 sensors placed in sand containers buried near water troughs, with thresholds for barley straw bedding. Data updated every 5 min and was interpreted based on alarm thresholds derived from literature and own research, adapted to species and production systems. THIcare is accessible via web browsers on PCs and mobile devices and is freely available in the Apple Store (iOS) and Play Store (Android). Users can select language and a demo version for testing (User: demo.thicare@uab.cat; Password: techcare2020). In conclusion, THIcare is an objective and user-friendly tool for assessing environmental welfare in sheep and goat farms, with planned future expansions to other species and production systems.

Developing an innovative telemedicine concept for the hessian sheep and goat health service to optimize herd care

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The present shortage of specialized veterinarians for small ruminants adversely affects their health and welfare. Additionally, there is no existing emergency service for their herd health, a service considered imperative to maintaining animal welfare, especially for rapid intervention in the event of suspected animal diseases. Therefore, there is a need to develop an innovative telemedicine concept to treat massive herd health problems connected with animal losses. The primary objective is to develop and implement a telemedicine application for mobile devices and desktop computers. The application aims to provide immediate remote specialized veterinary assistance for farmers and allow early detection of animal welfare and disease issues. A visit to the farm by a veterinarian would only be conducted when inevitable, minimizing unnecessary trips and thus contributing to climate protection. The application should enhance the work efficiency of veterinarians in herd health services and permit to reach the limited number of specialized veterinarians. The telemedicine application relies on the "Animal-Record" application, a protocol application for recording performance data, diseases, treatments, and animal assessments. This application can be utilized both online and offline if there is no network coverage. The telemedicine application enables interactions between veterinarians and farmers through video calls, text chat, and the exchange of photos for initial situation assessment. Furthermore, with the core functionality of animal assessment recording, farmers will also be able to capture additional information such as genetic defects, wool defects, and animal welfare indicators for a careful evaluation by the health service. The collected data will then be linked with genomic information to gain new insights about the complex genetic architecture of health traits and genetic defects. This approach aims to offer an efficient and convenient tool for farmers and farm veterinarians.

Panting score as indicator of heat stress in hair sheep: preliminary results

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Hair sheep exchanges heat with the environment differently than wool sheep, but the respiratory rate is an important indicator of heat stress and the main thermoregulatory mechanism of sheep in high-temperature environments. In a pasture production system, there may be little or no shade available, and in response, they may increase their respiratory rate to improve heat dissipation. However, when field assessment is difficult, the panting index can be a reliable alternative for monitoring stress levels. This study aimed to assess the panting score of hair sheep exposed to direct solar radiation. Seventeen Santa Inês ewes were kept in a 20 × 20 m pen corral without shade from 07:00 to 18:00. Over three days, vaginal temperature (VT, °C) was monitored at 10-minute intervals using mini biologgers (accuracy ± 0.06 °C), respiration rate (breaths·min⁻¹) at 30-minute intervals, and panting score (PS) every 2 hours, based on observation of panting using a scale from 0 to 4 where 0 presented no stress, normal respiration rate and 4 for extreme stress, open mouth with tongue fully extended for prolonged periods. Air temperature (AT, °C) and relative humidity (UR, %) were recorded at 10-minute intervals using a data logger, and the temperature and humidity index ($THI = 0.8 * AT + (\%UR / 100) * (AT - 14.4) + 46.4$) was calculated. Pearson's correlation coefficient was calculated at the 5% significance level. Throughout the day, the animals were exposed to AT from 17.36 to 34.87 °C, THI from 65.36 to 100, which altered respiration rate (15 – 269 breaths·min⁻¹) and VT (38.62 – 41.5 °C), as sheep were experiencing heat stress. Pearson's correlation coefficients suggested a strong relationship between the respiration rate and PS ($r = 0.79$; $P < 0.0001$). PS could be a reliable tool for assessing and monitoring thermoregulation during heat stress in hair sheep under tropical summer conditions and can be easily measured compared to respiration rate.

Should we choose between performance and welfare: case study of a citrus extract

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Optimizing broiler production efficiency has led to animal welfare concerns, generating important economic losses for producers. Hence there is interest for solutions combining productivity and animal welfare for producers. Studies have shown that maintaining intestinal health is closely linked to both productivity and well-being. The present study evaluated the effect of a Standardized Natural Citrus Extract (SNCE) on both broiler chickens growth performance and welfare. To this aim, 756 one-day-old Ross 308 males were randomly divided into 2 groups: a control group (CTL) fed a standard diet; a citrus group (SNCE) supplemented with 250 g/ton of feed of SNCE. Growth performance (bodyweight, feed intake (FI), feed conversion ratio (FCR), mortality, European Efficiency Index (EEI)) were weekly recorded until 35 d; welfare indicators (footpad dermatitis (FPD), litter quality, lameness (gate score), and hock burn) were blindly assessed at 35 d, according to the Welfare Quality Assessment Protocol. Results showed that birds from SNCE group were heavier compared to CTL group (2364 g vs. 2202 g, $p=0.001$); and FI and EEI were higher in the SNCE group (3288 g and 461, respectively) compared to the CTL group (3096 g and 426, $p<0.001$). However, no differences were observed as for FCR (1.44 vs. 1.42 for CTL vs. SNCE groups, respectively; $p>0.05$) and mortality (3.03% vs 2.75% for CTL vs. SNCE groups, respectively; $p>0.05$). Regarding welfare, the occurrence of FPD was reduced in the SNCE group compared to CTL group (3.1% vs. 20.8%, $p<0.001$). As a consequence, the rate of broiler chickens with gait score 0 (lameness) was higher in the SNCE group compared to CTL group (96.9% vs. 84.9%, $p<0.001$). Based on these results, we suggest the use of SNCE as feed additive to improve growth performance while maintaining the welfare of broiler chickens.

Session 7

Theatre 1

Innovative Processing Strategies for Enhancing Protein Utilization in Poultry Nutrition

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The use of technologically and fermentatively processed proteins is an important aspect in poultry nutrition. Given the economic pressures on the EU farming industry, optimizing protein sources is critical, as feed costs account for more than 70% of poultry production expenses. One key aspect discussed is the use of processed plant proteins, such as rapeseed and legumes, which have undergone breeding advancements to reduce anti-nutritional factors (ANFs). Despite progress, limitations remain due to residual ANFs that impact digestibility and market value. Various feed processing techniques, including hydrothermal treatment, have been shown to mitigate these factors. Heat treatments such as pelleting, extrusion, expansion, and autoclaving can deactivate protease inhibitors, tannins, and lectins, improving nutrient availability. However, these processes may also alter fiber solubility, increasing digesta viscosity and potentially impairing nutrient digestibility. Innovative feed processing technologies, including fermentation and enzymatic pre-digestion, offer promising solutions to improve protein digestibility and reduce ANFs. Fermentation with microorganisms has been shown to enhance the nutritional quality of feed by increasing beneficial microbial populations, lowering pH, and improving gut health. Enzyme treatments help break down complex fiber structures, improving nutrient absorption. Experimental findings demonstrate that fermentation and enzymatic pre-digestion significantly enhance starch and protein digestibility in both broilers and turkeys. Trials conducted with fermented pea-based diets revealed improvements in feed conversion efficiency and body weight gain. Additionally, fermented feeds contributed to a notable reduction in foot pad dermatitis in turkeys, highlighting the potential benefits of alternative protein sources in maintaining animal welfare. In conclusion, integrating innovative technologies is necessary to enhance feed efficiency and sustainability in poultry nutrition. By leveraging fermentation, enzymatic treatments, and targeted processing methods, the industry can move towards more sustainable, cost-effective, and high-performing feed solutions for poultry and other monogastric animals.

Estimation of standardized ileal digestible calcium and phosphorus requirements for broilers from 21 to 28 days of age using response surface methodology

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This study aimed to estimate the standardized ileal digestible (SID) calcium (Ca) and phosphorus (P) requirements in broiler chickens using response surface methodology (RSM). A total of 464 21-day-old Ross 308 male broilers were weighed and randomly assigned to nine treatments based on body weight, with six replicates per treatment (except for the central treatment, which had ten replicates). The experimental diets consisted of SID Ca and P, each at five levels: 3.20 and 5.90, 3.84 and 4.70, 3.84 and 7.10, 5.40 and 4.20, 5.40 and 5.90, 5.40 and 7.60, 6.96 and 4.70, 6.96 and 7.10, or 7.60 and 5.90 g/kg, respectively. On days 28 and 35, body weight and feed leftovers were recorded to calculate growth performance. Tibia bones were collected to analyze tibia ash, Ca and P content, and bone mineral density (BMD). Tibia bone breaking strength (BBS) in 35-day-old broilers was assessed additionally. The statistical analysis of the experimental data was conducted using Design Expert 13 software based on RSM with a central composite design. In 28-day-old broilers, no significant differences were observed in growth performance or tibia Ca and P content among experimental diets, whereas tibia ash and BMD showed significant differences with a quadratic effect. In 35-day-old broilers, significant differences in G:F, tibia ash, BMD, and BBS were observed with a quadratic effect. The multi-objective optimization analysis demonstrated that SID Ca at 6.96 g/kg and P at 5.56 g/kg were required to achieve optimal tibia ash and BMD in 21 to 28-day-old broilers. Furthermore, 6.29 and 6.54 g/kg of SID Ca and P were required to achieve optimal G:F, tibia ash, BMD, and BBS in 35-day-old broilers. The findings of this study suggest that estimating the optimal requirements of SID Ca and P for broiler chickens using RSM while considering multiple analytical factors is a feasible and practical approach.

Session 7

Theatre 3

Effects of insect meal as a substitute for commercial antibiotic growth promoter on gut microflora, haematology, and performance parameters in broiler chicks at stater phase

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Effects of *Macrotermes subhyalanus* meal as a substitute for zinc bacitracin (commercial antibiotic growth promoter [AGP]) on bacteria composition, haematology, apparent digestibility and performance parameters were evaluated in Ross 308 broiler chicks at 14 days/stater phase. Three dietary treatments, NC (negative control; commercial broiler diets with no insect meal and AGP), PC (positive control; commercial broiler diet with only AGP), and IBD (commercial broiler diet with only *M. subhyalanus* meal), respectively, were used in this study. 150-day-old unisex broiler chicks were randomly assigned to each of the three treatments, with 10 chicks per replicate and 5 replicates per treatment in a completely randomised design. Data on caecal bacteria composition, apparent digestibility, blood profiles, and growth parameters were measured per fortnight. Collected data was subjected to the analysis of variance using SAS software. The results of the present study revealed that Firmicutes were the predominant phylum over Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria, Verrucomicrobiota, and Vertebrata in broiler chicks fed diet IBD during the starter phase. Furthermore, Firmicutes diversity was predominantly higher in broiler chicks fed diet IBD followed by those on a diet PC. Furthermore, dietary IBD reduced ($P < 0.05$) apparent dry matter (83.50%) and crude fibre digestibility (75.62%) while apparent crude protein (CP) digestibility was significantly improved (86.24%). Plasma enzyme alanine aminotransferase was also significantly reduced by diet IBD (0.87 U/L). However, plasma protein uric acid (0.27 mmol/l) was ($P < 0.05$) elevated in chicks fed diet IBD. Body weight gain (483.28g/b) also increased significantly and feed conversion ratio (1.12) was improved in chicks fed diet IBD. In conclusion, *M. subhyalanus* inclusion in broiler diets has produced satisfying results on caeca bacteria composition, CP digestibility, and growth performance without causing negative effects on the birds' metabolic pathways and health status. Keywords: insect meal; gut macroflora; blood profile; apparent digestibility

Behavior and Stress in Slow-Growing Chickens: Effects of Live and Dehydrated Black Soldier Fly Larvae

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The use of black soldier fly larvae (BSFL) as environmental enrichment in poultry farming has been shown to improve welfare and behavior in commercial broiler lines. However, their effects on slow-growing genotypes, such as the Italian breed Bianca di Saluzzo, remain unexplored. This study evaluated the behavioral and welfare responses of slow-growing male chickens to the administration of live (LL) and dehydrated (DL) BSFL. A total of 144 Bianca di Saluzzo (a native slow-growing chicken breed) males were divided into three groups: control (C, standard feed + no larvae), dehydrated larvae (DL, standard feed + 4.12% dehydrated BSFL as fed), and live larvae (LL, standard feed + 15.38% live BSFL as fed), and reared until 147 days of age. Explorative and agonistic behaviors were assessed through video recordings, ethological tests (avoidance distance and tonic immobility), and physiological stress markers (heterophile/lymphocyte ratio and excreta corticosterone metabolites, ECM). The results indicate that live BSFL and dehydrated BSFL supplementation significantly reduced aggressive behaviors compared to controls ($P < 0.05$), while indoor exploratory behavior was higher in DL than LL groups ($P < 0.01$). A significant reduction in fear responses was observed in the avoidance distance test with fewer birds tended to be observed within 1 m of the operator in C groups compared with DL groups ($P = 0.065$). ECM levels were significantly lower in LL and DL groups compared to controls ($P < 0.001$). A trend was observed for the H/L ratio ($C < LL < DL$, $P = 0.051$). These findings suggest that BSFL supplementation, whether live or dehydrated, can serve as an effective environmental enrichment tool for slow-growing chickens, contributing to improved welfare outcomes, stress reduction, and social stability. The use of DL, despite being a non-moving stimulus, appears to provide comparable benefits to LL, offering a practical alternative for farm management.

Session 7

Theatre 5

Effect of 5 and 10% cricket (*Acheta domesticus*) meal dietary inclusion levels in broilers: growth performances

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The establishment of alternative, sustainable proteinaceous feedstuffs for monogastric nutrition is a pressing challenge requiring viable short-term solutions. Insects, a natural part of the food chain of different animals, are gaining interest as an alternative, more sustainable and nutritional valuable ingredient for both human and animal diets. This study aimed to determine the optimal inclusion level of *Acheta domesticus* (house cricket) meal as a substitute for soybean meal for broilers. Seventy-two 1-day-old male Ross 308 chicks were housed in groups of two chicks in cages and randomly assigned to three experimental diets ($n = 12$). Isoproteic and isoenergetic diets were prepared with 0% (control, C), 5% (G5) and 10% (G10) of cricket meal and provided ad libitum. To assess growth performance parameters, both liveweight (LW) and feed intake (FI) were measured weekly. This allowed the calculations of average daily gain (ADG) and feed conversion ratio (FCR). Data analysis was performed using the Generalized Linear model in SAS software considering the cage as the experimental unit. A tendency ($p = 0.079$) was observed for greater final LW with C, G5 and G10 having respectively 1198 g, 1330 g and 1318 g. No significant differences ($p > 0.05$) were observed for FCR, AVG and FI. These results suggest that *A. domesticus* meal can be included up to 10% in the broilers feed, adequately replacing soybean meal, without negative impacts on productive performance. Further research is needed to evaluate the effect of these inclusion levels on carcass and meat traits, thus assessing its potential as a soybean meal replacer in the framework of poultry production. Keywords: Insect; Feedstuff; Protein; Broiler; Sustainability.

Effect of 5 and 10% house cricket (*Acheta domesticus*) meal dietary inclusion levels in broilers: body and organ measurements

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Insects are gaining interest as alternative proteaginous sources to replace conventional feedstuffs, such as soy-bean meal, imported from third countries with high environmental costs and burdens. In addition to its low environmental impact, high biomass and local production, insects are valuable sources of nutrients and bioactive compounds. This study aimed to assess body and organ traits of broilers fed diets containing 5% and 10% of *Acheta domesticus* (house cricket) meal. One-day-old male Ross 308 chicks were randomly assigned to three experimental groups, each with 12 replicates of two chicks, housed in cages. Three isoproteic and isoenergetic diets with 0% (control, C), 5% (G5) and 10% (G10) of cricket meal were provided ad libitum. After 35-days, animals were euthanized. Carcasses were dissected and organs removed, emptied and weighed. The length of intestinal compartments was measured and the viscosity of digesta contents in small intestine was analyzed. Carcass yield was evaluated by weighing eviscerated carcasses and the main retail cuts: breast, legs and wings. Data analysis was performed using the Generalized Linear Mixed model of SAS software. The group G5 and G10 had higher carcass yield ($p < 0.0001$) and G10 higher breast muscle yield ($p = 0.0052$). Leg yield was lower in G5 and G10 ($p = 0.0011$). Relative organ weight of crop, heart, pancreas, spleen and liver was similar. Gastrointestinal organs weight and length reduced in G10. Duodenum+jejunum viscosity did not differ; however, ileum viscosity ($p = 0.0420$) was higher in C, lower in G5, with G10 showing no differences from the other two groups. Cricket meal dietary inclusion up to 10% in broiler diets suggests improved intestinal efficiency and enhanced carcass and breast muscle yields.

Session 7

Theatre 7

Effects of extruded *Spirulina* with or without a super-dosed enzyme mixture on growth performance, gastrointestinal metrics and gut microbiota in broilers

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This study evaluated the potential of extruded *Limnospira platensis*, alone and with super-dosed enzymes, as a promising feed ingredient for broiler chickens. focusing on growth performance, gastrointestinal metrics, and caecal microbiota composition. A total of 120 broilers were divided into four groups: a control diet (maize-soy-bean meal) (CTR), a 15% unprocessed *L. platensis* diet (MA), a 15% extruded *L. platensis* diet (MAE), and 15% extruded *L. platensis* supplemented with a 0.21% enzyme mixture diet (MAEM). Growth performance was monitored from day 21 to 35, gastrointestinal metrics were evaluated post-mortem, and caecal microbiota was analysed using 16S rRNA sequencing. Results showed that the MA diet negatively impacted growth due to reduced feed intake, probably due to feed adhesions around the beak. In contrast, the MAEM diet showed no significant differences compared to the CTR diet regarding final body weight, feed intake or feed conversion ratio. Concerning gastrointestinal metrics, *L. platensis* dietary inclusion decreased liver weight while the MA diet increased jejunum and ileum length all compared to CTR. Nevertheless, duodenum + jejunum and ileum viscosities were increased in *L. platensis* diets. Additionally, *L. platensis* incorporation altered caecal microbiota composition, with the MAEM diet creating distinct microbial communities. In conclusion, extruded *L. platensis*, especially when combined with enzymes, is a promising and viable feed ingredient for broiler chickens, enhancing its nutritional benefits. Acknowledgments: This research had financial supported by FCT grants, Lisbon, Portugal (UIDB/00276/2020 to CIISA, LA/P/0059/2020 to AL4AnimalS, UI/BD/153071/2022 to M.S., SFRH/BPD/116816/2016 to J.P., UIDB/04129/2020 to LEAF, and 2022.11690.BD to A.M.). Also by the Portugal2020 project (P2020/17/SI/70114/2019) and associated researcher contract to M.M..

Effects of *Euglena gracilis* administration in poultry dietC. Cenati¹, A. Pea¹, V. Perricone¹, E. Petrosillo¹, A. Agazzi¹, K. Parati², G. Savoini¹¹ University of Milan, Department of Veterinary Medicine and Animal Sciences, via dell'Università 6, 26900 Lodi, Italy; ² Istituto Sperimentale Italiano Lazzaro Spallanzani, Località la Quercia, 26027 Rivolta d'Adda, Italy

Microalgae, such as *Euglena gracilis* (EG), are photosynthetic organisms that promote gut health in animals through their antibacterial, anti-inflammatory, and antioxidant properties. This study aimed to assess the effects of EG on growth performance, relative organ index, and intestinal morphology in broilers. A total of 240 male 1-day-old broiler chickens (ROSS308) were divided into two homogeneous groups, receiving either the basal diet (CTR) or the basal diet supplemented with 190 mg/kg feed of EG (TRT). Each group was composed of 120 chickens (12 replicates, 10 birds/replicate). Initial body weight was determined at the beginning of the trial and growth performance were evaluated at days 11, 20, and 40. On each of these days, one animal per replicate was sacrificed. Liver, spleen and Bursa Fabricius were collected to determine Relative Organ Index (ROI) and samples of jejunum, duodenum and ileum were collected for morphological evaluations. Data were analysed using one-way ANOVA (GraphPad Prism), with statistical significance set at $P < 0.05$. EG was produced at the Istituto Sperimentale Italiano Lazzaro Spallanzani (Rivolta d'Adda, CR), with the following chemical composition: DM 94.24%, CP 56.46%, CF 1.24%, EE 4.42%, and ash 11.89%. No significant differences were found in growth performance at any timepoint ($P > 0.05$). Similarly, there were no significant differences in ROI between the two groups ($P > 0.05$). Intestinal samples are currently being analysed for morphological changes. Overall, the administration of EG under optimal environmental conditions didn't benefit growth performance. Further investigation under more challenging conditions may provide valuable insights into its potential to support animal health. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions.

Session 7

Theatre 9

Impact of Untreated, Extruded, and Enzyme-Treated *Chlorella vulgaris* on Broiler Breast Meat QualityA. Mendes^{1,2,3}, M. Spínola^{2,3}, O. Madacussengua¹, C. Martins^{1,4}, D. Carvalho⁵, J. Ferreira^{2,3}, A. Fontes^{2,3}, M. Mourato^{1,4}, A. Almeida^{1,4}, J. Prates^{2,3}, M. Lordelo^{1,4}¹ LEAF, ISA-ULisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal; ² CIISA, FMV-ULisboa, Av. Univ. Técnica, 1300-477 Lisbon, Portugal; ³ All4Animals, FMV-ULisboa, Av. Univ. Técnica, 1300-477 Lisbon, Portugal; ⁴ TERRA, ISA-ULisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal; ⁵ ISA-ULisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal

This study evaluates the effect of high *Chlorella vulgaris* (MCV) inclusion on broiler breast meat quality. A total of 120 male Ross 308 broilers (14–35 days old) were assigned to four dietary treatments (10 replicates each) and fed ad libitum: CTR (maize-soybean meal), CV (15% MCV), CVEnz (CV + 0.3% pancreatin), and CVExt (15% extruded MCV). On day 35, one bird per pen was slaughtered and breast meat samples were collected for meat quality analysis. Data were analysed using SAS software. MCV inclusion did not affect pH but led to decreased L* (lightness) and a* (redness) and increased b* (yellowness) compared to CTR. Extrusion reduced parameter b*. Compared to CTR, total lipids decreased in all MCV-fed groups, while carotenoid levels were higher but decreased with extrusion. Cholesterol levels were unaffected. Compared to CTR, α -tocopherol and γ -tocopherol + β -tocotrienol content were lower in the CV group, while β -tocopherol decreased across MCV groups. After 8 days, malondialdehyde levels were lower in all MCV groups relative to CTR. On the contrary, MCV inclusion increased magnesium, phosphorus, and potassium while reducing sodium contents. MCV diets increased C12:0, C14:0, C15:0, C16:0, C18:1c11, C20:3n-3, C20:5n-3, C22:5n-3 while decreasing C18:2n-6. All MCV diets enhanced breast meat quality by improving oxidative stability, mineral profile, and n-6/n-3 fatty acid ratio, with a noticeable shift in colour towards a more yellow hue. Further research should optimize MCV incorporation and processing for cost-effectiveness, enhancing its benefits to meat quality and consumer acceptance.

Effect of the administration of natural antioxidant with a nano-sized vesicle in water in comparison with an in-feed supplementation on laying hens performances

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The aim of this study was to evaluate the effect of nano-vesicles containing paprika extract supplemented via drinking water on laying hens' performances and on egg quality. 1,000 Rhode Island laying hens housed in cages of 20 birds were divided into 5 experimental groups (commercial farm, Brittany, France). From 49 to 52 weeks of age, all the birds received a diet without paprika extract. Then, for 4 weeks, a group control received the same feed without paprika extract, 2 other experimental groups received a supplementation with paprika extract in feed at a low (0.5kg/t) and high (1kg/t) inclusion rate and the last 2 groups received the same paprika extract supplemented in the drinking water through a nano-vesicle technology (Laboratoires Nano, Toulouse, France) incorporated at a low (1.5mL/L) and high (3mL/L) inclusion rates. Zootechnical performances were monitored weekly by Zootests (Ploufragan, France). The data collected were analyzed with a mixed linear model to determine the effects of the treatments on the laying performances and egg quality. The birds receiving the paprika extract in feed at the high inclusion rate and in drinking water at the low inclusion rate had better laying rate compared to the control (+3.4 and +4.6% respectively), with no effect on the feed intake resulting in a better Feed Conversion Ratio (-4.9% and -4% respectively). Egg quality was not affected by the treatments except for the yolk pigmentation. The birds supplemented in feed at both inclusion rates laid eggs with a higher Roche Scale score than the control and water-supplemented groups. Thus, we concluded that supplementing laying hens with nano-sized paprika extract in the drinking water at a low inclusion rate might be an interesting strategy to improve laying hens' performances without impacting the egg quality.

Session 7

Theatre 11

Meat quality assessment on broilers fed with high levels of *Limnospira platensis*, untreated, extruded or extruded with a super-dosed enzyme mixture

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The growing demand for food and feed requires sustainable protein alternatives to soybean meal, ensuring food security and reducing environmental impact. This study evaluates the inclusion of 15% *Limnospira platensis* — untreated, extruded, or with super-dosing enzymes—in broiler diets, focusing on meat quality and oxidative stability. In a 35-day trial, 120 broilers were assigned to four groups (10 birds each): a soy-maize diet control (CTR), control with 15% *L. platensis* (LP), control with 15% extruded *L. platensis* (LE), and control with 15% *L. platensis* + 0.21% of a commercial enzyme mixture (LEM). *Limnospira platensis* supplementation began on day 21 and continued until slaughter on day 35. During the trial, growth performance was monitored. Breast and thigh meat were collected post-mortem and analysed for meat quality and oxidative stability. Dietary of *L. platensis* did not affect lipid oxidation meat, but increased yellowness and improved lipid profile quality. It enhanced omega-3 levels and optimized the n-6/n-3 ratio, offering better nutritional value and potential health benefits. The study concluded that extruded *L. platensis* with enzymes improves digestibility, and nutrient utilization, enhancing meat quality. These findings support its use as an alternative feed ingredient for a more eco-friendly poultry production. Acknowledgments: This research was financial supported by Fundação para a Ciência e a Tecnologia grants, Lisbon, Portugal (UIDB/00276/2020 to CIISA, LA/P/0059/2020 to AL4AnimalS, UI/BD/153071/2022 to Maria P. Spínola, SFRH/BPD/116816/2016 to J.M.P., UIDB/04129/2020 to LEAF, and 2022.11690.BD to A.R.M.). Also by the Portugal2020 project (P2020/17/SI/70114/2019) and associated researcher contract to M.M.C..

Are there credible alternatives to soyabean meal for broiler diets?

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Soybean meal has significant environmental impacts, creating a need to reduce its use in broiler diets for sustainability. A systematic review and meta-analysis identified alternative proteins from the circular bioeconomy to replace soybean. Dependent variables included feed intake (ADFI), weight gain (ADG), feed conversion ratio (FCR), mortality, and diet digestibility, while independent variables were protein type, inclusion level, breed, and region. Mixed-effects and linear models were used, with region as a random variable. Analyses were conducted per broiler stage and overall. Breeds were grouped as Ross, Cobb 500, and 'Other.' Alternative proteins were categorized into eight groups: aquatic, DDGS, processed animal proteins (PAPs), microbial, rapeseed, seed, leaf, and mixed sources. Proteins were ranked based on inverse variance-weighted relative FCR. A total of 102 studies were identified. PAPs were prevalent in Europe (44%) and Asia (33%), DDGS in the Americas (50%), and seeds in Africa (56%). Only 29.6% of studies covered all three broiler stages. All performance indicators had a three-way interaction ($p < 0.05$) in the starter stage, ADG and FCR in the grower stage, and none in the finisher stage, indicating reduced sensitivity to dietary substitution with growth. Breeds responded differently to protein type and inclusion level in the starter and grower stages. Finisher ADG and FCR showed a two-way interaction between protein type and inclusion level. DM digestibility decreased ($p < 0.05$) with higher inclusion levels, but differences ($p > 0.05$) among alternative proteins were not significant. Higher inclusion levels reduced ($p < 0.05$) CP digestibility. PAPs, microbial, and seed proteins had similar digestibility ($p > 0.05$) but differed ($p < 0.05$) from DDGS. Mortality rate was influenced by protein type ($p < 0.05$) but not inclusion level or breed ($p > 0.05$). Seeds had lower mortality ($p < 0.05$) than DDGS and microbial protein but did not differ ($p > 0.05$) from other proteins. The results suggest that one size does not fit all when it comes to substitution of soyabean in broiler diets, but both broiler breed and stage of growth need to be considered. Ranking results identified aquatic, PAPs, and mixed proteins as the best-performing alternatives to soybean.

Session 7

Theatre 13

Circular Nutrition: Transforming Mushroom Waste into Quail Feed Benefits

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The circular economy in animal nutrition repurposes by-products into valuable feed ingredients, reducing waste while enhancing food production sustainability. Agaricus bisporus mushroom waste contains bioactive compounds with antioxidant properties that may improve meat quality and skeletal integrity in poultry. This study assessed the effects of incorporating Agaricus bisporus powder into quail diets on oxidative stability in meat and bone strength. A total of 300 one-day-old Japanese quail (*Coturnix coturnix japonica*) were randomly assigned to five dietary treatments: a control diet (0% mushroom powder) and diets supplemented with 0.25%, 0.50%, 0.75%, or 1.00% Agaricus bisporus powder. The birds were fed ad libitum for 42 days. At the end of the trial, meat and bone samples were analysed for oxidative stability. Lipid oxidation in the breast meat was determined using the thiobarbituric acid reactive substances (TBARS) assay, while bone strength was assessed by shear force and shear stress measurements. Dietary Agaricus bisporus supplementation significantly improved oxidative stability in meat by reducing malondialdehyde (MDA) levels at 1 and 5 days post-mortem ($P < 0.01$). The highest inclusion level (1.00% MP) resulted in the greatest reduction in lipid oxidation. Bone mechanical properties were also improved, particularly at 0.75% MP, where shear force and shear stress values increased significantly ($P < 0.01$) compared to the control. These results indicate that Agaricus bisporus powder is a promising feed additive, enhancing meat oxidative stability and bone strength in quail. Utilising mushroom by-products in poultry feed aligns with circular nutrition principles, reducing waste while improving animal health and product quality.

Effect of 5 and 10% cricket (*Acheta domesticus*) meal dietary inclusion levels in broilers: meat traits

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The increasing demand for poultry meat is pressuring the sector to identify alternative ingredients with high nutritional and functional values and lower environmental footprint. Insects are naturally consumed by birds, and are promising proteinaceous alternative feedstuffs, locally produced and with low environmental impact. Thus, this study assessed meat traits of broilers fed diets containing 5% and 10% of *Acheta domesticus* (house cricket) meal as a protein source. A total of 72 1-day-old male Ross 308 chicks were housed in cages, each with two chicks, and randomly assigned to three experimental groups (n = 12). The diets were formulated to respect the protein and energy levels for the strain and age of the broilers, with the inclusion of 0% (control, C), 5% (G5) and 10% (G10) of cricket meal. The animals were fed ad libitum. After 35 days of feeding trial, broilers were euthanized and breast muscle meat traits (proximal composition, pH, color, drip-, thawing- and cooking loss, as well as texture parameters (hardness, chewiness, springiness and cohesiveness)) were assessed. Data were analyzed by Generalized Linear model of SAS software. No significant difference ($p > 0.05$) was observed for protein content in dry matter nor ash content. Drip-, thawing- and cooking loss, pH and texture parameters were similar for all groups ($p > 0.05$). Concerning color, no significant differences were found, except for b* (yellowness, according to CIELAB system) that was significantly higher in G10 ($p = 0.0002$), indicating higher meat yellowness. Findings suggest that cricket meal may be incorporated up to 10% level into broiler diets with no negative impact on meat traits. Keywords: Insect; Alternative Feedstuff; Sustainability; Meat Quality; Color.

Session 7

Poster 15

Effect of insect meal in the starter diet on the proximate and fatty acid composition of broiler meat

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The increasing costs of poultry diets due to dependency on external ingredients make it necessary to investigate new sources of protein. In the EU, insects have been approved as part of broiler feed in recent years. Nevertheless, insect meal can be expensive and might not be available in large quantities. The composition of the insects can vary depending on various production factors, even for the same species. This project has been developed to assess the effect of different insect meals in the starter diet of broilers on the proximate and fatty acid composition of the meat. A total of 1400 one-day-old male chicks were housed in 28 pens (50 birds/pen), randomly assigned to one of four treatments depending on the composition of the isonitrogenous and isoenergetic starter diet: T1: Control; T2: 5% Tenebrio meal A; T3: 5% Tenebrio meal B; T4: 3% *Acheta* meal. Animals received the starter diet for 14 days, after which they were given a common grower diet for all treatments without insect meal, based on wheat, soybean meal and corn. Animals were slaughtered at 42 days old, and 12 randomly selected carcasses were sampled per treatment. The breast proximate composition and fatty acid profile were assessed in frozen samples kept in vacuum packaging. Data were analyzed with a General Lineal Model with SPSS (29.0) and differences between treatments were assessed with a Tukey test. No differences were found in the amount of fat or protein in the breast. However, the inclusion of Tenebrio meal in the starter showed a tendency ($P=0.079$) to increase the percentage of saturated fatty acids (SFA). Insects also showed a tendency to enhance the polyunsaturated fatty acids (PUFA) profile ($P=0.069$) by reducing the composition of monounsaturated fatty acids, especially in T3 ($P<0.05$). No differences were found in the ratio PUFA/SFA between treatments, but T1 had lower n-6 PUFA/n-3 PUFA than T2 ($P=0.009$). In conclusion, even with a common grower diet, the inclusion of insect meal in the starter can modify the fat composition of the breast. This variation will depend on the species and the way insects are produced.

Breeders supplemented with 1,25-dihydroxycholecalciferol glycoside improve the intestinal health of broilers upon reaching 21 days of age

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This study aimed to investigate the effects of 1,25-dihydroxycholecalciferol glycoside (1,25(OH)2D3-G) supplementation from *Solanum glaucophyllum* (waxy-leaf nightshade) in powder form, standardized with pre-gelatinized wheat starch and wheat middlings on intestinal histomorphometry in broilers from breeders supplemented or not with the same additive. A total of 1,152 one-day-old male Ross 308 AP chicks were distributed in a completely randomized design with a 2 x 3 factorial arrangement. One factor was the inclusion or not of 1,25(OH)2D3-G (100 and 0 mg kg⁻¹) in the breeders' diets from 21 to 30 weeks of age. The other factor involved three levels (0, 50, and 100 mg kg⁻¹) of 1,25(OH)2D3-G inclusion in the broilers' diets from 1 to 21 days of age. The study totaled six treatments, with eight replicates and 24 birds per experimental unit. The experimental diets were iso-nutritive and isocaloric, based on corn and soybean meal. The data were analyzed for normality and homogeneity of variance. Then, a two-way ANOVA was applied, with mean comparisons using the F-test for breeders and the Student-Newman-Keuls test for broilers, adopting a 5% significance level. Broilers from breeders supplemented with 1,25(OH)2D3-G reduced crypt depth and increased the absorption area and villus-to-crypt ratio in the jejunum at 21 days of age. It is concluded that breeder supplementation with 100 mg/kg of 1,25(OH)2D3-G improves the intestinal health of broilers at 21 days of age.

Session 7

Poster 17

Estimation of calcium and phosphorus standardized ileal digestible requirements on bone characteristics of broilers from 10 to 21 days of age using response surface methodology

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Calcium (Ca) and phosphorus (P) are the major minerals composing bones, playing an essential role in skeletal development. This study estimated the standardized ileal digestible (SID) Ca and P requirements based on bone characteristics, utilizing response surface methodology (RSM) to account for the interaction between Ca and P, and to consider multiple analytical factors simultaneously. A total of 480 10-day-old Ross 308 male broiler chickens were weighed and randomly allocated to nine treatments with five replicates (except the central treatment with eight replicates) based on body weight. In this experiment, SID Ca and P were set at five levels each, and the central composite design, an RSM experimental design, requires only nine treatments. Experimental diets contained SID Ca and P concentrations of 2.80 and 5.50, 3.44 and 4.44, 3.44 and 6.56, 5.00 and 7.00, 5.00 and 5.50, 5.00 and 4.00, 6.56 and 4.44, 6.56 and 6.56, or 7.20 and 5.50 g/kg, respectively. On day 21, the tibiae were collected to analyze ash, mineral contents (Ca and P), and bone mineral density (BMD). Statistical analysis was conducted using Design Expert 13. The single-objective optimization using RSM estimated the optimal SID Ca and P requirements as 7.20 and 7.00 g/kg for tibia ash, 5.75 and 5.87 g/kg for tibia Ca content, 7.20 and 7.00 g/kg for tibia P content, and 7.20 and 6.96 g/kg for BMD, respectively. In the multi-objective optimization of all criteria, the optimal SID Ca and P concentrations were determined to be 7.13 and 6.80 g/kg, respectively. These results demonstrate that RSM is an efficient method for simultaneously estimating SID Ca and P requirements while considering various response criteria.

Effect of exogenous emulsifier and different oil inclusion levels on nutrient and energy utilization in broiler chickens

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The current study examined the influence of soy oil inclusion level and emulsifier supplementation on apparent ileal digestibility (AID) and total tract retention (TTR) of nutrients and energy in broiler chickens. A completely randomized design was used, with a 4 x 2 factorial arrangement of 8 treatments (8 replicates per treatment, 6 birds per replicate) involving 4 soy oil inclusion levels (0.0, 1.5, 3.0 and 4.5%) and with (500g/ton) or without emulsifier supplementation. Feed intake and excreta output were recorded for each cage over 4 consecutive days from d 20 to 24 post-hatch for measuring TTR of nutrients and nitrogen-corrected apparent metabolizable energy (AMEn). To measure ileal digestibility of nutrients ileal digesta were collected from the lower half of the ileum of birds. The ileal digesta were pooled within a cage, freeze-dried, ground and then stored at 4 °C until laboratory analysis. Each replicate cage was considered as an experimental unit. The data were analyzed by two-way ANOVA to determine the main effects and their interaction using the JMP18 software. In general, TTR of nitrogen (N) and AMEn and energy metabolizability increased ($P < 0.001$) with increasing fat level. Birds fed 3.0 and 4.5% soy oil showed higher TTR of N, AMEn and energy metabolizability values compared to those that did not receive supplemental soy oil. Emulsifier supplementation significantly ($P < 0.05$) increased the TTR of N and fat and AMEn compared to the diets with no emulsifier. Emulsifier effect tended ($P = 0.075$) to be significant for AID of N, with diets having emulsifier tended to have higher AID of N compared to those not having emulsifier. No interaction was observed ($P > 0.05$) between soy oil inclusion and emulsifier supplementation, for any parameters measured, showing that emulsifier effect was similar with all soy oil inclusion levels. The current results showed that supplementation of emulsifier to broiler diets, regardless of oil inclusion level would support improving nutrient and energy utilization.

Session 8

Theatre 1

Application of Neogen's GGP Equine 70K SNP Array

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Neogen's GGP Equine 70K array supports a broad range of applications, including research and discovery of new traits, parentage analysis, and hereditary disease and trait screening. The Royal Dutch Sport Horse (KWPN) has effectively integrated SNP genotyping, the application underscores the transformative impact of SNP genotyping on equine breeding, genetic research, and health management, shaping a data-driven future for the horse industry. The primary application is parentage verification (i), with all foals and their parents genotyped since 2021 using the GGP Equine 70K array, in line with ISAG guidelines. In 2023, the Royal Friesian Horse Studbook (KFPS) adopted the same system through KWPN's genotyping framework, resulting in 63,000 KWPN and 10,000 KFPS horses genotyped to date. Beyond parentage, SNP genotyping is used for breeding value estimation (ii), significantly improving the accuracy of estimated breeding values (EBVs) for health, conformation, and sport performance traits, as observed in other species employing genomic selection. Another major application involves monogenic trait detection (iii), such as Warmblood Fragile Foal Syndrome (WFFS), where carrier frequencies have decreased from 5.8% to 4.4% in dressage horses and from 3.8% to 3.0% in show jumping horses since 2021. While carriers are not excluded from breeding, genotyping enables informed mating decisions, contributing to this decline. Similarly, KFPS uses SNP data to manage dwarfism, hydrocephalus, and the chestnut factor. Finally, SNP genotyping plays a crucial role in research applications (iv), especially genome-wide association studies (GWAS) for traits like Grand Prix-level performance and disorders such as osteochondrosis and kissing spines. A U.S. study (Patterson Rosa et al., 2022) identified a SNP associated with kissing spines, which appears to have a similar effect in KWPN horses, with ongoing research aiming to pinpoint the underlying functional mutation. In conclusion, the early adoption of genomics in horse breeding enables KWPN to make informed decisions when it comes to breeding high-performing, healthy horses.

Evolution to Genomic Selection with the EQUIGENE SNP Array in Purebred Spanish Horse

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The Purebred Spanish (PRE) horse is a breed with a population exceeding 290,407 animals distributed across 71 countries and a structured breeding program established for more than 20 years. This program includes selection criteria based on morphological traits (linear conformation score) and traits related to classical dressage aptitude. The newly developed Axiom EQUIGENE 90K array (90,926 SNPs) integrates informative and high-throughput imputation markers from various density arrays, ISAG-recommended SNPs for parentage verification, and markers identified in literature, international databases, and genome-wide association studies relevant to PRE horses and related breeds. This array enables applications in parentage testing, heritable disease detection, analysis of economically important traits, and genomic selection. Initial genomic evaluations in PRE horses utilized data from 6,752 animals genotyped with the Axiom EQUIGENE 90K, selected for their impact on genetic evaluations. A single-step genomic BLUP approach was applied to assess the impact of genomic data incorporating reliability (R^2) using single-step restricted maximum likelihood (ssGREML) compared to traditional REML methods across all evaluated traits (61 morphological, 7 reproductive, and 1 dressage traits). Heritability estimates remained consistent between ssGREML and REML. Reliability improvements with ssGREML ranged from 9.6% to 14.1% for morphological traits, while gains for reproductive traits were lower (1.8%–3.96%), and dressage-related traits showed a 2.96% improvement. Selection response gains with ssGREML varied from 2.1% to 8.8% for morphological traits, 1.6% to 5.9% for reproductive traits, and 2.96% for dressage-related traits. These findings confirm the enhanced reliability and genetic progress achievable through genomic selection in equine breeding programs, particularly for the PRE horse.

Session 8

Theatre 3

Evaluating the impact of a new stallion performance testing concept on genetic progress using stochastic simulation

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Starting in 2025, revisions to the stallion performance testing framework in Germany aim to improve the accuracy and relevance of evaluations, ensuring they better reflect the demands of modern equestrian disciplines and breeding objectives. The system follows a multi-stage phenotypic selection and points-based approach, assessing stallions from 3 years old based on their achievements in dressage or show jumping. Stallions qualify for breeding (Stallion Book I (SBI)) through either stationary tests (50-day performance test (HLP50)) or field tests (Evaluation Test and Tournament Sport Test), which could be combined with a stationary 14-day test (VA14)). A key change is the Evaluation Test, replacing previous sport tests for 4- and 5-year-old stallions, and the expansion of tournament sport test results. These modifications are expected to have a significant impact on breeding stallion generation intervals and long-term genetic improvement, potentially affecting the qualities and capabilities of future generations of sport horses. To assess the effectiveness of different scenarios for registering a stallion in SBI, a comprehensive stochastic simulation of a standard German horse breeding program was conducted using the MoBPSweb (Modular Breeding Program Simulator) over 20 years. Changes to stallion selection protocols were evaluated, including variations in testing duration (3–6 years) and the integration of diverse performance metrics such as standardized tests, additional evaluations, and competition success. Genetic progress was measured in genetic standard deviations (gSD). Preliminary results indicate that while the new HLP concept with a 3-year generation interval achieves high genetic progress (0.54 gSD, HLP50) compared to traditional testing over 6 years (0.48 gSD), the VA14 test remains valuable. An extended VA14 combined with field tests over a 5-year selection period appears most effective for long-term genetic gain, achieving the highest genetic progress (0.57 gSD) among all scenarios.

Young Horse Competition results boost genetic evaluation for dressage performance

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The breeding goal of the Royal Dutch Sport Horse (KWPN) is top-level (Grand Prix) sport performance. Since this trait is only measurable later in a horse's life, predictor traits are crucial in genetic evaluations. Breeding organizations vary in how they handle Young Horse Competition (YHC) results—ranging from ignoring them to publishing a dedicated index—though full inclusion in the Best Linear Unbiased Prediction (BLUP) would be preferable. Some concerns about using YHC results include differences in scoring compared to regular dressage judging and a general misunderstanding of genetic correlations among breeders. This study applies LR-validation (Legarra & Reverter, 2018) to assess whether YHC results improve predictions of sport performance. Data from 11,159 YHC starts (7,899 horses, spanning 22 years) were analyzed. The best model included random effects for animal, permanent environment, judge, and rider (minimum three horses per rider; otherwise, a 'dummy' rider was assigned). Estimated heritability was 0.15, with repeatability at 0.35. Sport performance data included 76,062 single observations per horse (highest ranking), with a heritability of 0.22. The genetic correlation between YHC results and sport performance was 0.90. The LR-method compared genetic predictions using partial and complete datasets. Validation animals—those whose highest ranking changed in the last three years or were newly recorded—were assessed using three criteria (rIH, bias, and slope), once with and once without YHC results. The model including YHC results performed better in all criteria, though differences were small. These findings confirm that YHC results are valuable early predictors of sport performance and should be integrated into multivariate genetic evaluations.

Session 8

Theatre 5

Unraveling the Hidden Genetic Load: Analysis of Inbreeding Depression in Dressage Performance of Pura Raza Española Horse

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Inbreeding depression, associated with increased homozygosity, constitutes a critical issue in closed populations such as the Pura Raza Española (PRE). High levels of inbreeding have been identified in the breed, with an average coefficient of 7.4%. Average inbreeding ranged from 7.012% (for the 26,512 animals with walk, trot and canter data) to 6.957% (for the 43,619 animals with point per reprise data). This study analyzed 43,838 dressage performance records from 4,546 PRE horses, focusing on walk, trot, canter, and total points per reprise (PPR). Wright's classical inbreeding (F) and partial inbreeding coefficients (Fij) were computed for 406,545 individuals, partitioning inbreeding by common ancestors. Regression analyses showed detrimental inbreeding effects for all traits, with slopes ranging from -0.4 (trot, canter) to -5.5 (PPR). Heritability values were moderate to high (0.09 for walk, 0.11 for trot, 0.33 for canter, and 0.25 for PPR). Inbreeding load ratios ranged between 0.14 (trot) and 0.89 (PPR), exceeding the additive genetic variances in all cases. In addition, the inbreeding load genetic values estimation allows us to identify individuals that, used as common ancestor, will transmit an increase in the phenotypic value of its inbred descendants for a certain trait. These findings highlight the importance of incorporating inbreeding depression load estimates into selection programs to reduce potential deleterious effects on dressage performance. Thus, identifying and selecting breeding animals with a suitable inbreeding load genetic value for key traits, PRE breeders can enhance the overall fitness and competitiveness of future generations in dressage disciplines.

Association of infrared imaging in young horses with genetic functional longevity in show jumping

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A specific protocol was developed to identify early criteria of functional longevity in show jumping horses. Surface temperatures of young horses (aged 2-4) were measured using infrared images captured by a thermal imaging camera. The study included 921 horses, selected as progeny of two extreme groups of stallions: 61 with favorable and 80 with unfavorable estimated breeding values calculated from 202,320 horses for functional longevity defined as the number of years spent in competition adjusted for performance levels. For each horse, six images were captured: the back, a front view of the forelimbs, a rear view of the hindlimbs, right and left side views, and the head. Four categories of temperature variables were defined: temperature of regions of interest (ROIs), temperature differences between ROIs, asymmetry between left and right or front and back of the same ROIs, variability (difference between maximum and mean) within ROIs. An animal mixed model was used to estimate heritability, accounting for age, sex, coat color, weight, visit, and using a relationship matrix spanning five generations. A multivariate partial least squares (PLS) logistic regression was performed to predict the group of sires based on temperature variables adjusted for fixed effects from the mixed model. Key findings included: high heritability for general temperature (0.53), relative temperature to the body of fetlocks (0.47), knees (0.55), feet (0.46), and, to a lesser extent, hocks (0.21), lower heritability for differences between zones of interest (0.02 to 0.23), except for the difference between knees and feet (0.63). Asymmetries and temperature variability showed low heritability. One principal component in the PLS analysis was significant: cooler eye temperatures (p-value: 0.01), smaller asymmetry in mean hindfoot temperatures (p-value: 0.02), and low variability in dorsal temperatures (p-value: 0.03) were associated with the group of stallions exhibiting longer longevity in show jumping. These results suggest potential early criteria for selecting horses with better longevity in show jumping.

Session 8

Theatre 7

Genetic parameters of clinical and radiological findings of candidate stallions presented for licensing for German Warmblood breeding

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Sustainability and long-term success of sport horse breeding requires proper consideration of health aspects. In Germany, the central equine health database is the core infrastructure supposed to foster development of new, health-targeted breeding tools. Harmonized screening protocols including standardized clinical and radiological examinations could deliver valuable information for genetic and genomic analyses if used for collection of data on a large enough and representative sample of the whole population. However, such screening is hard to establish and does not yet exist for riding horses. Therefore, the aim of this study was to evaluate the usability of health data on a preselected horse sample for breeding purposes. Examination results of 1,692 stallions presented for licensing at German Warmblood studbooks in 2018 to 2020 had been entered into the health database and made available for genetic analyses. The most commonly recorded clinical findings and radiological findings in the limbs were selected for further analyses, considering different levels of grouping vs. refined trait definitions. Variance components were estimated in single- and multiple-trait linear animal models using pedigree information on 4 ancestral generations (17,406 horses in the relationship matrix) and VCE6 software. Most of the findings were minor, and only two clinical and four radiological findings had prevalences of > 10% including, e.g., swellings / exostoses in Mc/Mt and changes of bone contours at P2. Heritability estimates were low for all traits, ranging from < 0.001 to 0.089 (clinical) and 0.118 (radiological), and had high standard errors ($SE \leq 0.059$). Plausible patterns of h^2 and rg were found for radiodense bodies in different limb joint locations, consistent with previously described advantages of considering joint (fetlock, tarsus), limb (front, hind) and location within joint (dorsal, palmar/plantar). Preselection had likely impacted the results implying slower, but possible breeding progress through systematic use of health data on selection candidates rather than population-wide data.

Genomic insights into limb conformation defects in Pura Raza Española horse

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Conformation is a major selection criterion in horse breeding, affecting functionality, sport performance, as well as health, and appearance. Reducing the prevalence of conformation defects, in particular limb defects such as the knee and hock, is a challenge for breeders and their genomic background remains understudied. This study aimed to identify genomic regions associated with these defects in Pura Raza Español (PRE) horses using wssGBLUP (weighted single-step genomic BLUP) analysis. A total of 58,930 horses were evaluated in lateral and rear views of hock and knee angles, with 4,000 genotyped using the Axiom EQUIGENE array. After quality control (CR >0.95, MAF >0.01), 77,584 SNPs were retained. Traits were assessed as continuous variables scored at nine levels (-4 to 4) for both lateral view and rear view hock and knee defect (LVHD and RVHD, LVKD and RVKD, respectively) and classified into opposite defects (multinomial variables) scored from -4 to 0 and from 0 to 4 (closed/open, convergent/divergent hock, buck/calf knee, bench/knock knee). Hock defects were more prevalent than knee defects in the PRE population studied. Heritability estimates for the continuously evaluated traits ranged from 0.18 (LVHD) to 0.25 (RVHD), and from 0.13 to 0.42 for the multinomial evaluated traits, with the lowest for bench knee and the highest for divergent hock defect. The genome-wide association study identified 8 genomic regions associated with hock defects and 17 with knee defects, with two common regions for both defects on ECA10 and ECA19. Additionally, genes related to musculoskeletal diseases, bone malformations, joint disorders, cartilage defects, and bone fragility were identified. These findings enable us to implement selective breeding strategies to reduce limb defects in PRE horses and provide insight into genetic and molecular mechanisms involved. Further research is needed to enhance understanding of conformation defects in horses.

Session 8

Theatre 9

Breeding against chronic progressive lymphedema in Belgian Draft horses: can radiography provide an improved phenotype?

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Chronic progressive lymphedema (CPL) is a systemic disease that plagues several breeds of draft horses. It affects the lower limbs and afflicts progressive swelling, nodulous formations and severe skin folding. Therefore, Belgian Draft horses are screened through palpation of the four limbs for their CPL status graded from 0 to 4 (De Keyser et al., 2014). Previous research has shown a clear heritable component in the development of CPL, but collecting sufficient reliable phenotypes has proven difficult and makes the development of a breeding program challenging. Since 2011, radiography has been used to evaluate the distal limbs of candidate sires, providing a detailed assessment of the number of skin folds of the limbs. Therefore, this new phenotype could aid in CPL characterization and be incorporated into the breeding program. For this study, radiographs of 216 two-year-old candidate sires were available. On average, 7.6 folds (SD=3.9) were counted per limb, with rear limbs having significantly more skinfolds compared to the front limbs (+2.1 folds). On average, the palmar/plantar side had seven times as many folds as the dorsal side. No significant differences were found between right and left limbs. These scores were compared to the clinical evaluation through palpation (ordinal scores from AA to D). Correlation between clinical assessment and the radiographic number of skinfolds were ± 0.58 (p-value < 0.01). Combinations of these traits will be included in a genetic model to estimate genetic parameters for CPL in the Belgian Draft horse.

Detrimental Alleles Detection in Norwegian Fjord horse

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The Fjord horse is a Norwegian native breed that have undergone several population bottlenecks and is endangered. The mean foaling rate of the breed is 10% lower than in comparable breeds using the same breeding methods. Additionally, a marked decrease in the number of covers for the past few decades has resulted in a decreasing population size. The breeding association has made grants available to incentivize the covering of 3 to 5-year-old mares in order to improve foaling rates. Sparse documentation about the genetic health limits the possibilities to maintain a sustainable population. This study aims to test whether segregating recessive lethal alleles can be detected and explain the reduced foaling rate in the breed. High-Density Single Nucleotide Polymorphisms (Illumina 670K SNP chip) from ~1300 samples will be utilized to genetically characterize the Norwegian Fjord horse population. The analyses will be a homozygote-missing based detection from a previously developed bioinformatic pipeline (<https://github.com/NattyandMinnie/Master-Thesis>). Preliminary results on ~800 samples show several candidate recessive loci, including six loci (ECA9:36505547-36977686, ECA11:26775261-26853544, ECA11:27142204-27611490, ECA11:36620429-26747778, ECA12:20440094-20444108, ECA20:991548-1061005) with known effect on fertility or early lethality in humans or model animals. The detected recessive detrimental alleles will be further characterized with whole-genome sequences on 25 samples and confirmed through a complex segregation analysis to model the variants impact on fertility.

Session 8

Theatre 11

Responsible and qualified horse breeding to protect health – a collaborative project to reduce squamous cell carcinoma of the eye in Austrian Haflinger and Noriker

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Ocular squamous cell carcinoma (OSCC) is a common cancer of the equine eye. The etiopathogenesis is multifactorial, including exposure to ultraviolet radiation and a lack of pigmentation as contributors increased risk. A high incidence of OSCC in the Haflinger and Noriker breeds is evident from the patients at the Vetmeduni Vienna. In 2017, a missense variant in the DDB2 gene was discovered, and held responsible for the increased risk for OSCC in the Haflinger breed. Genetic testing is recommended. Is this finding the quick solution to the problem? Genetic testing of disease-reported variants can influence a horse's treatment and prognosis; test results should also impact mating decisions – however, this will affect the genetic composition of the breed in future generations, particularly in terms of genetic diversity. Breeding associations are faced with the challenge of finding sustainable strategies to ensure the health of individual animals as well as the entire population. The enormous economic value of horses and their ideological significance as well as public interest further complicate the actions. Here, we present results from a joint effort of geneticists, veterinarians and breeders to create a data-driven basis for responsible breeding recommendations to improve horse health. The project involved the determination of the frequency of the risk allele in the Austrian Haflinger and Noriker populations. Furthermore, the impact of the risk allele on the progression of the disease was evaluated with clinical examinations in the field. Based on collected data, breeding strategies and their consequences on genetic diversity were evaluated and discussed. The project underlines importance of evidence-based decision making, education and communication to safeguard animal health. Supported by DAFNE – Nr. 101905.

Does nose shape have a predictive value for equine personality?

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The Prader-Willi Syndrome gene region includes a tandemly repeated regulatory RNA coding gene (SNORD116). The copy number (CN) at this locus is assumed to be related to differences in behaviour and craniofacial shapes in mammals. In the Holstein horse, there are horses with and without a Roman nose shape (convex nasal profile). Our aim was to test whether nose shape and SNORD116 CN have a predictive value with regard to personality traits. 103 horses on six farms were evaluated with an owner directed personality survey using a 5-point scale and a 3-point scale. 16 behaviour traits (BTraits) were assessed, and the principal component analysis method followed by factor analysis with orthogonal transformation used to associate these traits with personality components (PComps). Two components (nervousness and curiosity) were retained based on the eigenvalue criterion and scree test. Eight BTraits were loaded on the component 'nervousness' and five BTraits were loaded on the component 'curiosity'. Scores indicating the individual level of nervousness or curiosity were calculated for each horse. In addition, nose shape was assessed and hair samples were used for CN analysis. Data was split into training (80%) and test (20%) datasets. A random forest model was used to evaluate the predictive value of nose shape, SNORD116 CN, age, sex and owner for both BTraits and PComps. Hyperparameter optimization was applied with a 10 times 3-fold cross-validation, selecting the best configuration based on the Root Mean Squared Error for PComps and Area Under the Receiver Operating Characteristic Curve (AUROC) for BTraits. In the test set, prediction quality was better for 'curiosity' ($R^2 = 0.27$) than for 'nervousness' ($R^2 = 0.16$). Among individual BTraits, AUROC values in the test set ranged between 0.44 (attachment to people, adaption to novelty) and 0.84 (agitation when alone). Nose shape and SNORD116 CN consistently ranked among the most important predictors, indicating their role in equine personality variation with possible implications for breeding, training and management.

Session 8

Poster 13

Genomic evaluation for height of withers and cannon bone circumference in Heavy Warmbloods – First Results

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As in other horse breeds in Germany, height at the withers (WH) and cannon bone circumference (CBC) in Heavy Warmbloods are the only metric traits that are recorded and WH was also used as a reference trait in genomic selection in riding horses. In Saxon-Thuringian Heavy Warmbloods (STHW), breeders attach great importance to both measurements as they influence the caliber and are therefore decisive for the breed type. In addition to their suitability as a driving horse, size and bone strength also clearly distinguish the STHW from warmbloods or riding horses. Based on the Illumina Equine80select bead chip, 1,106 genotyped animals with 71k SNPs and 2,654 animals with measurements for both traits (born after 1989) were available after quality control filters. Using the BLUPF90 family programs (Misztal et al., 2014), heritabilities and genetic correlations were estimated applying a 2-trait animal model with sex and birth year, as well as their interaction as fixed effects, age in months as a covariate and a random animal effect. Heritability estimates for both traits were within the expected range, i. e. 0.64 for WH and 0.60 for CBC (se=0.04). Genetic and phenotypic correlations were 0.59 and 0.48, respectively (se=0.047 and 0.020). Contrary to an ever increasing genetic trend in the population for WH, the phenotypic trend in stallions remains almost constant. In mares, on the other hand, both the genetic and phenotypic trends are increasing. The current mare birth years phenotypically and according to gEBV resemble the level of the stallions. For CBC there is practically no difference between the sexes in the gEBV, while phenotypically the difference is 0.7 cm. Results from GWAS reveal one potential region around the LCORL gene influencing WH on ECA 3 and 2 to 3 regions for CBC on ECA 3, 6, and 9, which could contain potential candidate genes.

Genetic trends in the endurance performance of horses in Spain

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The Spanish Arabian Horse Breeder Association (AECCA) is currently developing a breeding program aimed at improving endurance performance and conformation. The Arabian breeding program was established in 2005 and the first genetic evaluation for endurance was published in 2012. The data used in the routine genetic evaluation are collected during the Young Horse competitions (YHC) and in the races organized by the Royal Equestrian Federation (REC). Two genetic indexes are developed: one for young animals including the placing, the race time and the recovery time, and another for adult animals including placing and ranking. The objective of this study was to evaluate the genetic trends for the endurance traits evaluated after 20 years of the breeding program establishment. The data contained 4035 records from YHC and 10210 records from REC. The pedigree used had 16382 individuals (59% Arabian horses). The models included the sex, the age, distance as covariate and event as fixed effects and the rider, rider-horse or environmental permanent effect as additional random effect depending on the trait. The models were solved using threshold methodology for placing, Thurstonian method for ranking and classical linear methods for times. Heritabilities for racing time, recovery time and placing in YHC were 0.14 (0.03), 0.25 (0.03) and 0.04 (0.02), respectively. For REC, heritabilities for ranking was 0.13±0.02 and for placing was 0.11 (0.02). The phenotypic trends for recovery time and placing showed an improvement in both traits after 2017 in YHC. This positive trend in the phenotype was observed also in REC for placing. In terms of genetic trends, compared to the average of individuals born in 2005, animals born in 2018 have moved the average by 0.33 genetic standard deviations for placing and 0.12 for recovery time. These results show that the Arabian programme in Spain is working well, and that genetic progress is being disseminated throughout the population.

Session 8

Poster 15

Y chromosome reveals shared ancestry of Noriker and Haflinger patriline

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The paternally inherited, male-specific part of the Y chromosome (MSY) facilitates tracing the origin and distribution of sire lines. Recently, fine-scaled MSY haplotyping was established as a paternal ancestry prediction method in horses (Radovic et al., 2024). Here, we investigated Haflingers and Norikers, two renowned Austrian Alpine breeds. Haflingers have seven bloodlines, all tracing back to stallion 249 Folie, born in 1874 from a Tyrolean mountain mare and an Arabian sire. The Noriker, one of the oldest mountain draft breeds, originates from ancient Roman war horses and now comprises five distinct sire lines. MSY haplotypes (HTs) were determined in a dataset of 80 Norikers and 91 Haflingers representing all sire lines in those breeds. MSY HT determining markers were genotyped using KASPTM method, and ten different HTs were detected. Both breeds clustered across daC_Ad-h and daC_Ao-nM1 MSY haplogroups (HGs), whose common ancestor lived about 1,000 years ago. However, the breeds had distinct HT patterns within the shared HGs. In Haflingers, daC_Ad-hC HT predominated in 44 males across five lines (B, M, N, S, St and W), followed by daC_Ao-nM1a2 in two lines (A and N). In Norikers, daC_Ao-nM1a2 was most common and noted in 55 males from four sire lines (Elmar, Diamant, Schaunitz and Vulkan), followed by daC_Ad-hA/B HTs in Nero and Schaunitz lines. The MSY HTs observed in both breeds link to Coldblood breeding and Spanish ancestry, a finding that supports the use of heavy horses to consolidate these breeds. Remarkably, we found no HTs of Arabian origin in Haflingers, which contradicts historical claims. Furthermore, discrepancies between paternal lineage records and MSY HT results were identified. For example, 13 males from a Haflinger St subline carried the unique HT daC_Ao-aA2, suggesting a recent undocumented introgression. Overall, this project highlights the utility of MSY haplotyping to uncover the origin and history of horse breeds. Radovic et al., 2024. The Global Spread of Oriental Horses Through the Y Chromosome, PNAS, 121(49), e2414408121. <https://doi.org/10.1073/pnas.2414408121>

Using x-rays to study severity of CPL in Dutch Draught horse population

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Chronic Progressive Lymphedema (CPL) is expected to be threatening the already endangered Dutch Draught horse population with incidence rates around 80%. CPL causes the lower legs to start swelling, skin folding and progressively resulting in open wounds. Treatments can reduce inflammation and swelling, however no cure has been found so far. Several studies have been performed to detect the genetics of the disorder, each with varying success. Genetic studies have been strongly hampered by the limitations of the data structure and phenotyping, which is mainly done via palpation. Drawbacks to this method are 1) subjectivity, 2) no repeatability and 3) feathering obscures mild cases. Phenotyping based on x-ray is expected to be more accurate. The aim of this study is to infer the variation in CPL in Dutch draught horses based on x-rays and to determine its suitability for genetic studies. A total of 247 x-rays were collected from 108 individuals – 52 females (0-18 years), 56 males (52 stallions 3-8 years and 4 geldings 7-19 years). Scores were from 0 (no CPL) to 4 (extreme CPL). Variance between horses was studied based on x-rays of front leg. Only 9% (n=12) of the horses showed no symptoms of CPL, all younger than 5 years. Extreme CPL was scored for 18 horses (14%). Variance within horses was studied based on x-rays of all 4 legs per horse (n=41). Different scores were found between left and right legs for 25% of the horses. Whether this is caused by real differences in left and right severity in CPL or it might be caused by subjectivity in scoring needs further investigation. Variance in progressiveness was studied based on repeated x-rays of stallions aged 3 and 4 years (n=16). Most stallions (n=10, 63%) increased with 1 score, 4 stallions had the same score and 2 stallions increased more than 1 score. The preliminary results indicate high incidence with varying severity for CPL in the Dutch Draught horse population. Additionally, phenotyping CPL with x-rays prove to generate useful and repeatable information.

Session 9

Theatre 1

Digital Tools Supporting Grazing Sheep Farming

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The use of digital tools could optimize farm management by facilitating data collection and processing. While previous studies have identified tool types, needs, motivations, and barriers to adoption across sectors, they do not explore in detail current uses, frequency of use, user profiles, or reasons for discontinuation, particularly in grazing livestock systems. This study aims to explore these aspects, focusing on sheep farming across four French regions. 63 sheep farmers were interviewed in four French regions. The collected data were analyzed using descriptive statistics and a multivariate analysis was carried out to establish a typology of farms and examine the relationship with the use of digital tools. Results show that 49% of farmers have a positive perception of digital tools, 25% a negative perception, and 25% a neutral perception. Digital tools, categorized into seven categories, are not widely used in sheep farming, although 87% of the farms surveyed use at least one tool. The most widely used categories are farm management software (73%) mainly for animal management, and online exchange platforms (57%) for information or social interactions, while on-board sensors (4%) and barn monitoring tools (9%) are the least used. The frequency of tool use varies by category and function, with farmers being the main users. Motivations for adoption vary according to the tool, each responding to specific needs such as reducing work intensity or saving time. However, farmers reported difficulties in using some tools, including problems with maintenance, networks or reliability. Although few tools have been entirely abandoned, some shortcomings were identified by farmers, leading them to stop using specific technologies. The multivariate analysis identified three main farm groups with different perceptions and levels of adoption of digital tools, each with their own specific uses. The next step of this work will be to conduct an in-depth survey to analyze how farmers appropriate the tools they use. Keywords: sheep farming, grazing systems, digital tools, adoption, motivations and uses.

DIGI-Rangeland: Digital innovation and technology network for rangeland livestock farming systems and areas in Europe

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Rangeland livestock farming systems (RLFS) play a significant role in achieving the sustainable development goals in Europe. However, these systems face numerous challenges that threaten their viability. The use of innovative technologies takes part of a wider bundle of measures required to improve the future viability of such farming systems. The DIGI-Rangeland project aims to create and expand an innovative network focused on digital technologies and innovations (DTI) tailored for farmers and other land-users in rangeland territories. Using a multi-actor approach in 10 countries, the project will facilitate knowledge sharing among EU stakeholders and foster cross-fertilization between rangeland areas actors around the use of DTI. By identifying the needs and barriers faced by practitioners the project will identify, evaluate and promote effective DTI solutions to ensure the resilience and sustainability of RLFS across Europe. Based on examples from the first workshops, we present the EU network, its objectives and structure as well as the first elements resulting from the identification of the challenges and needs of the stakeholders.

Session 9

Theatre 3

Bluetooth technology to assess sheep access to winter feeding supplementation in extensive systems

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Sheep production in extensive systems is subject to several challenges linked to welfare, climatic and geographical conditions, animal performance, rural connectivity and social fabric, to name a few. The TechCare project, funded by the EU H2020 research and innovation programme (grant 862050) identified undernutrition during winter as one of the main welfare priorities for meat sheep in UK systems, where animals are grazed outside most of the year on poor quality mountain pastures. The introduction of the Internet of Things (IoT) and low power wide area (LPWA) networks alongside with Bluetooth Low Energy (BLE) technology present interesting opportunities to monitor animals in extensive systems. This abstract presents results from a trial held in Scotland in Jan-Feb 2022, where 100 grazing ewes (50 Scottish Blackface, 50 Lleyen) were fitted with BLE beacons (Feasybeacons 4.2). Receivers (14) were positioned above feed resources (4 on hay feeders and 4 above molasses buckets) and on posts (6) in a 40 ha mountain pasture. Each beacon transmits a unique ID, with the BLE receivers reporting these IDs along with the beacon's RSSI (Received Signal Strength Indicator). Over a 5-min duty cycle, the BLE reader was programmed to report the identity and RSSI of 16 beacons with the strongest signal for that duty cycle. Results showed that the technology allows for a clear differentiation in different groups of ewes, and the BLE system was able to detect daily rhythms in the ewes. Additionally, the readers were able to detect the presence or absence of particular ewes in their vicinity over time. This could allow the development of thresholds (green/amber/red) to identify animals which are always or never read by the BLE readers, or which present changes in their daily rhythms, due for example to welfare issues or disturbance. The technology shows promising opportunities for sheep monitoring in extensive areas.

The use of Ultra High Frequency (UHF) technology to investigate feed resource use by sheep as a potential welfare indicator

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Potential uses of UHF (Ultra High Frequency) RFID (Radio Frequency Identification) tags in small ruminants have been explored as part of the EU Horizon 2020 funded project TechCare. The value of using this technology to assess animal behaviour and potentially detect any changes, in an extensive system, was investigated. UHF tags were attached to 49 mixed-age Lleyen ewes in late January 2023. The ewes were grazing in an unimproved field, of approximately 15.6ha, with access to additional hay and high energy feed buckets. A UHF reader was set-up in the field attached to two antennas placed above the feed buckets. Visits by each ewe to the feed buckets were monitored during the day (with the power pack removed and charged overnight before being deployed again the next day). The animals were monitored daily (Monday to Friday) for one month. Live weights and body condition scores were collected at the start of the trial and then again fortnightly until the end of the trial. Weather data (rainfall and temperature) was also available from a nearby weather station. Preliminary results, using the first week of data collected, indicate that weather conditions potentially affect the number of visits made to the feed buckets with less visits recorded on days when the rainfall was higher. In addition, the age of the ewe was also significant ($p < 0.05$) in relation to the number of visits recorded during the week of data collection. Younger ewes recorded fewer visits when compared with older ewes. The relationship between the number of visits recorded with live weight and body condition score changes was also investigated, but no significant effect was observed. Although the UHF technology has allowed us to do some preliminary investigations into ewe behaviour and potential links to other welfare indicators, the issues experienced with the equipment, particularly those associated with battery life and water damage, would need to be considered for future trials using the equipment in extensive systems.

Session 9

Theatre 5

Effects of a virtual fence on heart rate variability and use of pasture

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Virtual fences (VF) may offer a potential labour-saving solution for pasture management. Animals wear a GPS controlled collar that emits an audio signal when they approach the virtual boundary. If they do not stop, an electric pulse is delivered. The animal needs to associate the audio signal with the electric pulse to avoid it. Studies on effects of VF on animal welfare remain limited. We examined the effects of VF on the autonomic nervous system using heart rate variability (HRV) and analysed pasture use. In a cross-over design, 20 Blobe goats in two groups experienced being pastured (4.5 h per day, 2-6pm) in a paddock fenced completely with an electric fence (control) or with VF in two 12-day periods. Behaviour on pasture was video recorded and analysed for use of four zones: Warning Zone (0 – 3 m from the VF), Observation Zone (3 – 9 m), Transition Zone (9 – 10.5 m), and Front Zone (>10.5). HRV was measured in the barn (11 am – 13 pm) in undisturbed, lying goats. LMMs were calculated with treatment, period, day, and all interactions as fixed, and animal nested in group as random effect. Goats in the VF treatment spent less time in the Observation and Warning Zones but more time in the Front and Transition Zones (all $p < 0.001$), with no differences during the initial training days. Increased use of the Transition Zone in later days suggests that goats may have learned to associate it as a 'safe area'. Resting heart rate was higher ($p = 0.013$), and the HRV parameter RMSSD/SDNN ratio was lower ($p = 0.009$) after seven days of VF exposure compared to control, indicating increased sympathetic activation and heightened stress. The results suggest that goats exposed to VF experience behavioural adaptations in pasture use that may indicate location learning, and physiological stress responses. Further studies are needed to assess long-term welfare impacts, including in different goat populations and over extended recording durations.

Monitoring environmental welfare conditions in commercial dairy sheep and goat farms by using wireless sensors
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As a part of the TechCare project (www.techcare-project.eu), animal-based indicators and PLF technologies were prioritized by stakeholders of the sheep and goat value chain. Based on obtained results, cheap weather stations (Froggit HP1000SEPro) with sensors for pen temperature-humidity (°C and %RH, DP-50; n = 8), air quality (AQI, DP-200 PM<2.5 µm; n = 2) and bedding moisture (BQI, % capacitance, DP-100; n = 8) were implemented in 12 intensive dairy sheep and goat commercial farms in Spain. Wireless sensors (Bluetooth, 868 MHz) were installed at key places of each farm and data were automatically transmitted to the cloud every 5 min via Wi-Fi and accessed by a free App (Ecowitt Weather). To facilitate data interpretation according to critical environmental welfare thresholds, the thermohydrometric index ($THI = 0.8 \cdot T + RH/100 \cdot [T - 14.41] + 46.4$; Mader et al., 2005) and a user-friendly application based on coded colors and alarms were used (THIcare® App; Caja et al., 2025). Collected daily environmental data (24-36 mo according to farm) were correlated to individual milk yield (7 farms with electronic meters) or to total milk from the tank (5 farms). Effects on rates of pregnancy, abortion and post-partum mortality by periods in all farms were also studied. The system demonstrated robustness, with an 83% sensors' survival rate in 3 yr and failures 4-17%. Indoors THI converged to outdoors THI as values increased, meeting at $THI = 85$. Thermoneutral comfort was shown for $50 < THI < 65$. THI quadratically correlated with milk production, explaining more of 30% change, after removing the effect of lactation stage. $THI > 60$ had negative effects on conception rate according to the photoperiod, the THI explaining 40% of variation during the increasing photoperiod (spring-summer). Heat stress ($THI > 65$) negatively impacted post-partum mortality, explaining 55% of its variation. In conclusion, the implemented sensors were robust and useful for identifying optimal conditions for sheep and goats based on their physiological state, allowing the implementation of protective measures, particularly in extreme summer and winter when housing is less efficient.

Session 9

Theatre 7

Preliminary study on THI, animal welfare and zootechnical performance in dairy ewes: first lessons from a dataset of a network of 11 farms in France.

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Future climate projections indicate rising temperatures and increased climatic variability. In the dairy sheep sector, limited research exists on how these climatic changes affect animal welfare and zootechnical performance in dairy sheep. As part of the H2020 TECHCARE project, a network of 11 dairy sheep (Lacaune breed) farms located in the French Occitanie region was equipped with external weather stations and indoor sensors in sheds. Key environmental parameters, including outdoor and indoor (shed) temperature and humidity, have been recorded hourly since early 2024. A welfare assessment protocol was conducted five times during lactation on a sample of ewes from each farm. The primary objective is to analyse the variation of the Temperature Humidity Index (THI) throughout the year, considering geographic and housing conditions. Additionally, we investigate the relationship between THI, welfare indicators, and zootechnical performance as a preliminary step toward establishing threshold values for future studies. This study aims to monitor THI variations across different farm locations and housing conditions while assessing their impact on dairy ewe welfare and performance. The findings will serve as an initial framework for defining critical THI thresholds, which could support future climate adaptation strategies in the dairy sheep sector.

Do feed conversion efficiency and its inverse, feed conversion ratio, differ in their distributions for dairy cows?

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Efficiency can be calculated from dry matter intake (DMI) and energy corrected milk (ECM) as feed conversion efficiency (FCE, ECM/DMI), or its inverse: feed conversion ratio (FCR, DMI/ECM). Inversion breaks the distributional assumption for linear normal models (LNM). This study investigates deviation of FCE and FCR from normal distribution. Cattle Feed InTake (CFIT) technology was used to record DMI, and ECM was recorded monthly, in 12 dairy farms with Holstein or Jersey cows. Individual cow solution for each parity (1 to 3) and week in lactation (1 to 45) were obtained using a linear mixed model to account for permanent environment effects and known animal effects. Cow solutions were used untransformed and log transformed. Within breed and parity, a single week solution was randomly selected in a random sample of 500 cows, and the observed and normal distribution densities were estimated and divided by their standard deviation, to make variables comparable. The sum of squared residuals (SSR) was calculated with a linear regression of observed against normal distribution densities. For Holstein cows the SSR for FCE were 0.0035 (0.0038), 0.0042 (0.0049), and 0.0050 (0.0075) for first, second and third parity, respectively. The SSR for FCR were 0.0061 (0.0038), 0.0097 (0.0049), and 0.0130 (0.0075). For Jersey cows the SSR for FCE were 0.0132 (0.0115), 0.0082 (0.0106), and 0.0023 (0.0039). The SSR for FCR were 0.0143 (0.0115), 0.0181 (0.0106), and 0.0093 (0.0039). For both breeds, a small change was observed in the SSR between untransformed and transformed FCE distributions, however log transformation resulted in lower SSR for FCR distributions compared to the normal distribution. Graphical inspection showed an observed distribution narrower and left skewed compared to the normal distribution for FCR. We conclude that FCE is more likely to fit a LNM than FCR.

Session 10

Theatre 2

Impact of indoor forage feeding strategies on utilization in dairy goats: a collaborative effort between research and practitioners

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In France, most goat farmers house their animals indoors for at least part of the year, feeding them often self-produced forages, variable in quality. Goats are sensitive to forage feeding methods, prompting farmers to seek guidance suited to diverse systems. To address this, a project combining research and farmers' collaboration was undertaken. It included 2 online surveys and 8 focus group sessions with farmers and advisors to assess and update current recommendations. To support these updates, 11 trials, conducted in 4 experimental stations following a crossover design, evaluated the effects of various forage feeding strategies—frequency, order, and proportion of refusals—on goat behaviour and performance. Additionally, earlier studies were analysed to explore the impact of refusal proportion. Surveys revealed that farmers generally believe that increasing feeding frequency or proportion of refusals increases intake and production, while no consensus emerged on the optimal order when two forages are fed daily. The trials confirmed that greater refusal proportion enhances intake and diet quality, depending on the forage type. Feeding frequency had no impact on intake and production. Goats showed strong forage preference when two forages are fed daily, being able to wait for the preferred forage during hours every day. These findings encourage farmers to revisit their practices, showing that simplified management can align with technical performance.

Effect of tannins and drying methods on in vitro gas and short-chain fatty acid production of rapeseed meal
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This study evaluated the effects of different tannin types, concentrations, and drying methods on in vitro gas and short-chain fatty acid (SCFA) production in rapeseed meal (RSM). Quebracho (QUE) and chestnut (CWE) tannins were added at 50 and 100 g/kg, mixed with distilled water (1:2.5 w:V), and subjected to three drying methods: freeze-drying (LYO), air drying at 50°C (LOW), and 80°C (HIGH). RSM without tannins and drying was used as a negative control (CON-). Tannin addition combined with drying significantly ($p < 0.05$) affected in vitro gas and SCFA production. All tannin-treated samples, regardless of drying method, showed reduced in vitro gas production at 24 h (117–156 ml/g DM) compared to CON-. Acetate production decreased across LYO, LOW, and HIGH treatments (1.84–3.17 mmol/g DM), and propionate and butyrate production also declined, with the lowest values observed in CWE 100 LYO (0.57 mmol/g DM) and QUE 100 HIGH (0.24 mmol/g DM), respectively. Total SCFA production was lowest in QUE 100 LOW (2.86 mmol/g DM), while the acetate-to-propionate ratio decreased across all drying treatments (2.40–4.52). The findings suggest that combining CWE or QUE tannins with drying methods further reduces in vitro gas and SCFA production in RSM. However, it remains unclear whether this effect is due to reduced dry matter and crude protein degradation or a negative impact on rumen microorganisms.

Session 10

Theatre 4

Evaluation of protein kinetics of different protein sources using an in vitro assay

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Identifying how protein is digested may help to optimize the formulation of innovative diets to maximize performance and limit the amount of undigested protein reaching the hindgut. This study aims to compare the protein digestibility kinetics of different protein sources: soybean meal (SBM), extruded SBM (Promax 48) and potato protein (PP), using an invitro simulation to mimick the gastrointestinal digestion of weaned piglets. Six replicate samples were incubated at 39°C for 7.5h: the 1st incubation step was performed at pH 3.5 for 1.5h after the addition of pepsin to simulate the gastric phase and the 2nd step was performed at pH 6.8 for 6h after addition of pancreatin and bile extract to simulate the small intestinal phase. To classify whether a protein is fast, slow or resistant, dry matter and crude protein content from the supernatants were analyzed at time 0, 0.5, 2, 4 and 6h of incubation, considering fast protein being digested between 0 to 0.5h, slow protein being digested between 0.5 and 4h and protein not digested at 4h is considered resistant protein. In SBM samples, the average percentage of protein digested increased from 63.5% at 0h to 71.8% at 6h post incubation. In extruded SBM, the average percentage of digested protein increased from 67.5% at 0h to 79.4% at 6h. The percentage or protein digestibility of PP raised from 25.2% to 59.5% during the 6h of incubation. Differences were found in protein digestibility response between ingredients. Higher fast protein was observed in extruded SBM followed by SBM and potato protein ($78.8 \pm 0.03\%$ vs $69.9 \pm 0.02\%$ vs $37.5 \pm 0.02\%$, respectively) while higher slow protein was observed in PP compared to SBM and extruded SBM ($12.7 \pm 0.03\%$ vs $2.3 \pm 0.05\%$ vs $1.2 \pm 0.02\%$, respectively). Thus, PP shows the most resistant protein ($49.9 \pm 0.04\%$), compared to SBM ($27.7 \pm 0.03\%$) and extruded SBM ($20.0 \pm 0.03\%$). These results highlight the different response of feed ingredients to protein digestibility which may be taken into consideration when formulating diets. By generating a broad database of protein quality in common feed ingredients, diet formulation will better match requirements of young animals.

Water consumption of dairy and meat ewes during end of gestation and suckling period

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For dairy cows and goats, the French INRAE Feeding System for Ruminants has predictive equations for water consumption. In those equations, the dry matter (DM) intake is the prior factor explaining the total water ingestion. In the goat equation, the other variables considered are milk production and DM and mineral content of the diet. For cows, an additional quantity of drunk water is calculated when the temperature exceeds 15°C. For dairy or meat sheep, there is no equation yet. The present work was performed within a project studying the intake capacity of ewes during end of gestation and suckling period. Within 8 research or school farms, we measured forage intake according to the weight, the physiological stage, the litter size and the hay quality for different breeds. We also measured concentrates and water consumption. The present article synthesizes the water consumption of ewes during the first trials realized in 2023 and 2024 on 5 different farms and on one dairy breed and 4 meat breeds. Each site was equipped with water meters and temperature sensors. Water consumption was recorded daily per group including 6 to 47 animals according to the site. Data were averaged on weekly basis in order to smooth out daily variations. According to the site and the breed, average water consumption varied between 4.6 and 6.9 L per animal per day during end of gestation, and to 7.8 to 10.2 L per animal per day during the suckling period. Based on kilogram of DM consumed, water consumption varied from 2.3 to 3.7 L for end of gestation and from 2.6 to 3.6 L for suckling period. Data observed are coherent regarding usual references (ANSES 2010, INRA 2022). Behind those means, we observed a variability, especially during period with temperature variations and high indoor temperatures. The effect of temperature has not yet been precisely estimated, but subsequent tests should enable this effect to be approached. CIBRésil project Supported by Casdar funds.

Session 10

Theatre 6

Fibre and Feed Processing: Optimising digestive efficiency, nutrient utilisation and gut health in non-ruminants

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Industrial compound feeds have transformed livestock performance by offering high quality mixtures. At the same time, the inclusion of fibre-rich co-products from the food and biofuel industries has gained traction, supported by innovative feed processing techniques to enhance their nutritional value. This study examines the influence of dietary fibre or feed processing on digestive efficiency, nutrient utilisation and gut health in non-ruminants, with a focus on the selection of fibre types to optimise outcomes. Dietary fibre plays a multifaceted role in non-ruminant nutrition, influencing digestion, metabolism and gut microbiota composition. While fibre can dilute energy density, it promotes satiety, short chain fatty acid production and serves as a substrate for probiotic microbes, enhancing their selection and survival. To maximise biomass resources, fibre-rich ingredients are increasingly being incorporated into diets, but their physical and chemical complexity requires accurate characterisation. Traditional analytical approaches, such as crude fibre and detergent fibre methods, inadequately measure the soluble fibre fraction (e.g. pectin), which is critical for microbiota interactions. Total Dietary Fibre analysis provides a comprehensive approach, distinguishing between soluble and insoluble fractions and facilitating the selection of fibre types tailored to support beneficial microbial populations. The compound feed industry faces the dual challenge of balancing cost effectiveness with high nutritional standards. Advances in feed processing, such as particle size reduction, can improve the digestibility of fibre-rich co-products while promoting a favourable gut environment. This study highlights the importance of defining fibre sources, chemical composition and inclusion levels to improve nutrient absorption, energy efficiency and gut health. The use of fibre-rich co-products in non-ruminant diets requires precise fibre characterisation, tailored feed processing strategies and strategic selection of fibre types to support beneficial microbial communities. Future research should refine analytical methods and investigate fibre-microbe interactions to maximise the benefits of fibre-rich diets.

Cutting time (AM vs. PM) of grassland forage: Holstein Cows' responses to two hay variants.

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Feeding dairy cows hay-based diets has been re-emerging in the alpine region. However, the lower energy density of hay, when compared to concentrates, can limit cows from attaining their full potential. Advanced harvest and conservation techniques could counteract this by improving nutritional quality of hay. Non-structural carbohydrates, such as sugars, accumulate through photosynthesis and serve as easily available energy for cows, however, they are partly consumed by plants through respiration overnight. The aim of this study was to investigate whether cutting time (AM vs. PM) of grassland forage impacts nutritive value of hay, and consequently intake and performance of dairy cows. 18 lactating Holstein cows were divided into two feeding groups and fed either AM hay or PM hay ad libitum, as well as 3.6 kg (DM) concentrate per cow and day. Data collection (18d) commenced after an adaptation period (10d) to the altered feeding conditions. Chemical analysis revealed that hay made from PM-cut grass (n = 10) had a higher water-soluble carbohydrate content (+21 g/kg DM) compared to hay made from AM-cut grass. When fed to cows, however, cutting time had no effect on dry matter intake (on average 23.3 kg DM/d) and daily milk yield (on average 28.3 kg energy corrected milk). Technical limitations of the hay drying facility may have led to microbial respiration during the drying process, resulting in oxidation of the sugar dissolved in the cells. Results indicate that optimised drying conditions for grassland forage mown in the evening could further contribute to increasing hay quality. In practice, this could lead to more efficient utilisation of grassland as a feed resource and thus help to reduce the use of concentrates per kg of milk.

Session 10

Poster 8

Feed sorting by particle size in dairy cows fed different silages and concentrates in total mixed rations

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Feed sorting should be minimised to assure that the nutrient supply predicted matches what the animal actually ingests. The aim of this study was to quantify to what extent cows sort feed by particle size when different silages and concentrates are fed. Twenty-four lactating Danish Holstein dairy cows were used in a cross-over design with two periods of 21 d each. Each cow was fed two out of four diets. The four diets were two by two factorially arranged with two forages, either grass-clover or maize silage (G or M; 55.0 and 54.2% of diet DM, both chopped at 11 mm, respectively), and two concentrates, either rolled barley or rolled dried beet pulp pellets (B or D; 21.5% of diet DM). The diets were mixed as total mixed rations (TMR), and had an average DM content over the trial of 50.6% for GB, 51.0% for GD, 45.4% for MB and 45.6% for MD. In each period a sample from each TMR and the matching residue (aiming for 10% DM feed residue) from each cow after 24 h from a single feeding were run in a Penn State Particle Separator. The proportion of fractions (>19, >8, >1.18, and <1.18 mm) was calculated based on fresh weight. The proportions recovered for the TMR were for each sieve with a generalized linear mixed model assuming gamma distributed residuals, including the individual and all possible interactions as fixed effect of silage (G vs M), concentrate (B vs D), and random effect of testing day (1 to 4). The model means for the proportion by particle size from largest to smallest were 35.3, 20.2, 36.2, 8.26% for GB, 36.8, 26.7, 30.0, 6.44% for GD, 0.90, 44.2, 45.3, 9.66% for MB, and 0.91, 51.7, 37.9, 9.51% for MD. There was a significant effect of silage for particle >19 mm, effect of both silage and concentrate for particles >8 and >1.18 mm, and a significant interaction between silage and concentrate for particles <1.18 mm. The differences between the TMR and residue proportions for each sieve were analysed in a model similar as the previous, with addition of the random effect of the cow, and assuming normally distributed residuals. The fixed coefficients from the model were tested against zero. No coefficient was found different from zero, and therefore there is no indication of cows sorting by particle size.

Effects of Whole Maize Grain and Expander Use on Broiler Performance

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The first weeks of a broiler's life are critical for optimizing growth and nutrient absorption. Reducing the use of energy-intensive machinery, such as the mill, and instead utilizing the secondary grinding effect of the equipment from the mixer to the pelletizer can help support the production of affordable compound feed. This study investigated the effects of whole maize grain inclusion after the mill in the production line on broiler diets and the use of expander (Exp) prior to pelleting. We hypothesized that whole maize grain would not adversely impact broiler performance and that the use of Exp would improve nutrient digestibility. A trial with 432 male Ross 308 chicks was conducted for 42 d using a 3×2 factorial, testing three levels of whole grain inclusion (0, 50, and 100%) and two feed processing methods (with or without Exp), using Tukey to separate means ($P < 0.05$). Diets were formulated according to breeder specifications, and weekly performance parameters – including body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) – were evaluated through 35d. During the 1-14 d, the BWG had an interaction between whole grain and Exp. BWG was reduced with 100% whole grain inclusion (491g), while Exp was improved (523g). Whole grain inclusion affected FI with lowest level on 100% inclusion (511g). The FCR showed interaction and reflected the improved performance when Exp prior to pelleting in combination with 100% whole grain was used. No differences were observed between 14-28d. At 1-35d there was an interaction for BWG and FI, without difference on exp group, but decreased for 100% whole grain inclusion without Exp, with no influence on FCR. Whole grain inclusion increased the abdominal fat at 35d. In conclusion, the use of expander enhanced broiler performance by improving nutrient availability, especially during early growth. While whole grain inclusion did not affect overall performance, high inclusion levels may reduce FI and increase fat deposition, requiring further evaluation. These results support the conclusion that exp results in better overall broiler performance.

Session 11

Theatre 1

Sustainable Livestock Farming: Assessing Grassland Adaptation Strategies to Climate Change

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Grasslands play a crucial role in livestock farming, providing forage for ruminants while delivering essential ecosystem services. However, climate change presents significant challenges to grassland productivity, with rising temperatures, altered precipitation patterns, and extreme weather events affecting forage availability and quality. This study examines the literature on grazing livestock farming systems, focusing on grassland adaptation to climate stress relevant for Irish agriculture. One key strategy is the use of multispecies swards, which enhance resilience and productivity by combining grasses, legumes, and herbs. Experimental studies show that diverse species mixtures outperform monocultures under drought conditions, maintaining higher yields and demonstrating faster post-drought recovery. The drought-tolerant grass cocksfoot outperforms other grasses due to its deep-rooted water extraction at low soil water potentials. Deep-rooted species such as chicory and red clover improve water-use efficiency and nitrogen cycling, reducing reliance on fertilizers. Plantago, more drought-tolerant than legumes, serves as an intermediate species in terms of resilience and productivity. However, long-term research is needed to assess species persistence under typical Irish grazing systems. Breeding strategies also play a pivotal role in climate adaptation. Advances in plant genetics, including *Festulolium* hybrids and clover breeding, offer promising solutions for improving drought tolerance, persistence, and forage quality. However, long-term sustainability requires further research under real-world grazing conditions. Adjusting stocking rates based on local climatic and soil conditions, along with optimized grazing management, further enhances sustainability by preventing overgrazing and ensuring pasture resilience and farm forage self-sufficiency. This study highlights the importance of integrating diverse swards, nitrogen management, enhanced disease resistance, optimized stocking rates, and targeted breeding programs to improve the sustainability of livestock farming. Future research should focus on long-term trials to assess the persistence and performance of these adaptive strategies.

Do multispecies swards increase resilience of Dutch dairy farms?

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Multispecies swards (MS) are being promoted for their potential to improve productivity, biodiversity, ecosystem services, and resilience in Dutch dairy farms compared to perennial ryegrass (PRG) monocultures. The environmental and economic effects of MS and their impact on farm resilience have, however, not been quantified. Therefore, our objective was to assess the effects of adopting MS on resilience to drought of a representative Dutch dairy farm on sandy soil. A linear programming model with a nutrient balance and life cycle assessment module maximized farm income by optimizing farm configuration for three types of grassland (only-PRG, a combination of PRG and MS, and only-MS) and for two years with different precipitation levels (average and dry). Parameters for the grassland types in an average and a dry year were obtained from the literature and experts. In an average year, PRG and MS (ratio 1: 4.1) and only-MS increased income (+€20.400 and +€18.100 per year) and reduced N surplus (-11 and -10 kg N per ha per year) and greenhouse gas emissions (-53 and -90 g CO₂ eq. per kg milk), but increased hired labor (+160 and +123 hours per year) and exported manure (+328 and +328 kg P₂O₅ per year) compared to only-PRG. Comparing a dry year to an average year, income decreased considerably less with PRG and MS (ratio 1:4.1) and only-MS (-€36.300 and -€29.000 per year) than with only-PRG (-€ 73.100 per year). Increases in N surplus were less with PRG and MS and only-MS (+49 and +24 kg N per ha per year) than with PRG only (+83 kg N per ha per year). The P₂O₅ surpluses followed similar trends. The better performance with PRG and MS and only-MS in a dry year was explained by a higher on-farm protein production due to legumes in the MS, less manure export, and lower amounts of purchased mineral N fertilizer, maize and grass silage. In conclusion, adoption of MS offered advantages under average years and increased farm economic resilience to drought considerably.

Session 11

Theatre 3

Multifunctionality of mountains livestock systems: a multi-approach framework for the requalification of an abandoned pasture in the Italian Eastern Alps

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Extensive livestock systems maintain mountain pastures that provide provisioning and non-provisioning ecosystem services. We present here the preliminary results of a multi-approach pilot project (2022-2024) to requalify an abandoned alpine pasture in Monte Coppolo (Italian Eastern Alps) through grazing by Lamon sheep, a critically endangered local breed according to FAO's DAD-IS. In 2022, pre-grazing pasture soil microbial communities (sequencing and real-time PCR to quantify N cycle genes) and vegetation (botanical composition and primary productivity as indexed by NDVI) were characterized. Sequential grazing in four one-hectare sections was then introduced in 2023 (30 heads) and 2024 (85 heads), and soil microbial communities and vegetation were compared in grazed and non-grazed areas using exclusion cages. Sheep flocks were monitored with GPS collars with activometers to estimate grazing spatial intensity and sheep activity. In 2024, a photo-trapping system was implemented to monitor the grazed plots. Gene abundances and taxonomical and functional profiles of soil microbial communities varied with vegetation type but not with grazing intensity or across the summer. GPS tracking and NDVI indicated that grazing intensity positively influenced vegetation regrowth. Photo-trapping and GPS tracking confirmed a circadian activity rhythm of the sheep with peaks in the early morning and late evening. Photo-trapping also verified the presence of wolves and the effectiveness of electrified nets to prevent predations. These findings highlight the effectiveness of a multi-approach framework to characterize the interconnected alpine pasture components, providing practical information for the sustainable management of these areas with local breeds. They also evidence the fundamental role of livestock grazing for the conservation of alpine grassland biodiversity. Actions supporting the economic sustainability of low input grassland-based livestock farms, in particular smallholders, are needed.

Fattening Holstein×beef breed on continuous pasture in mountainous regions – implications for productivity and meat quality

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Ecological issues as well as consumer demand for sustainably produced agricultural food rise the trends to fatten beef cattle on pasture during the grazing season. Furthermore, in central Europe, dairy breeds (Holstein) from dairy farms are hardly in demand for beef fattening. One strategy to reduce the socially undesirable export of dairy calves is to sire these dairy cows with beef breeds. Therefore, the present study was conducted at AREC Raumberg-Gumpenstein (Austria) to compare Holstein×Angus (HF×AA) steers and heifers with Simmental (FV) steers on pasture. At the beginning of the fattening trial animals were on average 6.2 months old and had 180 kg live weight. During grazing period (beginning of May until October) animals were kept on continuous pasture (6-8 cm sward height). Afterwards they were finished in barn on a grass silage-hay diet and low concentrate amounts for on average 2.5 months. Animals were slaughtered at a target live weight of 400 kg, which is typical for Austrian suckler beef production. Statistical analyses were performed using SAS software and the procedures MIXED and GLM. The average age at slaughter was 14.8 months for the HF×AA animals and 15.8 months for the FV steers ($P=0.02$). Average daily weight gain (ADG) during pasture period was 750 g for FV steers and HF×AA heifers, but 870 g for HF×AA steers ($P=0.21$). Compensatory growth potential during barn fattening was evident (ADG 1.100 g, $P=0.84$). FV steers tended to better slaughter performance (carcass conformation, muscle size, valuable cuts). Between HF×AA steers and heifers minor differences in slaughter performance were observed. The greater fat storage of the heifers was clearly visible. Regarding meat quality, the HF×AA animals performed better than the FV steers. This was particularly evident in the shear force (meat tenderness). The meat of the heifers showed the lowest grill loss and the highest intramuscular fat (IMF) content. FV steers had lowest IMF contents (1.2%, $P=0.001$), but enhanced proportions of omega-3 fatty acids and PUFA. The fattening of HF×AA crosses on pasture would be a new Austrian beef product with excellent production and product quality.

Session 11

Theatre 5

Effects of high-altitude grazing on dairy cow behavior using precision technologies

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Dairy farming practices at high-altitude settings significantly impact cows' behaviour and productivity. Proper management of animals promotes the maintenance of forage quantity and quality. Precision Livestock Farming (PLF) technologies provide valuable tools for understanding how different factors affect cows' behaviours, aiming to optimize herd and pasture management. The study was conducted during July and August 2024 on 10 Brown dairy cows on an alpine pasture in Northern Italy (>1700 m asl). Milk production was assessed every two weeks. Each cow wore a noseband halter, equipped with a pressure sensor, a 3-axis accelerometer and a data logger for monitoring feeding behaviour. Pasture botanical composition was assessed, grass samples were collected and analysed using near-infrared spectroscopy and forage values (FV) were calculated. Statistical analyses were conducted using SAS with the GLM procedure. The rumination and grazing behaviour of the animals, 5 primiparous and 5 multiparous cows with an average milk production of 16.4 kg/day (SD 4.26), were monitored. Primiparous cows ate and ruminated for a longer time throughout the season compared to multiparous ones ($P<0.001$ and $P<0.05$, respectively). Rumination time was higher in pastures with low FV compared to those with high FV (23% vs 19% of the day, $P<0.006$). A similar trend was observed for eating behaviour (21% vs 18% of the day, $P<0.03$). Dry matter of pastures significantly influenced both behaviours ($P<0.001$). Milk production was significantly associated with rumination time ($P<0.001$): high-producing cows ruminated more than low-producing ones (23 vs 18% of the day). The use of PLF technologies may help the management of grazing cows in mountain areas, aligning grazing practices with pasture-specific conditions to improve productivity and sustainability of alpine dairy farming. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – Missione 4 Componenti 2, Investimento 1.4 – D.D. 1032 17/06/2022, CN000000022).

Pasture-use intensity and Vegetation: Insights from GPS Tracking and Remote Sensing from an alpine pasture

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Understanding the relationship between grazing patterns and vegetation productivity is essential for the sustainable management of alpine pastures, their biodiversity and ecosystem services. This study integrates GPS tracking and remote sensing to assess the impact of dairy cow grazing on vegetation condition in alpine pastures. The study area is a summer farm pasture (171 ha) located in the eastern Italian Alps at an average elevation of 1900 m a.s.l. (SD: 90). The herd (150 Livestock units) was conducted to different pasture's sub-areas each morning after the milking and returned to the barn for the evening milking. After that, it roamed freely during the night. We monitored 12 cows with GPS collars (one position every two minutes) from July 3 to September 5, 2023. We used NDVI (Normalized Difference Vegetation Index) from Copernicus SENTINEL-2 to index vegetation conditions at the beginning (June 22), during (July 17 and August 21) and after (September 25) the grazing seasons. We divided the pasture into a 25 m grid and associated with each cell the average NDVI (to index vegetation biomass) on each date and, on September 25, the NDVI difference from June 22 ("NDVI difference", to index residual vegetation), the normalized positions count to index grazing intensity in the periods between each date, and slope, aspect, and prevalent vegetation type to consider permanent environmental features. Based on NDVI difference, we found a significant relationship between pasture use intensity and vegetation productivity, varying by vegetation type, with a general regrowth pattern in intensively used areas at the end of the season. A resource selection function approach revealed that the spatial selection patterns of farmer-animals varied between dates, with the preference for higher productivity areas at the beginning and end of the season and for lower ones in the middle, with a general avoidance of steep areas. These results indicate the potential for using vegetation indices to estimate the impact of grazing intensity on pasture conditions, highlighting the usefulness of remote sensing and GPS tracking for monitoring this agroecosystem and its productivity.

Session 11

Theatre 7

Facilitating collective action for ecosystem services in rural landscapes: the case of Alblasserwaard

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Agricultural landscapes, particularly livestock agroecosystems, have the potential to deliver a wide range of ecosystem services. However, many ecosystem services are undersupplied resulting in biodiversity loss. To solve the undersupply of ecosystem services, the most common initiatives include government policy and private initiative. Alternative governance arrangement, such as collective action, are understudied. The main objective of this article is to explore the potential of collective action to facilitate delivery of and payment for ecosystem services (PES) at the landscape level to enhance biodiversity. Using a co-creation approach, we conducted workshops and interviews to map the ecosystem services, and their beneficiaries and producers. We also ranked the delivery and demand of ecosystem services and identified conditions for collaboration based on Ostrom's design principles. We find a mismatch between the delivery and demand of ecosystem services, particularly food, clean air, biodiversity, and health. Beneficiaries perceive that too much food is produced for the world market. Clean air, biodiversity and health are underprovided relative to demand. Producers and beneficiaries recognize the need for collective action and were able to agree on conditions for collaboration. There was a shared interest in exploring the potential of a follow-up initiative in the form of a landscape fund.

Shading the future: The potential of solar grazing to mitigate heat stress in sheep

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Free-field photovoltaic systems (FFPV) are becoming increasingly important but require large areas of land. Therefore, energy and agricultural production are often combined on the same area in the form of solar grazing, e.g. grazing with small ruminants. Although some studies have found that sheep spend more time under the modules, it remains an open question to what extent this spatial behaviour is influenced by different microclimatic conditions. In order to provide an answer to this question, we analysed the daily air temperature and the behaviour of sheep in an FFPV during two periods in the year 2023. We set up a trial site (2,000 m²) in an FFPV on grassland in 2023, consisting of two equally sized sectors (with/without modules). The average daily temperature in the sectors with and without modules was obtained from TMS-4 sensors (TOMST, Prague, Czech Republic). Therefore, eight sensors were installed in each of two transects adjacent to the trial site. One transect was in an adjacent open area without modules and the other was under modules. During two grazing periods (8 days each), 10 sheep were fitted with GPS collars to provide minute-wise data on their location. The average daily temperature in the sectors with and without modules was then correlated with the location of the sheep. The average daily air temperature under the modules was always lower than in the sector without modules. The correlation shows that sheep spent more time in the sector with modules when the temperature increased in both periods. The presence of the modules offers the sheep protection from the sun and can reduce heat stress, thus, contributes to the welfare of the sheep. In the context of climate change and rising temperatures, solar grazing can be a sustainable and animal-friendly method of outdoor livestock production.

Session 11

Theatre 9

Harnessing sun and pasture: Exploring the potential of Agrivoltaics for dairy farming

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Agrivoltaic systems are becoming increasingly popular as they mitigate land-use conflicts by producing renewable energy and agricultural products on the same site. However, knowledge on the effects of elevated photovoltaic (PV) modules on grassland productivity and diversity as well as grazing livestock is limited. In this study, we investigated the effects of elevated PV modules (lower edge approx. 210 cm above ground level) on the grassland vegetation and cattle behaviour (n=8) in two treatments by comparing two areas with elevated PV modules (each 10 x 5 m) with two control areas (same size) on peat soil grassland (total pasture size 1.63 ha) in northwest Germany during 15 days in May 2024. Measurements included vegetation composition, chlorophyll content, compressed sward height (CSH), and spatial behaviour of cattle (monitored with GPS collars). Higher chlorophyll levels and lower CSH were observed under PV modules. There were more plant species in the control areas, but some trampling-resistant species were only found under the PV modules as cattle spent more time under PV modules (1.1% of daily time) than in control areas (0.6%). These low percentages of the total time per day per animal are due to the fact that the animals were allowed to move freely on the pasture. Despite these restrictions, it can be said that the animals did not avoid the two areas with PV modules compared to the two control areas, but rather preferred them. Furthermore, there were no injuries to the animals or damage to the PV modules. Therefore, elevated PV modules appeared to be suitable for construction on pasture, with potential benefits for animal welfare. Significant differences in vegetation-related data were observed between treatments. This study provides initial insights into the effects of elevated PV modules on the underlying vegetation and grazing livestock, paving the way for a multifunctional land use approach.

Nutritional and non-nutritional factors associated with bulk tank milk fat concentration in commercial spring-calving grazing dairy herds

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The objectives of this experiment were to 1) identify associations between nutritional and non-nutritional factors and bulk tank milk fat concentration and 2) develop a multivariable model capable of predicting herd-level milk fat concentration for commercial spring-calving grazing dairy herds. An observational experiment comprising of 25 commercial spring-calving dairy herds was conducted over a 2-year period. Farms were visited 10 times per year which coincided with each grazing rotation. During each visit, grassland measurements and pasture samples were collected from the next two paddocks scheduled for grazing, along with samples of other dietary ingredients and bulk tank milk. Using the data from 12 of the 25 herds, univariate analysis was performed to identify the relationship between each explanatory variable and milk fat concentration. Variables which had a univariate analysis of $P < 0.2$ were offered to a multivariable linear regression model and backward stepwise elimination was performed until the remaining variables had $P < 0.05$ and the most parsimonious model was achieved. The predictive performance of the final multivariable linear regression model was evaluated using the data from the remaining 13 herds. While several univariate relationships were identified, the backward stepwise elimination process revealed that grazing rotation and the herd's milk fat concentration predicted transmitting ability were the key factors associated with bulk tank milk fat concentration. When the model was applied to the evaluation dataset of 13 additional herds, the model predicted bulk tank milk fat concentration with an R^2 of 0.73 and a RMSE of 0.23%. The slope between observed and predicted milk fat was 1.17 (SE = 0.06) with an average bias of -0.04% and an RPE of 1.01. While the association between grazing rotation and bulk tank milk fat concentration is likely multifactorial involving many nutritional and non-nutritional factors, the positive relationship between milk fat concentration predicted transmitting ability and bulk tank milk fat concentration highlights the important role of genetics on milk fat production in pasture-based systems.

Session 11

Theatre 11

Animal response of dairy cows grazing supplementary crops during the summer

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Currently, the marked seasonality of the pasture during the summer in southern Chile affects the production of the dairy herd. The aim of this study was to evaluate the productive response of dairy cows grazing chicory (*Chicorium intybus* L.) or forage rape (*Brassica napus* spp. *biennis*) during the summer. Forty-eight Holstein Friesian cows in mild lactation were assigned to continuous treatment for 60 days. Cows were paired by milk yield, days in lactation, parity and live weight, and then randomly allocated to one of two different dietary treatments. Cows from both treatments were offered 18 kg dry matter (DM). Treatment one (Ch) corresponded to 45% chicory, and treatment 2 (FR) corresponded to 45% forage raps. Both treatments received the same remaining 55% of feed, which included commercial concentrate (5 kg DM), grass silage (4 kg DM) and soybean meal (1 kg DM), with the first being offered in individual feeders and the others in a feed yard. Milk production was recorded at each milking (07:00 and 14:00 h). Milk samples were collected 4 times during the experiment, at morning and afternoon milking to estimate the protein, fat and urea in milk. The effect of treatments on milk production and composition and body weight were analyzed using a linear-mixed model. Daily milk production for both treatments was 22.7 ± 1.03 liters, without differences among them ($P > 0.05$). Likewise, the concentrations of fat, crude protein and total solids between the two treatments were also not different ($P > 0.05$), averaging $4.24 \pm 0.11\%$, $3.39 \pm 0.08\%$ and 1.71 ± 0.07 kg, respectively, except for milk urea which was higher in FR ($P < 0.001$). We conclude that the inclusion of chicory in the diets of dairy cows on summer grazing presents productive levels like those of forage raps, without presenting differences in the composition of the milk. Project financed by FONDECYT N° 1220559.

Could bite composition of grazing dairy cows inform on animal performance, milk quality or enteric methane emission?

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Grazing selection is a driver of the quality and quantity of ingested herbage and could thus be related to performance. During summer 2023, we compared the composition of selected herbage, estimated through simulated bites (SB), of two groups of 14 dairy cows grazing two paddocks differing for plant diversity level (DIV+/DIV-). We used ANCOVA and MANOVA to test the effect of the proportion of grass leaves/stems, legumes and forbs in the SB on dairy performance, milk fatty acid (FA) profile, urea excretion and enteric methane (CH₄) emission. Grass leave SB proportion was higher in DIV- (65.9% vs 22.8%) while forbs' one was higher in DIV+ (25.5% vs 6.5%). Within paddocks, differences in diet composition were driven by individual selection. In DIV-, milk yield (MY) was positively related to the SB proportion of legumes while protein content was negatively linked to the proportion of forbs. In DIV+, MY increased with grass stems proportion while fat and protein content decreased with grass stems and forbs proportions in SB. In DIV-, the level of saturated FA decreased with legumes proportion, while the level of polyunsaturated FA increased. Grass leave proportion in SB was determinant in DIV+, acting in the opposite way. Urea excretion in DIV- increased in milk and urine with legumes proportion in SB (reflecting SB higher nitrogen content), but decreased in DIV+ urine with grass leave proportion. The CH₄ emissions did not differ between paddocks, even though DIV+ cows had a higher proportion of fibrous grass stems in SB which should have led to higher CH₄ emissions. However, the high fibre content could have limited the dry matter intake. Furthermore, DIV+ cows also had a higher SB proportion of forbs, rich in tannins that may contribute to a potential mitigation effect. Within paddocks, the SB composition was not related to CH₄ emissions. Further investigations are ongoing to explore if the spatio-temporal distribution of grazing activities recorded through sensors, can provide insights into SB composition, individual performance and environmental impacts.

Grazing stocking densities and cattle urine impact on soil compaction and nitrous oxide emissions under temperate conditions

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Intensive grazing can offer some potential productive and environmental benefits but relies on high stocking density (HSD), which can cause soil compaction, leading to conditions that activate denitrifying bacteria and result in high nitrous oxide (N₂O) emissions. Nitrogen input from cattle urination events amplify these emissions, however more data is required to determine how these changes under various levels of compaction. In this study, we aimed to quantify soil compaction and N₂O emissions following a grazing event using three stocking densities (control, low (LSD) and high (HSD) with 0, 10 and 100 cows/ha/day, respectively) and a controlled urine application on a temperate Southwest UK pasture. Following the grazing event measurements were taken for 12 consecutive weeks: compaction was assessed using a penetrometer and soil greenhouse gas emissions (GHGe) were monitored with static chambers. The penetration resistance after the grazing event was 1379±368 Kpa for the control, 1544±429 Kpa for the LSD, and 1767±490 Kpa for the HSD. Differences in penetration resistance were found between the HSD and LSD ($p<0.05$), and HSD and control ($p<0.001$), with a tendency between the control and LSD ($p=0.07$). Cumulative N₂O emissions at the end of the 12-week period were 493 g N₂O-N/ha for the control with urine, 804 g N₂O-N/ha for the LSD with urine and 1237 g N₂O-N/ha for the HSD with urine, with differences found between the control with urine and HSD with urine ($p<0.05$). The N₂O emissions from the stocking densities that were not treated with urine were however similar. This suggests that denitrification in soils is enhanced under animal treading from high stocking densities when there is a nitrogen excess, such as with urine. The developing results are informative for improving pasture management under cattle grazing and provide data for models simulating soil mechanisms in these systems.

Forage species and grazing frequency effects on warm-season perennial grasses nutritive value and gas production

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Warm-season perennial grasses are a primary source of nutrients for livestock in tropical and subtropical regions worldwide. Despite their superior herbage accumulation, these grasses may have decreased nutritive value, which can negatively impact animal performance and contribute to increase greenhouse gas emissions. The objective of this study was to test the effects of grazing frequency and forage species on forage nutritive value and gas production. Treatments were the factorial combination of four forage species/cultivars, “Camello” brachiariagrass (*Urochloa* spp.), Spain and Mombaca guineagrass (*Megathyrus maximus*), and Jiggs bermudagrass (*Cynodon dactylon*), and two grazing frequencies, 2 or 4 weeks, distributed in a randomized complete block design with four replicates. There were species and grazing frequency effects ($P < 0.05$) on CP and IVTDOM concentrations. Jiggs had greater CP than other species (14.1 vs. 12.1%). Mombaca and Spain had greater ($P < 0.05$) IVTDOM than Jiggs and Camello (61.1 vs. 55.5%). Forage grazed at 2 weeks had greater CP and IVTDOM than 4 weeks interval (15.0 vs. 10.2% CP, and 61.5 vs. 56.0% IVTDOM). There was no difference ($P = 0.26$) in gas production among species; however, Jiggs had greater ($P < 0.05$) gas production:IVTDOM than Spain, Mombaca, and Camello (234 vs. 200 mg/g IVTDOM). Jiggs had less ($P < 0.05$) methane production than other species (14.6 vs. 18.0 mg/g DM); however, there was no difference in methane:IVTDOM among species (mean = 26 mg/g IVTDOM). Forage grazed at 2 weeks had greater ($P < 0.10$) gas production than 4 weeks (137 vs. 127 mL/g DM) but there was no difference in gas production:IVTDOM (mean = 210 mg/g IVTDOM). The selection of species with greater digestibility and grazing with shorter regrowth interval may result in greater animal production with no increase in gas emissions.

Pasture productivity of a mixed Vs. a polyphyletic pasture during spring and summer

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The objective was to determine the variations in the yield of a mixed pasture (MX) composed by *Lolium perenne* L. and *Trifolium repens* L. Vs. a polyphyletic (POL) composed by *Lolium perenne* L. and *Trifolium repens* + *Bromus valdivianus* Phil. during the spring-summer season in southern Chile. Eighteen hectares were divided into 3 random blocks in which on each block there were six hectares, 3 sown with a MX pasture and 3 sown with a POL pasture. The blocks were distributed in different sectors. After sowing, a homogenization cut was made and, subsequently, every 7 days the yield was determined using a 0.24 m² metal ring where, the vegetative material inside was cut at ground level, weighed in the field and taken to the Forage Laboratory, where it was weighed fresh and taken to a forced ventilation oven at 60 °C for 72 hours or until reaching a constant weight and, by weight difference, the % of dry matter (DM) was calculated. Subsequently, with the %DM and fresh forage yield, the DM yield (g DM m⁻²) was estimated and extrapolated to kg ha (kg DM ha⁻¹). The growth rate (GR – kg DM ha⁻¹ day⁻¹) was also calculated and the growth curve was constructed until defoliation, which took place between 24 to 30 days during spring and 35 to 40 days during summer. Yield, GR and DM accumulations were analyzed using a generalized linear model, comparing the two types of pastures. The data showed that during spring there were no statistical differences ($p > 0.05$) between the variables evaluated with an average of 2600 kg DM ha⁻¹ cut⁻¹ for MX and POL pasture, and a GR of 75 kg DM ha⁻¹ day⁻¹. The main differences occurred during summer, where the POL pasture had a GR of 35 kg DM ha⁻¹ day⁻¹ and a yield of +350 kg DM ha⁻¹ than the MX pasture ($p < 0.05$). The DM accumulation curve did not show differences between MX and POL during spring, but there was in summer. In conclusion, pastures that include *B. valdivianus* are a good forage alternative during the summer period. Project financed by FONDECYT 1240451.

Grassland management and biodiversity measures on organic dairy farms in the mid-mountain border region of Bavaria and Upper Austria

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Grassland habitats on dairy farms represent important ecosystems for numerous plant and animal species, significantly shaping the landscape of the Bavarian-Upper Austrian border region. However, ongoing structural changes in agriculture and the impacts of climate change are putting many of these high-biodiversity sites at risk. As part of the Interreg Bavaria-Austria project “Biodiversity in grassland” (grant number BA0100015), four organic dairy farms with different grazing practices are demonstrating targeted measures for the conservation and management of species-rich grasslands, while simultaneously optimizing grassland utilization and reducing management costs. In 2024, grassland vegetation monitoring of each field before the first cut, together with insect monitoring using “Bartak” type malaise traps during three periods from June to August, was carried out on each farm. Insect samples were further analysed using DNA metabarcoding to generate DNA barcode sequences and compared with reference libraries. The results of the vegetation monitoring on the dairy farms indicated an average plant diversity with 23 species, ranging from 21 to 27 plant species per farm. On average, 478 insect species, ranging from 389 to 604, were recorded per farm throughout the entire observed period, with Diptera being the most common. Certain species listed in the German Red List database were recorded on every farm. No significant differences in the number of insect species were found among the three observation periods. The initial results of biodiversity monitoring provided the first insights into grassland vegetation and insect species diversity on organic dairy farms in the region, highlighting the important role of ruminants in maintaining species-rich grasslands.

Session 11

Poster 17

Pasture quality of recently rewetted peat grassland

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Rewetting of peat grasslands is expected to reduce of greenhouse gas emissions. However, agricultural use, such as pasture for beef cattle, should remain viable. This study evaluated the nutritive value of four peatland pasture fields (PF 1, 2, 3, and 4) during the 2024 grazing season (from May 15 to October 11), rewetted shortly before the season started (PF 3) or up to four years ago (PF 4). At the beginning of each grazing period (GP) for each PF (when possible), the sward was sampled at 10 locations. The samples were then pooled to form a composite sample, which was analysed using an extended Weender analysis. The field areas were 0.77, 0.42, 0.99, and 1.01 ha for PF 1, 2, 3, and 4, resp. The number of GPs was 3, 4, 5, and 4 for PF 1, 2, 3, and 4 resp. PFs sampled during a single GP were: 2 and 4 in GP 1; 1 and 4 in GP 2; 1, 2, and 4 in GP 3; 2, 3, and 4 in GP 4; and 3 in GP 5. Among the nutritive parameters analysed, only ash-free neutral detergent fiber (aNDFom), water-soluble carbohydrates (WSC), and enzymatically soluble organic substance were significantly ($P < 0.05$) affected by GP (1, 2, 3, >3). PF had a significant effect ($P < 0.05$) on crude ash, crude protein (CP), and ELOS. The mean metabolizable energy for ruminants across all samples was 10.04 ± 0.30 MJ/kg dry matter (DM). The mean content of CP varied between 113.00 ± 14.73 (PF 2) and 167.50 ± 21.92 g/kg DM (PF 1). The mean aNDFom content ranged from 588.00 ± 15.56 (GP 1) to 622.00 ± 46.76 (GP >3). The mean WSC content varied between 86.54 ± 25.62 (GP >3) and 157.85 ± 14.64 g/kg DM (GP 1). Due to high water levels above the ground, parts of three PFs could not be grazed during certain GPs or even throughout the entire grazing season (PF 4). For this reason, PF 1 (in particular) could be grazed only for shorter periods than fodder availability would have allowed. The mean duration of GPs, when sampling performed, was 7.4 ± 4.4 calendar days, ranging from 3 to 15 days. In conclusion, the nutritive level of all fields was acceptable for beef cattle. However, the periods of high wetness restricted the grazing area or duration. Further sampling in the coming years will help verify these findings or detect changes.

The biology of aging in dairy cows

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Dairy cows are said to have a lifespan of approximately 20 years but their lifespan in the dairy herd is considerably shorter with most cows leaving the herd before their maximum lifespan. Three factors have come together to create the current economic situation that favors keeping older cows in the herd. First, a low beef cow inventory has facilitated record-high market prices for cull dairy cows and dairy crossbred calves. At the same time, highly effective reproductive programs on dairy herds have enabled dairy producers to achieve high levels of reproductive performance. High fertility in both cows and heifers has been combined with sexed semen and genomics to produce replacement heifers from a limited number of animals with the remaining cows or heifers bred to beef to produce crossbred calves. Collectively, these factors have led to a low number of replacement heifers and historically high replacement heifer prices. These trends are expected to remain for the foreseeable future. Most summaries will cite mastitis, lameness, poor reproduction, low milk production and disease as the primary reasons for culling. The odds ratios for all major dairy diseases except metritis increase as a cow ages. Fertility decreases with increasing parity. Despite the clear evidence that there is aging-related disease and infertility, there is very little information on the biology of aging in dairy cows and the reasons behind age-related decline. One of the most-well described endocrine processes in aging across all mammalian species is “somatopause” which is the process of age-related decline in the growth hormone, insulin-like growth factor endocrine system. Somatopause also occurs in dairy cattle and may explain, in part, age-related decline. Other processes including systemic inflammation, myeloid bias, the loss of muscle mass, stem cell depletion, and cellular senescence are very-well described in other mammalian species but unexplored in dairy cattle. Genetic selection for health, fertility, and longevity increases the survival of cows in dairy herds by improving body condition and reversing some of the endocrine changes seen in older cows. Aging is an active area of research. Scientific discoveries made in humans, laboratory animals and (or) other species may provide insight into methods to increase the lifespan of dairy cows.

Session 12

Theatre 2

Genomic blueprint of cow longevity

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Improving dairy cattle sustainability involves selective breeding to improve productivity, fertility, welfare, health, efficiency, robustness, and livability. This should be done without increasing animal footprint and natural resource utilization. Intensive selection for production performance is frequently associated with elevated susceptibility to diseases and shorter lifespan. In the US, genomic evaluations for livability started in 2016, with the trait included in the \$Net Merit index (\$NM) in 2017. The relative emphasis on livability in the \$NM is 4.3%. Although the emphasis is higher than for many traits, the most important feature in dairy operations is the cows' ability to stay alive in the herd while producing milk. For that, productive life fulfills the purpose. This trait was included in the \$NM in 1994; its current relative emphasis is 15.1%. Productive life has increased since 1960, with a recent jump in genetic gain since implementing genomic selection. In terms of genetics, productive life increased around 4 months from 1975 to 2008 and 8 months from 2009 to 2020. This is a considerable achievement given the heritability of this trait is low ($h^2 = 0.08$). Genotyping a bull in such a case may be equivalent to collecting phenotypes for almost 100 daughters. Although genomic selection has effectively identified bulls that will have daughters that stay longer in the herd while producing milk, management decisions have shortened the cows' lifespan. The average productive life may be around 3 to 4 years, and about 65% of the cows that leave the herds have a termination code “lactation ended normally”, meaning there were no health or reproductive issues. Extending productive life can result in better welfare and reduced environmental footprint. Additionally, this trait is strongly correlated with fitness traits like fertility. In this talk, we will review the benefits and opportunities for improving functional longevity, together with lessons learned from genomic selection. Finally, we will discuss whether intensive genomic selection for production traits can have a negative impact on cow longevity and welfare.

What is the longevity of dairy cows in France? Current situation and outlook

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Improving the longevity of dairy cows is a major challenge for the sustainability of dairy systems. The goals of this study were to describe the longevity of French dairy cows and to identify the factors affecting it. The current state of dairy cow longevity and its evolution at national level were studied by using several indicators. The identification, animal flows and performance data of French national databases of 2,325,535 dairy cows from 12,933 herds, culled between 2010 and 2022, were analysed for this purpose. The cows that finished their careers in 2022 had a median lifespan (LS) and cumulative lactation length (LL) of 2,005 days and 949 days respectively. Their median milk production over their career (MC) was 24,065 kg of milk per cow, or 11.5 kg of milk per day of life on average. Between 2010 and 2022, the LS of dairy cows fell slightly (-60 days) and MC increased by 1,593 kg, or 1 kg of milk per day of life. These median and mean values for LS, LL and MC mask significant inter-individual differences. In 2022, the difference in LS and MC between the 1st and 3rd quartile of the population studied were respectively 1,033 days and 24,983 kg of milk. Younger age at first calving and higher milk productivity in first lactation seem to have a positive influence on MC to the detriment of LS. This study highlights the significant potential for improving the longevity of French dairy cows. It needs to be supplemented by an approach at farm level. But it already highlights the importance of choosing and equipping ourselves with suitable indicators and benchmarks to draw up a relevant assessment for the farm and identify the levers for improvement.

Session 12

Theatre 4

Perspectives from Canada on Dairy Cows' Longevity

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Our focus is ensuring the sustainable life of dairy cattle, or, how to keep healthy, comfortable animals longer in the herd, encompassing research on animal welfare for better management and facilities, together with our understanding of cow longevity. Our approach is to develop methods to remotely assess changes in cow welfare, and to evaluate how health issues and other risk factors are linked to cow longevity. We aimed to exploit the potential of the considerable dataset regularly collected on Canadian dairies via the Canadian Dairy Network (Lactanet). We have assessed the impact of health events such as lameness and mastitis, finding that primiparous cows afflicted with these diseases have reduced milk yields and gross profit, and higher risk of culling than healthy cows. We also unveiled valuable insights on early-life variables that are linked with future productivity and longevity, such as weight at birth and at weaning, weaning age, and serum IgG levels; these represent early indicators that can be used to select replacement animals based on their potential, and reduce economic and environmental losses incurred by keeping animals that will likely never be profitable. Our work has also led to innovations in the remote detection of welfare. On a herd basis, the analysis of DHI indicators routinely collected on dairies was used to develop a composite herd welfare index. On an individual cow basis, we have tested the use of spectral data from milk samples as a source of information on cow-specific changes in welfare status; this approach was successful in linking milk components with improvements in specific outcomes of cow welfare. Overall, these innovations show that the potential to conduct remote assessments is present and could thus be extended to more specific topics (e.g., lameness in individual cows) and other aspects (e.g., economic and environmental performance of cows and farms).

Impact of a milk based fixed effect on stayability evaluation in Italian Holstein cattle

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The aim of the present study was to investigate the effect of milk yield on breeding value of longevity in the Italian Holstein population. This investigation was provided in the context of the LATteco2 project (National Rural Development Programme – PSRN). This study defined stayability as a binary trait, where 1 are survived animals and 0 are non-survived animals. The dataset included 7,987,171 cows with a first calving, raised in 36,677 herds and offspring of 9,201 sires. Target breeding goal was defined as the survivability at third lactation. Thus, two traits were identified: stayability from first to second lactation (stay12) and stayability from second to third lactation (stay23). The estimation of breeding values was performed using a single-trait animal model (M1) for each stayability trait; it considered as random effects the herd-year of calving as contemporary group, the additive genetic animal, and the residual. Fixed effects were the change in herd size compared to the previous year (HSC) and the variation in cows' milk production from the contemporary group (DM). The utilisation of the DM effect resulted in the transformation of stayability into a functional stayability. EBVs from M1 were compared with EBVs from a single-trait animal model without the DM effect (M2). Moreover, both a Gaussian (g) and a threshold-liability (t) approach were tested for the two models. These comparisons enabled an assessment to be made between functional stayability and stayability. Sires' rank correlations between models were the following: 0.985 for stay12 and 0.899 for stay23 in M1g vs M2g, 0.981 for stay12 and 0.892 for stay23 in M1t vs M2t; 0.996 for stay12 and 0.998 for stay23 in M1g vs M1t, and 0.998 for stay12 and 0.999 for stay23 in M2g vs M2t. These findings indicate only a marginal re-ranking of bulls, with implications for genetic selection in the use of a milk correction effect.

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Theatre 6

Economic and environmental impacts of extending dairy cow longevity in Danish Holstein Herds

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Extending dairy cows' longevity to dilute emissions from their youngstock periods is of interest regarding GHG emission mitigation and the concurrent effects on farm economy, and dairy food production. Here we simulated a set of longevity-extending strategies to estimate their consequences on enteric methane emission, gross margin, and edible protein yield. These strategies included improved reproduction, improved health, extended lactation, and a strategy combining the former. The simulation was conducted in 10 Danish conventional Holstein herds by increasing their relevant performance figures to the 90th (or 10th for health problem risk) percentiles of the Danish national distributions for conventional Holstein herds or by postponing the start of inseminating primiparous cows to day 150 after calving. Results showed a large between-herd variation in response to the different longevity-extending strategies. On average across herds, these strategies decreased herd enteric methane intensity by 0.7 to 6.3 %. These strategies also favored gross margin which increased by 0.2 to 4.6 % on average across the 10 herds. The change in herd edible protein yield varied from -1.2 % (i.e. negative effect on protein yield from improved reproduction) to +1.5 % (positive effect on protein yield from improved health) across herds. These findings revealed that extending dairy cows' longevity can benefit both emission mitigation and farm economy in dairy herds while potentially maintaining dairy food production.

Trends for longevity of dairy cattle in the Netherlands

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Longevity of dairy cattle is an important trait from an economic and welfare perspective, as well as from a societal and government perspective. For a farmer it is beneficial to get older cows, as it will reduce costs for rearing. Dutch government aims to reduce environmental impact from livestock, and for that is also beneficial to have older cows. Older cows produce on average more, and feed is converted more efficient. Over the past 25 years all statistics on longevity metrics are favorable. These metrics are available in the annual statistics for herdbook cows. Productive life increased by 337 days to 1,445 days for cows culled in 2024. The increase in productive life was constant up to 2008, followed by plateau until 2015, a reduction from 2016 to 2018, followed by a steep increase. Main reason for this steep increase since 2018 was that farmers needed to reduce cow numbers to be below the threshold of their phosphate quota. Until 2015 milk quota were in place in EU, and in the Netherlands a new quota in the form of phosphate came in place in 2018. For farmers it became beneficial to limit the amount of young stock, to be able to produce more milk within a certain phosphate quota. Since 2018 productive life increased faster than in the past. Together with reduced amount of youngstock, the rearing period reduced by 40 days to 763 days. Number of calvings increased by 0.8 to 3.9 calvings. Lifetime production increased in 25 years by 14,329 kg to 38,283 kg of milk (with 4.40% fat and 3.58% protein), resulting in 1,684 kg fat and 1,369 kg protein. Production per day of life increased by 4.8 kg to 17.1 kg of milk. Genetically, longevity increased by 600 days, meaning that the full genetic potential is not yet utilized. The strong increase of genetic trend in longevity was supported by selection on udder health, claw health and feet & legs. Longevity is a result of management (e.g. feeding, housing and culling decisions), environment, and genetics. Long term trends show that all these factors together resulted in significant improvements for longevity and also lifetime production.

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ADSA Discover Conference on Dairy Cattle Lifespan: Agreements, disagreements, and areas for further investigation

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In the fall of 2023, the American Dairy Science Association (ADSA) held a 3-day conference titled Dairy Cattle Lifespan: New Perspectives. The theme of the conference has continued to be much debated in the past year. Extended dairy cow lifespan is often said to be good for sustainability, and the image of the dairy sector. In practice, however, the topic is complex, and simple perceptions do not capture the breadth of the underlying questions. The conference was designed, therefore, to take an in-depth look at dairy cow lifespan and explore new perspectives in the modern era of dairy production. Speakers and attendees explored the many facets of lifespan including the underlying biology, genetics and genomic selection and important management factors including nutrition and housing. Importantly, economics and the environmental impact were assessed for herds under different production systems. Industry panels provided diverse ideas about goals for lifespan in different countries. The conference was successful in that there were topics that were generally agreed upon (replacement of an individual cow should be based on her competitive economic merit; long lifespan in itself should not be the goal; longevity is not a good measure of welfare). Disagreements existed on the importance of lifespan to consumer perspectives of dairying as well as its environmental impact. These disagreements identified important knowledge gaps and opportunities such as quantifying maturity and future cow performance, and the importance of early lactation culling. Rapidly changing genetics, technological advances that affect heifer supply (embryo transfer, sexed semen etc.), new lucrative markets for dairy farms (including meat from cull cows and the sale of beef-dairy crossbred calves) and legislative action on climate change will impact dairy farms and their decisions to keep or cull older cows. Despite advances in husbandry, precision dairy farming technologies and decision support tools, when dairy cattle should be replaced and what replacement rates should be remains an area for further investigation.

Environmental and Economic Impacts of Extending the Productive Lifespan of Swiss Dairy Herds in Diverse Husbandry Systems

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Ruminants are major contributors to greenhouse gas emissions due to methane production and biodiversity loss through land for feed and fodder production. Prolonging the productive lifespan of dairy cattle is a promising approach, as it reduces the need for replacement animals, and the related environmental impact during the first two unproductive years. Yet, herd-level productive lifespan affects both milk productivity and economic impacts on farms. Impacts of increasing dairy cow productive lifespan on greenhouse gas emission, land use and the gross margin contribution are quantified with different representative Swiss farms. These represent different agro-ecological zones with different dairy breeds in both conventional and organic productions systems. To derive herd level yearly average milk yield from herds with short, average and long lifespans for each representative farm Swiss herdbook data was used. Greenhouse gas emissions, land use, and farm profitability were assessed using the FiBL FarmLCA tool. Prolonged productive lifespan reduces greenhouse gas emissions per unit of milk by decreasing the need for replacement animals, even when the prolonged productive lifespan comes with a reduced herd level milk yield. Land-use per dairy production unit is always reduced as well. Economically, short lifespan of dairy cows always yields lowest while average lifespan yields for most farms the highest gross margin per cow. In case of long lifespan, income lost from fewer culled cows is not offset by additional revenue. This perspective, however, ignores that the spare agricultural land could be used differently and compensates for economic loss further. In intensive systems like Holstein herds in the lowlands long lifespan yields the highest gross margin. Extending dairy cow lifespan offers clear environmental benefits by lowering emissions and land use, supporting climate and biodiversity goals. Economic viability depends on effective land use strategies and making prolonged lifespan a profitable solution for reducing CO₂ emissions in the livestock sector.

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Individual cows responses to climatic perturbations using VAR models

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Within a dairy herd, response to climatic challenges may differ between cows and analysing the impact of heat stress for example on an animal basis is then of importance. In traditional approaches, animal responses to such a challenge are assessed against a reference value, based on thresholds or periods prior to this challenge. But post-disturbance response, may not return back to pre-disturbance level, questioning the relevance of a single, fixed cut-off over the time for considering the impact of a climate challenge. To address this limitation, a dynamic approach using VAR (Vector Autoregressive) models was used to assess individual animal responses to climatic perturbations (heat stress in this case) over time without assuming predefined thresholds or periods. Data were collected from June 16th to September 15th 2024, on 158 Holstein dairy cows (parity 1–5), across 2 commercial farms in western France. The cows were housed indoors year-round and monitored through behavioral data (standing, holding area time, drinking, eating detected by Alherd® device) and environmental data (temperature, humidity, wind speed, Wet Bulb Globe Temperature). Data were aggregated on a 4 hours basis. VAR models combined with Impulse Response Analysis (IRF) were used to analyze animal response dynamics, focusing on intensity (response magnitude) and lag, e.g. the delay between a temperature shock and the peak response. Results indicated a strong predictive performance ($R^2 > 0.7$, p -value < 0.05). The IRF diagnostic test revealed significant responses of cows to increased temperature, especially in drinking behavior. For instance, an increase of 7% (Farm 1) and 5% (Farm 2) was observed after 24-hours (lag 6 : 6x4h). Cumulative effects of temperature reached peaks of 44% at lag 13 (Farm 1) and 59% at lag 21 (Farm 2) for drinking, and 36% at lag 21 (Farm 1) and 9.7% at lag 13 (Farm 2) for standing behavior. In contrast, eating and lying behaviors showed minimal effects ($< 1.5\%$), indicating a limited response to the thermal shock. To fully assess this original approach and its perspectives, a comparison with commonly used methods will be performed.

Longevity and characteristics of cows with lifetime milk yield over 100,000 kg in Slovenia

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Extending longevity increases profitability as cows that stay in the herd longer produce more milk over their lifetime and reduce the need for replacement heifers. Longevity depends on genetic and environmental factors and age at first calving. Conformation traits of cows also affect longevity. Poor udder traits increase the risk of injury and mastitis, which can lead to early culling. Optimal body characteristics ensure longer life and higher productivity. Premature culling of dairy cows results in less lifetime milk production, fewer calves, higher rearing costs per liter of milk produced and a smaller proportion of older cows in the herd. Longevity also has a positive environmental impact, as it reduces emissions associated with raising replacement animals. A longer lifespan of cows enables more sustainable and efficient milk production. The lifetime production of dairy cows is a complex trait influenced not only by genetics but also by the environment in which a cow lives and the management practices of the farmer. Indeed, most of all the farmer's strategy towards keeping younger or older cows as part of the management of the herd is essential. In Slovenia, despite selection for longevity, the number of productive days across the breeds is decreasing (in 2010 and 2023 for Holstein-Friesian, Simmental and Brown, respectively, 1278 and 1191, 1377 and 1335, and 1577 and 1492 days), especially from 2020 on, although lifetime milk yield is increasing (+3 kg for Simmental and Brown and + 4.7 kg for HF per productive day in period 2010-2023). The trend and factors behind it will be explained. Moreover, we analyzed those cows that exceeded a lifetime milk yield of 100,000 kg of milk. It included 266 HF cows that exceeded 100,000 kg milk by the end of 2024. The average lifetime milk yield of these cows was 108,810 kg of milk with 3.84 % fat and 3.23 % protein. The average age of these cows was 11.7 year and, on average, they completed 9.21 lactations. These cows are off-spring of cows that also achieved relatively high lifetime milk yields, as these yields were on average 47,210 kg. A characteristic of HF cows that did exceed 100,000 kg of milk are, generally, better conformation traits, especially frame, udder, feet and legs.

Session 12

Poster 12

Analysis of predisposing factors for the incidence of foot lesions in dairy cows

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Recently, there has been an increase in foot problems as a result of the intensification of production in the dairy sector. In order for animals to express their full productive and reproductive potential, they need to be able to stand comfortably so that they can perform their normal functions. Lameness in dairy cattle causes significant losses, ranking third in economic losses and the most important pathology, ahead of mastitis and infertility. This work aims to verify the importance of implementing a preventive protocol for foot problems. The aim of this work is to verify the importance of implementing a preventive protocol for foot problems. It was carried out on the "Bracamonte Agropecuária Lda" farm, with a milking herd of 1,000 animals, and compared the results obtained from the conventional protocol for treating existing foot problems (treating animals only when they have visible pathologies/ grade ≥ 3 lameness) and the implementation of a new preventive protocol (elaborated: focus on footbaths, preventive actions and hoof correction, training actions, greater feed control, separation of problem animals and targeted treatment). Field sheets were created to observe foot problems, where lameness was classified from 1 to 5, according to the locomotion classification system used in cattle, and data was collected on production, reproduction, mastitis, foot history, treatment and future decisions (culling). Preliminary results show that the preventive protocol resulted in a significant reduction (-56%) in animals with serious foot pathologies, a reduction in antibiotics administered (-32%), a reduction in animal waste (-12%), a reduction in milker stress/time (-6%), which was consequently reflected in an increase in production (an increase of around 8L cow/day) and animal fertility.

Characterizing the NAD⁺ metabolome of dairy cows during the transition from gestation to lactationM. Da Silva^{1,2}, N. Potphode¹, M. Hubbard¹, A. Miles³, R. Baldwin³, E. Rico^{1,4}¹ University of Maryland, 8127 Regents Dr, 20742 College Park, United States, ² Bonn-Rhein-Sieg University of Applied Sciences, Von-Liebig-Straße 20, 53359 Rheinbach, Germany, ³ USDA ARS, 10300 Baltimore Ave, 20705 Beltsville, United States, ⁴ University of Pennsylvania, 382 W Street Rd, 19348 Kennett Square, United States

Dysfunctional energy metabolism is associated to metabolic stress and postpartum (pp) disease in dairy cows. Nicotinamide Adenine Dinucleotide (NAD) coenzymes are central catalysts of metabolism. In rodents, the depletion of the NAD⁺ metabolome is associated with reduced energy use efficiency, poor lactation performance, and impaired growth in offspring. Our objective was to characterize the NAD⁺ metabolome in blood and liver of cows of low residual feed intake (L-RFI; high efficiency; n=12) and high RFI (H-RFI; low efficiency; n=12) during the peripartum (21d pre- to 21d post-partum). Liver biopsies were performed at d -21 and d7 relative to parturition. Blood samples were collected weekly from d -30 to d 21, relative to parturition. The NAD⁺ metabolome in liver and blood was quantified using HPLC/MS. Data were analyzed under a mixed model with time and RFI class as fixed, and cow and enrollment block as random effects. Plasma glucose and insulin decreased postpartum ($P < 0.05$), while NEFA and beta-hydroxybutyrate (BHB) increased ($P < 0.05$). BHB were higher at d14 pp in H-RFI and NEFA were higher in L-RFI at d 14 and 21 pp ($P < 0.05$). Glucose was higher at parturition in L-RFI cows. The Low-RFI group tended to have higher total antioxidant capacity 21 days pp ($P = 0.08$). Hepatic NAD⁺ metabolites increased, while NAD precursors decreased pp ($P < 0.05$). Nicotinamide Mononucleotide was lower in H-RFI ($P = 0.04$) and tended to decrease pp in both groups ($P = 0.06$). The sum of hepatic NAD⁺ metabolites was unaffected by time or RFI ($P = 0.83$). The increase in hepatic NAD metabolites, coupled with a decrease in NAD precursors, agrees with a heightened demand for NAD biosynthesis during this period. However, no short-term shortage of NAD⁺ metabolites was apparent, and limited differences were observed between RFI groups. Future assessment over the totality of lactation may indicate whether hepatic NAD⁺ depletion develops progressively with sustained lactogenesis.

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Poster 14

Flavonoid-based feed additive improved resilience and metabolic efficiency in dairy cows' post-disease outbreak

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Effective health and welfare management is essential for sustaining dairy productivity, particularly during disease outbreaks. Phytochemicals, such as specific flavonoids, enhance immunity and overall cow well-being, potentially improving productivity. This on-farm trial evaluated the impact of flavonoid supplementation on dairy cow production following a bluetongue disease outbreak. The 16-week trial included 87 milking cows. For the first 8 weeks, cows (Control; n = 87, parity: 2.48; days in milk: 177.7) received a standard diet (CP = 166 g/kg DM, NEL = 7 MJ/kg DM). In the following 8 weeks, the same cows (Treatment; n = 87, parity: 2.46; days in milk: 193.8) were supplemented with 30 g/cow/day of a flavonoid-rich phytochemical feed additive (PFA; Dr. Eckel Animal Nutrition GmbH & Co. KG). Fat- and protein-corrected milk (FPCM), somatic cell count (SCC) and milk quality parameters were assessed, with data analysed using SAS software. Following the bluetongue outbreak, FPCM decreased by 7%. However, PFA supplementation resulted in a numerical increase in FPCM (33.7 kg vs. 35.3 kg; $P = 0.09$). Notably, milk protein significantly increased with PFA ($P < 0.01$; 3.33% vs 3.66%), suggesting improved protein metabolism. While there were no significant differences ($P = 0.137$) in milk fat, SCC, an indicator of udder health, decreased by 29% in the PFA group ($P = 0.269$). Additionally, milk urea concentration significantly decreased by 12% ($P < 0.01$), indicating improved nitrogen utilisation. Despite the initial disease-induced decline in lactational performance, flavonoid supplementation supported metabolic efficiency and resilience, contributing to enhanced milk protein content and nitrogen metabolism. These findings indicate that flavonoids may support recovery and overall cow well-being during health challenges, as evidenced by a 70% average milk yield recovery rate in cows severely affected by a bluetongue outbreak. Further research is needed to evaluate its long-term effects on dairy cows' health and productivity.

Clinical cytogenetics – a sustainable tool for genetic health of dairy cattle

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The improvement of genetic health in dairy cattle through clinical cytogenetic investigations is of economic importance and could contribute to the overall sustainability of the sector. Preventive chromosomal aberrations screenings in livestock species are in accordance with current European Union's strategies on animal health, that emphasize prophylactic prevention measures. Moreover, cytogenetic investigations is in accordance with the two main pillars of the agroecology principles, when livestock species are concern, namely: i) adopting genetic management practices that aim to improve animal health; and ii) decreasing the inputs needed for production. Subsequently, given that artificial insemination (AI) is used to a large extent in dairy cattle, with high breeding value (EBVs) bulls often having thousands of descendants, it is a well-documented that inherited chromosomal aberrations can be quickly distributed within populations; this can lead to great economical loses at farm level, mainly throughout poor survivability, productive and reproductive performances of the carriers. Current status of clinical cytogenetics programs in the Romanian dairy cattle sector are presented and discussed. In Romania, genetic screening of proven bulls used for AI has been routinely done as research efforts in the last decade, demonstrating the feasibility of integrating such approaches into breeding programs. In conclusion, the inclusion of clinical cytogenetics in future genetic improvement programs of dairy cattle, can result in an increase in the overall animal productivity and welfare, and could be integrated with agroecology strategies.

Resilience and productive performance of Holstein dairy cows under grazing (I)

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Dairy cows at grazing conditions are particularly required to be resilient to environmental disturbances. This study explores the relationship between animal performance and resilience for sixteen multiparous Holstein cows (5 ± 1.5 lactations, fall calving, body weight: 684 ± 77 kg; mean ± SD). Prepartum diet consisted of a corn silage-based TMR, and then during lactation cows grazed on seeded pastures and were supplemented with an energy concentrate at the milking parlor (3-4 kg DM/d) and a corn silage-based TMR at a feedpad (2-8 kg DM/d). Milk yield was recorded daily, and milk composition was determined monthly. Body condition score (BCS, 1–5 scale) was assessed at -30, 21, 100, and 200 ± 15 days relative in milk (DIM). The resilience was quantified as the natural logarithm of individual daily milk yield's variance (VarMYln). The VarMYln was finally expressed as the residual (ResVarMY) between observed and predicted VarMYln according to a linear regression where VarMYln = a + b·MYac + c·DIM, being MYac the accumulated milk yield during lactation and DIM the total DIM of each cow. The greater the ResVarMY, the lower the cow's resilience compared to her counterparts. Cows were ranked according to ResVarMY and extreme animals with the highest (High-Res, n=6) and lowest resilience (Low-Res, n=6) were selected. High-Res and Low-Res had significantly different ResVarMY (-0.309 vs 0.306 ± 0.04, mean ± SEM; P < 0.01, respectively). Results were analyzed with mixed models as repeated measures. No statistically significant difference was detected between High- and Low-Res cows for milk yield (30.3 vs 31.3 ± 0.98 kg/d, P=0.45), peak milk yield (41.4 vs 43.0 ± 1.14 kg/d, P=0.32), MYac (7,805 vs 7,841 ± 152 kg, P=0.87), or BCS (3.4 vs 3.2 ± 0.08). Milk lactose content and protein yield were higher (P<0.04), and protein content tended to be higher (P<0.07) in High- vs. Low-Res. These results suggest that High-Res cows may have a better nutritional status, as reflected in improved milk components synthesis.

The link between resilience and the metabolic profile of Holstein dairy cows under grazing (II)

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In grazing systems, dairy cows are required to be resilient to withstand environmental disturbances. The metabolic status of dairy cows affects their resilience, so we explore the association between resilience and the metabolic profile of sixteen multiparous Holstein cows (cow diets and methodology used to quantify resilience are described in abstract #786110791 as part of the same experiment). Milk yield was recorded daily, and milk analysis was determined monthly to analyze β -hydroxybutyrate (BHB) and Milk Urea Nitrogen (MUN). Blood samples were taken to determine concentrations in plasma of glucose, β -hydroxybutyrate (BHBp), nonesterified fatty acids (NEFA), cholesterol (CHOL), total protein, urea, albumin, creatinine and plasma activity of aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) at -30, 21, 100, and 200 ± 15 days refer to the calving. Data were analyzed with mixed models as repeated measures. No statistically significant difference was detected between high resilience (High-Res, $n=6$) and low resilience (Low-Res, $n=6$) cows for NEFA (0.22 vs 0.18 ± 0.04 mmol/L, $P=0.83$), BHBp (0.59 vs 0.64 ± 0.05 mmol/L, $P=0.46$), CHOL (82.5 vs 83.7 ± 8.8 mg/dL, $P=0.93$) or for total protein, urea, albumin, MUN and hepatic enzymes activity. The BHB in milk was higher ($P=0.05$) and glucose concentration and creatinine concentration tended to be higher ($P=0.09$) in High- vs. Low-Res cows. These results suggest that High-Res cows may have a better metabolic profile that enables them to cope with the metabolic challenges of lactation in dairy cows under grazing conditions.

Projecting milk yield based on mean rumination time using MilkBot lactation model

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The overall objective of this study was to find an association between cow rumination time (RT) and milk yield (MY) during the entire lactation period. Our hypothesis was that the mean rumination time (MRT) would impact the total MY produced throughout the lactation. Data were collected from 1,412 dairy cows at 9 commercial dairy farms during the 3 years period. Milk yield and behaviour data were obtained from Milk Manager Software (Dairymaster, Co. Kerry, Causeway, Ireland) at the end of the trial. The behaviour was measured using the behaviour monitoring collar (MooMonitor+, Dairymaster, Co. Kerry, Causeway, Ireland) of each animal 24 h/day. The cows had individual milk production records during the first 3 lactations that lasted at least 100 d with at least 1 measurement during the first 30 days in milk (DIM). We were especially interested in a high MRT above 550 min/d because we expected that those will be the high-yielding cows. The MilkBot lactation model was used to fit milk production curves. The MilkBot lactation model predicts MY as a function of time after parturition. Four parameters: a (scale), b (ramp), c (offset), and d (decay), control the shape of the lactation curves. The total MY produced during the entire lactation (305 days) was affected by the MRT. The differences between the highest and lowest MRT groups were 1,735 kg, 2,617 kg, and 1,941 kg in the first, second, and third lactation, respectively. The behavioral differences observed in this study provide new insight into the effects that MRT has on MY.

Breeding values for cow survival in a beef x dairy system

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The increasing use of beef sires in dairy herds has become a significant trend in the dairy industry, largely driven by advancements in reproductive technologies such as sexed semen. This has resulted in an excess of dairy heifers, prompting a shift towards beef-on-dairy breeding to optimise herd management and meat production efficiency. However, challenges exist, including cow survival, particularly survival to day 50, and potential genetic variation among Belgian Blue sires for this trait. This study investigates the genetic variation among 223 Belgian Blue sires used on 146,000 Holstein dairy cows with 210,000 calvings in the Danish beef-on-dairy system, specifically focusing on cow survival to day 50 after calving. We applied a four-trait multivariate model for calving ease (CE), calf survival (SCU), calf size (SCI), and cow survival to day 50 (SURV50), including fixed effects for herd-year, year-month, sex of calf, parity, and cow age, along with random additive effects of sire. The results showed high genetic correlations between survival traits (>0.9), for survival at day 50, day 75 and the survival to the next lactation. These correlations were estimated using a separate three-trait multivariate model for the three survival traits, incorporating the same fixed effects and random additive sire effects as the four-trait model. Estimated genetic parameters showed low heritability for all survival traits ($<1\%$). The genetic correlation between SURV50 and CE showed that easier calvings were associated with higher survival rates (-0.65 ± 16). Additionally, differences between the sires with the best and poorest breeding values for SURV50 had a difference of nearly 2%-points in survival rates. These findings enhance the understanding of genetic factors influencing cow survival in beef-on-dairy systems and could contribute to enhanced routine genetic evaluations for birth traits. Incorporating survival traits into breeding strategies may help improve both meat production efficiency and dairy herd sustainability.

Session 13

Theatre 1

Event detection, defining heat stress and detecting it in a way farmers can relate to

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Dairy cattle are selectively bred for high production levels under optimal conditions. However, defining the range of living conditions under which an animal can be considered to be operating optimally is a complex task. Within the SLU Infrastructure for Dairy Data Collection, Gigacow, we have developed models based on probability density functions to assess the impact of thermal conditions on milk production at the herd level. The analysis included 1,651,346 daily milk yield records from 2,179 lactations across 375 Nordic Red Dairy Cattle, 1,023 Swedish Holstein, 50 Swedish Jersey, and 151 dairy crossbred cows from nine Swedish dairy herds connected to the Gigacow network between 1 January 2022 and 18 August 2024. The distribution of milk yields at each milking under thermal and non-thermal stress conditions was analysed, enabling the estimation of herd-level losses at different thresholds using probability density functions. Variations were observed both between individuals within the same herd and across different herds in the overlap between the two density curves representing yield distributions. This suggests differences in individual heat resilience as well as potential variations in farm management practices. The models developed in this study enable advisors to provide herd owners with data-driven insights, allowing for the optimisation of farm management strategies, mitigation of losses, and evaluation of the effects of thermal stress on individual cows. By integrating sensor-based monitoring—such as temperature and humidity loggers within herd management systems, alongside activity trackers, these models can be further refined to investigate animal-environment interactions. This, in turn, supports precision livestock farming and breeding strategies, facilitating the selection of cows with greater heat tolerance and improved adaptability to environmental changes while maintaining high production levels.

Assessment of the Equivalent Temperature Index in livestock buildings thanks to machine learning tools
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Precision Livestock Farming (PLF) tools have become an integral part of sustainable livestock management, offering increased productivity while minimizing environmental impact. This study explores the application of the NeuralProphet model to predict Equivalent Temperature Index (ETI) in dairy cows, leveraging the development of powerful statistical algorithms and advances in artificial intelligence, particularly in the deep learning subset. The data used come from a smart monitoring system implemented in a pilot dairy farm located in Budrio (Emilia-Romagna, Italy) while the data of the main external environmental parameters were obtained from the weather service provider Open-Meteo, taking advantage of the performance of predictive models already tested in various applications. In this study, a predictive model for ETI was developed through an initial data exploration that includes preprocessing tasks such as outlier detection, unit adjustment, and calculation of the target variable. Exogenous variables were analyzed for sensitivity, resulting in the selection of 24-hour forecasts for outdoor temperature, relative humidity, solar radiation, and wind speed as model inputs. In addition, values for indoor temperature, relative humidity, and wind speed were included as initial conditions for model reproducibility in future studies. As a final step, the evaluation of the accuracy of the model was performed by comparing the indoor environmental parameters of the building provided by the model with raw measurements. As a result, a reliable tool was synthesized to support the decision-making process of farmers to improve livestock welfare and increase animal productivity.

Sensor-Based Prediction of Ketosis in Dairy Cows: Addressing Feature Selection and Class Imbalance
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We present a data-integrative predictive approach for classifying ketosis in dairy cows using dynamic sensor data. Our study addresses two critical challenges in predictive modeling: effective feature integration and management of class imbalance, both essential for developing robust, generalizable models for real-world implementation. Our analysis utilizes an extended dataset derived from the D4Dairy project. This dataset includes data from 34 farms (1,285 cows), with longitudinal records from 6 farms (224 cows), offering multi-faceted information at different levels of detail, depending on the available features for each observation. All farms utilized sensor systems, providing complete rumination data and partial data on eating behavior and activity levels. Ketosis status was determined through ketone blood tests (days 7 and 14 post-calving) and veterinary diagnoses. Additional features included health history, breeding values, dairy herd inspection (DHI) records, farm management data, environmental conditions, KETOMIR (mid-infrared test for ketosis prediction) results, and automated milking system (AMS) measurements. We evaluated three modeling approaches—logistic regression, Random Forest, and XGBoost—to assess performance across different feature sets. Our findings highlight complex feature interactions, where selective feature subsets sometimes outperformed more comprehensive models. To mitigate severe class imbalance, we applied downsampling techniques and threshold-based adjustments, optimizing F1-scores. Preliminary results indicate significant variations between models, downsampling strategies, and feature integrations across all test configurations. Our findings show that applying Random Forest with downsampling improves sensitivity at the cost of some specificity. In this setting, the model achieved a balanced accuracy (BA) of 0.65 (sensitivity [SE]: 0.54, specificity [SP]: 0.76), substantially outperforming sensor-only models (BA: 0.51, SE: 0.86, SP: 0.17).

Clarifying the long-term impact of diseases on lactation features in Holstein cattle with strategies for causal inference

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Disease represents a key constraint to farm sustainability. Previous study often focused on short-term milk loss during disease periods. However, milk yield is usually not fully recovered even after cured. Our study aimed to quantify the long-term impacts of diseases on lactation features through big data and causal inference strategies to improve knowledge and reduce disease burden. We collected high-throughput session milk yield and disease records (udder health, reproductive disorders, metabolic disorders, digestive disorders, and hoof health) for 37,246 Holstein cattle in China from 2020 to 2024. Three causal inference strategies were used to perform unbiased inference of causal effects on 305-day milk yield (MY305) and average coefficient of variation for session milk yields (CVM), including propensity score matching, overlap weighting, and convergent cross-mapping. Confounders included herd-year-season, parity, stillbirths, calving ease, the number of inseminations, and genetic levels of milk yield and resilience. The Cox model and genetic analyses were employed to clarify relationships among confounders, diseases and lactation features. Overall, cows with high parity, low resilience, difficult labor, and stillbirth (hazard ratio: 1.06-2.33) faced higher disease risks. High milk yield and inter-session variability were moderately genetically correlated with prevalence. Diseased cows showed a significant reduction in MY305, with causal effect estimates ranging from 345.76±0.81 kg (metabolic disorders) to 511.72±0.04 kg (reproductive disorders). After cured, long-term impacts of five diseases on milk yield ranged from 4.06 % to 8.42 %, with the second disease onset showing a weakened long-term effect (3.28%). Notably, CVM increased rapidly within five days before disease onset, ranging from 5.36% (metabolic disorders) to 10.36% (digestive disorders), suggesting its potential as an early monitoring tool for diseases. In summary, this study confirms the long-term impact of diseases on production performance and shows how data-driven causal inference and high-throughput data can enhance precision livestock farming.

Session 13

Theatre 5

Using collar-based algorithm to estimate individual feed intake and efficiency in commercial farms

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Feed efficiency (FE) is an important trait since it is established as hereditary and directly affects profitability and sustainability. A limiting factor to individual FE determination in commercial farms is the need for individual dry matter intake (DMI). Most DMI equations use data unavailable to many commercial farms and can be inaccurate in cows with relatively high or low DMI. Integrating eating behavior parameters from wearable sensors, may enable more accurate and reliable DMI prediction. Therefore, the objectives of this work were to develop an algorithm to accurately predict individual DMI based on AfiCollar neck-tag and milk data, and examine the potential of using FE, calculated with predicted DMI, to improve herd efficiency. Accordingly, weekly DMI data from five research farms in the US, Canada, Czech Republic and Italy (35 to 100 cows in each farm, overall, 350 cows wearing AfiCollar neck-tags) was collected. AfiCollar data measuring eating, rumination and panting time was processed and combined with basic cow data (DIM, lactation, gynecology status) and milk production. Machine learning methods were used to produce a model predicting weekly DMI which was trained on one research farm data (100 cows, 4500 weekly data points) and validated on the other four (250 cows, 12000 weeks). DMI predictions of the four validation farms were compared with the measured DMI, the R² and RMSE were 0.76 and 1.83 kg, respectively. To examine the heritability of FE, defined as energy-corrected milk/predicted DMI, data was collected from five Israeli commercial farms. Average FE of each cow between 100 to 200 DIM was calculated. FE of dams (n=280) was correlated with that of their offsprings (n=340), R² was 0.27 (p<0.0001). FE from 145 offsprings of 25 bulls with at least 5 offsprings, was collected, and the bull effect was determined significant using a F-test (p=0.01). These results demonstrate that a collar-based algorithm can accurately predict individual DMI. Moreover, the correlation between maternal and offspring FE suggests that using FE data to support breeding decisions should improve offspring FE and produce a more efficient and sustainable dairy herd.

Optimising foraging theory: does it apply to housed *Bos taurus* fed a total mixed ration?

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Optimal foraging theory suggests that animals prioritise high-quality feed components, leading to temporal variation in feeding behaviour post-feed delivery. This study examines whether feeding duration declines over time following a feed supply event in housed cattle (*Bos taurus*) consuming a total mixed ration (TMR). We hypothesised that feeding duration would be highest in the initial hours post-feed delivery as cattle selectively consume preferred feed components, declining thereafter as feed quality diminishes. Data were collected from five British Blue heifers weighting $368.6\text{kg} \pm 27.1\text{kg}$. Feeding behaviour was obtained using machine learning-based behavioural tracking synchronised with feed supply times. The two feeding supply events were identified as follows: on April 19, 2023, from 16:04:47 to 16:06:36, during which 18.9 kg of feed was added to the GrowSafe feeder and April 20, 2023, from 10:22:48 to 10:24:03, during which 179.4 kg of feed was added. Feeding duration was assessed hourly for six hours following two feed supply events to determine changes in feeding activity. Linear mixed-effects models (LMMs) were applied to evaluate feeding duration trends over time, with individual animal variation included as a random effect. Square-root transformations were applied to feeding duration data to improve normality. Model fit was assessed using marginal (fixed effects) and conditional (fixed + random effects) R^2 values. In the first feed supply event, feeding duration significantly increased in the third hour post-feed delivery ($p = 0.0188$) but showed no significant trend in later hours. In the second feed supply event, feeding duration exhibited no significant hourly variation. Conditional R^2 values (41.1% for event 1, 34.3% for event 2) indicate that individual variation strongly influenced feeding behaviour. Contrary to expectations, feeding duration did not consistently decline over time, suggesting that feed depletion may not be the primary driver of feeding patterns. These findings highlight the complexity of foraging behaviour in housed cattle and emphasise the need to consider individual feeding strategies when modelling TMR intake dynamics.

Session 13

Theatre 7

Feed efficiency and feeding patterns in growing pigs

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Understanding how feeding patterns are linked with productive traits provides innovative venues to prompt swine industry efficiency and profitability. On-farm automatic feeding systems allow to assess the feeding behaviour under commercial housing densities. The study objective was to describe the feeding patterns of different commercial crossbreed groups as well as their link with production efficiency traits. A total of 620 pigs from 8 different crossbreed types were raised in four trials from 80 to 165 days of age at IRTA pig experimental farm. Each pen was equipped with one automatic feeder for 12-14 pigs. Productive and behavioural traits were calculated for each animal over two periods: grower (105-125 days old) and finisher (145-165 days old). Mixed models, residual correlations, and multiple regression analyses were employed for data analyses. Among feeding profiles described for each group, two patterns appeared clearly differentiated, defined by: 1) numerous and short feeder visits at low feeding rate (g/min) (picky eaters), and 2) long and plentiful feeder visits at fast feeding rate (eager eaters). Pigs classified as picky eaters belonged to the lightest and leanest crossbreed group, while eager eaters were from the two heaviest genetic groups showing low feed conversion rates. The studied feeding behavioural traits showed associations between them: the number of daily visits to feeder negatively correlated with both average visit duration ($r = -0.68$) and feed intake per visit ($r = -0.70$), whereas feeding rate showed weak associations with the rest of traits. Looking at their relationship with production performance traits, feeding rate and feed intake per visit associated the most with body weight, daily growth rate and daily feed intake. Multiple regression analyses reported that these two variables jointly explained up to 20% of average daily feed intake variability and 14% of the body weight (at a fixed age) variability. These results highlight the tight link between feeding patterns and feeding motivation traits with both performance and feed efficiency traits, as well as its usefulness at characterizing pig performance during growing.

The use of Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy to determine chemical composition, apparent ileal nutrient digestibility, and digestible nutrient content of broiler diets

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Infrared spectroscopy has the potential as a tool for measuring not only nutrient composition but also nutritional value. The application of using Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS) in broiler diets remains largely unexplored. Therefore, we aimed to determine the gross chemical composition, coefficient of apparent ileal digestibility (CAID) of nitrogen (N) and energy, as well as the ileal digestible contents (IDC) of N and energy of broiler diets using ATR-FT MIRS. Ileal digestibility values were measured in two experiments with 56 and 20 diets, respectively. Mid-infrared (MIR) spectra of the 76 diets were collected using a Thermofisher Scientific Nicolet iS5 Spectrometer at MIR range of 4000-400 cm⁻¹ with 32 scans and at 2 cm⁻¹ resolution. Data were analysed using principal component analysis (PCA) and partial least squares regression (PLSR) for the development of calibration models. Prediction accuracy and performance were evaluated by coefficient of determination of prediction (R_{2p}) and relative performance deviation (RPD). Prediction was excellent for the gross chemical composition in terms of R₂ and RPD. Best models were generated for gross chemical composition of N, ash, dry matter, calcium, phosphorus, and organic matter (R_{2p} > 0.70 and RPD > 3.0). The models for gross energy and fat were inadequate for prediction (RPD < 2.0). The calibration model for CAID of N could be employed for screening purposes (R_{2p} < 0.56 and RPD < 2.29), whereas the calibration model for CAID of energy was unsuitable for prediction (R_{2p} = 0.48, RPD = 1.98). Prediction for IDC of N demonstrated accuracy (R_{2p} > 0.70, RPD > 3.0), while prediction for IDC of energy was poor and inappropriate for reliable forecasting (R_{2p} = 0.25, RPD = 1.72). Overall, the findings of this study indicate that ATR-FT MIRS could benefit the poultry industry as cheap method to determine the chemical composition of broiler feeds. However, further enhancements in calibration for CAID and IDC of nutrients are needed.

Deep Learning for Advancing Animal Breeding - A Study on Austrian Fleckvieh Cattle

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This study explores the application of deep learning models for predicting genomic breeding values using genotypic and pedigree data from Austrian Fleckvieh cattle. We investigate transformer-encoder and graph neural network (GNN)-based architectures to enhance predictive accuracy and assess their performance against traditional approaches like Single-Step Genomic Best Linear Unbiased Prediction (ssGBLUP) and machine learning methods such as XGBoost. Model performance was evaluated using mean squared error (MSE), Δ^2 score, and Pearson correlation, with regression and residual plots providing further insights. Results indicate that transformer-based models, particularly one utilizing a Hyena operator as a substitute for standard attention within a transformer, achieved the highest predictive accuracy, outperforming all other models across multiple metrics. Specifically, transformer-based models achieved the lowest MSE and highest R² score and Pearson correlation, while GNN and XGBoost models exhibited comparatively lower performance. The ssGBLUP benchmark model demonstrated competitive results but was outperformed by both transformer-based approaches in most metrics. Furthermore, an interpretability analysis using integrated gradients revealed associations between single nucleotide polymorphism (SNP) attributions and quantitative trait loci, highlighting the models' ability to map loci. This work contributes to advancing deep learning applications in genomic selection and underscores the potential of transformer-based models for handling high-dimensional genomic data effectively.

Seasonal Variability of Livestock Methane Emissions in the U.S.: A Comparison of Bottom-Up and Top-Down Estimates

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Methane (CH₄) emissions from livestock significantly contribute to agricultural greenhouse gas emissions, with seasonal variations influenced by ambient temperature, management practices, and manure handling. This study estimates county-level monthly CH₄ emissions from enteric fermentation and manure management in selected U.S. regions for 2020, following the U.S. Environmental Protection Agency (USEPA) methodology. Estimates integrate livestock data from the 2017 Census of Agriculture with climate data from the National Oceanic and Atmospheric Administration (NOAA). Comparison with USEPA's state-level inventories revealed notable differences. Enteric CH₄ emissions were, on average, 5% lower for dairy and 14% lower for beef cows, but 66% higher for other cattle categories. Manure CH₄ emissions showed even greater discrepancies, particularly for poultry and swine, averaging 66% higher than USEPA estimates. Bottom-up (BU) estimates from the current analysis were further compared with top-down (TD) inversion results using high-resolution regional modeling and NOAA's ground- and airborne-based CH₄ measurements. Three high-livestock-density regions were selected. The best agreement was observed in the Idaho, Nevada, and Oregon region, where TD estimates ranged from 0.02 to 0.08 Tg CH₄/month, while BU estimates were 0.03-0.04 Tg CH₄/month. Both approaches exhibited seasonal patterns, with stronger agreement in summer. Our results emphasize the need for seasonally resolved CH₄ inventories based on temperature-driven CH₄ conversion factors and livestock-specific characteristics. The comparison of BU and TD estimates in key livestock regions highlights potential biases in inventory methodologies, underscoring the need for further refinement in national CH₄ emission assessments.

Is a closer look always better? Evaluating scale and resolution in animal tracking studies

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Global positioning systems and remote sensing are available for some decades. Today, animal tracking and sub-sequential inference using spatial dataset has become standard in both grazing experiments and real-world farming. Over the years, energy efficiency and temporal frequency of tracking devices, the grain size of spatial data as well as computing techniques for their analysis have greatly improved. As technical restriction is falling as a limit of resolution, the question arises, which spatial and temporal resolution should ideally be used for animal tracking. Does a higher resolution necessarily provide better answers to the questions we are asking? What is the appropriate scale for the phenomena of interest and the uncertainty associated to them? We answer these questions using two state-of-the-art statistical models applied to tracking data from a grazing experiment with suckler cows on heterogeneous mountain rangelands. The first model is a log Gaussian Cox process (LGCP), which describes the observed distribution of steps in space and time. The second is a recently developed Gaussian field integrated step selection analysis (GF-iSSA), which evaluates properties of the selected location in each timestep given the area available to the animal in that timestep. To investigate effects of data resolution, we resampled the movement tracks to frequencies between 20 sec and 1 hour. Environmental covariates terrain slope and vegetation quality were resampled to grain sizes of 2, 10 and 20 meters. We found that the LGCP models were largely unaffected by temporal resolution, which was more important for the GF-iSSA models. Spatial resolution of covariance was important for both models and using too coarse grain drastically changed some of the estimated effects. We conclude that sampling effort should rather go into high-resolution co-variables than in high-resolution tracking data.

Assessing spatial sampling of PLF data – checks towards resolving power and sensitivity in pasture mapping
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Industrial PLF data are often not only approximate but also tend towards price-driven scarcity and irregular sampling over time. This holds true in particular for remote pastoral systems, where connectivity and energy supply impose additional acquisition limits. Yet, localization datasets are recognised as highly valuable primary information source for the agricultural stakeholder groups of farmers, consumers, and public administrations, because of their potential to support management, buying, and governance guidance. Such guidance can take several forms, e.g. indicators for space use for labelling purposes and compensatory payments; documentation for compliance with deforestation; animal welfare indicators; and direct warning and surveillance services. Accordingly, validation and assessment of spatial distributions reconstructed from affordable datasets, in terms of reproducibility and robustness for stakeholder guidance, remain important ongoing efforts. Which spatial distinctions can potentially be supported with a given, real-world data set? Can data from such devices, tracking a higher number of animals less often, support the same inferences as research devices on key individuals? As a contribution to comparability and validation, we put forward a potential adaptation of cross-disciplinary approaches to scaling, sensitivity and resolving power. Given data characteristic of either device and acquisition type, we test input recovery, investigate sensitivity, and approximate scales at which targeted distinctions appear to be supported. We qualitatively address effects of data normalization for stocking rate, effective sampling rate, as well as a priori variance estimates. To support and illustrate these considerations, we compare data from three summering pastures in the Swiss Alps, where herds were tracked with independent systems and sampling strategies simultaneously: low-frequency devices on tens of animals, and high-frequency devices on 2-3 animals.

Session 13

Poster 13

Assessment of individual expected lactation curves as indicator of resilience in dairy cows

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Comparing observed vs. expected production throughout lactation using automated milking system data provides a valuable framework for detecting perturbations and assessing resilience indicators. However, determining the expected production of individual cows from observed data remains a challenge. This study compares parametric and semiparametric models in Spanish Holstein cattle herds to refine resilience evaluation by assessing predictive performance and phenotypic correlations with productive traits. The study included 6.2 million milk records from 14,214 cows across 126 herds. Lactation curves were modelled using: (1) Quantile regression (QR, $\tau = 0.7$, fourth-order polynomial) and (2) A third-degree polynomial mixed-effects model (MM) accounting for systematic effects (herd-year-season, lactation-age, age-season, days in milk) on production and random individual curves. Perturbation points were removed to perform a 5-fold cross-validation to assess predictive accuracy of points defining the expected curve based on RMSE and correlation (r). The logarithm of the variance of milk expected from observed deviations (LnVar) and autocorrelations (ρ) were estimated as resilience parameters, and phenotypic correlations were evaluated with productive traits. The MM model showed lower accuracy (RMSE = 4.4, $r = 0.9$) compared to QR (RMSE = 2.5, $r = 0.94$), suggesting that QR better captures individual variability in expected production. LnVar correlated positively with milk yield (~ 0.5) and SCC (~ 0.19) but negatively with protein (~ 0.12) and lactose (~ 0.10) percentages, revealing that cows with higher milk yield exhibit weaker resilience. Results for ρ showed weak correlations overall. These findings highlight the importance of model choice in resilience assessment. QR offered superior accuracy, while MM accounted for systematic effects, providing biologically meaningful information. Additionally, resilience measures suggest that higher milk yield is associated with greater daily variability, which may reflect a metabolic trade-off, prioritising milk output. The project was funded by the R+D+i project TED2021-130759B-C31, MCIN/ AEI/10.13039/501100011033/ and by the European Union NextGenerationEU/PRTR.

Inter-quarter variability of milk β -hydroxybutyrate content in dairy cowsA. Hamon¹, K. Okoye¹, C. Krug², S. Dufour³, J. Guinard-Flament¹¹ PEGASE, INRAE Institut Agro, 65 rue de Saint-Brieuc, 35042 Rennes, France, ² Santé Publique France, 12 rue du Val d'Osne, 94410 Saint-Maurice, France, ³ Faculté de médecine vétérinaire, Université de Montréal, 3200 Sicotte, J2S 2M2 St-Hyacinthe, Canada

Milk β -hydroxybutyrate content (BHB) is commonly used to diagnose the risk of ketosis in dairy cows. The results of a recent study suggest the existence of a within-udder variability in milk BHB contents between quarters. So we aimed to study BHB inter-quarter variability in dairy cows in early lactation by comparing it to other milk components and to blood BHB. The analyzed data are from a previous experiment carried out on 13 commercial dairy farms in Quebec (CA) and performed on multiparous cows at wk 2 and 3 of lactation. The dataset consisted of 5,072 cisternal milk samples from each of the four quarters of 726 cows. Cows were sampled once or twice ($n = 184$ and 542 , respectively). Blood BHB analysis were assessed on the same day and time, immediately after collection. Cows were classified for blood BHB as blood BHB- (< 1.2 mmol/L) or blood BHB+ (≥ 1.2 mmol/L) and classified according to their number of quarters with elevated BHB (≥ 0.15 mmol/L) : 0Q, 1Q, 2Q, 3Q, 4Q. Milk BHB averaged 0.14 mmol/L ($SD = 0.11$, $min = 0$, $max = 2.12$ mmol/L). The coefficients of variation (CV) for milk components were calculated by dividing the SD of the four udder quarters of a cow by the mean of those four quarters. The highest mean of CV was that of SCC, at 77%, followed by milk BHB content (20%), milk fat and urea content (18%), and milk protein and lactose content (2.6%). Out of 726 cows, 176 were categorized blood BHB+ once and 109 cows twice. The majority of cows (58%) with blood BHB- presented no udder quarters (0Q) with elevated milk BHB. 14, 7, 8, and 13% of blood BHB- cows had, respectively, 1Q, 2Q, 3Q, and 4Q with milk BHB above 0.15 mmol/L. In cases of blood BHB+ cows, the majority of cows (74%) exhibited elevated milk BHB either in all four quarters (4Q) (46%) or in none (0Q) (29%) whatever the DIM. In conclusion, this study reveals inter-quarter variability of milk BHB levels underscoring the importance of accounting for this variability and its causes when using milk-based diagnostics.

Session 13

Poster 15

Integrating pedigree data into sow replacement model

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The sow replacement problem is today one of the most important challenges in sow herd management. Decision-making is currently based on a Markov decision process that comprehensive estimate characteristics of individual sows, e.g. parity, litter size and conception. Pedigree data plays a crucial role in animal genetics and breeding by providing information on genetic relationships among individuals, thereby enabling the accurate estimation of breeding values. However, traditional sow culling models neglect the pedigree information in biological models, resulting in inaccurate estimation of genetic values and reduced decision effectiveness. This study addresses this gap by integrating pedigree data into a sow replacement model, applying the Best Linear Unbiased Prediction (BLUP) method to enhance the accuracy of estimated breeding values (EBVs) for litter size. Our results demonstrate an 8% improvement on average in the precision of genetic evaluations of litter size from six pig farms, enabling more informed culling and replacement strategies. By bridging the disconnect between pedigree-based genetic assessment and herd management, our approach offers a practical pathway to maximize the long-term profitability of pig farms

Using Jupyter, Docker and Git for scientist group workflow in GESTE, a collective manure management model development.

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IFIP and INRAE have codeveloped a model called GESTE to implement and assess collective manure management at territory scale. This work has required a multidisciplinary approach with several engineers and researchers daily involved in its development. The model was implemented in Python language and Geopandas package was used to manage geographical data about farms and manure spreading plots. The partners involved faced difficulties to share and run the different versions of the model on each individual computer. Indeed, despite the massive development of communication technologies inside companies, the collaboration for coding remains difficult due to versioning troubles, compatibility between environments and software licenses. The first difficulty was due to package versions. To solve this issue, the model was containerized in a Docker container with defined Python, and its associated packages versions. The second difficulty was to define the code editor usage. Jupyter was chosen as an integrated development environment, easier to run into a container because it's a web-based software (running into web browser and no specific graphical user interface need). Thanks to this setting, everyone was able to work on the project, whichever its computer environment. The last difficulty managed was due to the long development process, which lasted two years and involved several people, this process led to multiple versions. The solution has been to create a Git repository for the management of model versioning. Finally, this developed workflow has enabled the GESTE project to move forward with efficiency with the combination of: the use of Python language inside Jupyter interactive development environment, its hosting inside a Docker containerization, and the management of versioning into a Git repository. This workflow facilitates group coding, even between different structures, which facilitating collaborative research projects.

Prediction of feed nitrogen and energy digestibility in pigs using fecal based NIR spectroscopy

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Feed efficiency measured as gain per unit of feed has been included in all pig breeding programs for decades. This measure of feed efficiency includes both the metabolic efficiency of directing absorbed nutrients to a desired expression of life, i.e. growth; and the ability of the pigs to digest and absorb as many nutrients from the feed as possible. Here we try to isolate the latter, i.e. the digestibility from the compound feed efficiency. In this study, fecal samples were collected from 28 digestibility trials with pigs with a large between-diet variability in digestibility. The fecal samples were subjected to chemical analysis, and phenotypes recorded were digestibility of organic matter (DOM; n=180), nitrogen (DN; n=202) and energy (DE; n=180). NIR spectra were recorded on the same fecal samples. NIR based prediction models using a 10-fold cross validation were developed in R using the R libraries Prospector and PLS. Preliminary results show that prediction model worked for DOM (R²: 0.79, Standard error cross validation (SECV): 0.14, Standard error of prediction (SEP): 2.62), DN (R²: 0.85, SECV: 0.29, SEP: 10.58), and DE (R²: 0.79, SECV: 0.15, SEP: 3.03). The results indicate that fecal based NIR spectroscopy could be a possibility to select pigs with improved digestibility of nutrients in pig breeding.

Livestock farming systems in next generations: can we imagine the future? Introduction

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It is clear that livestock production plays an essential role in circular agri-food systems. However, livestock farming needs to operate under a lower use of resources, and minimise its environmental impact. There are no 'one size fits all' perfect solutions to improve livestock systems, instead all livestock systems must move along pathways of progress by adopting a systemic approach which combines (i) innovations and science-based solutions and (ii) transition to more sustainable systems and value chains, supported by fair, inclusive and innovative governance. We would like to recall that in the last two years we have been addressing what do we mean by sustainable livestock systems, and in 2024 we were looking at more than food, in alignment with our vision paper. We think it is interesting to move towards a future vision of livestock farming systems in EU: where and how should livestock systems adapt to continue and fulfil their essential role while ensuring an equilibrium with resources. The theme should cover all the species and involve all the actors including farmers, farming related activities and suppliers, the by-product valorisation sector and the socio-economic aspects in enrolling in a collaborative discussion approach on where and how we see livestock farming systems in 2040, 2050, 2060. What will be the role of livestock in the next generations? How will farming systems evolve to ensure sustainability? Are we capable of imagining or designing the future livestock farming systems? What tools can we use to do so? We propose an integrated and multifactorial approach based on three potential Pathways of Progress for livestock that should be investigated simultaneously and with an integrated view: circularity, diversity and efficiency. In this theme the socio/economical aspects are an essential base and transversal area. Livelihood and economic growth aspects linked to livestock farming systems need to be addressed and developed.

Session 14

Theatre 2

Challenge voice – Thinking the future food systems

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Feeding a growing global population within planetary boundaries requires transformative approaches to redesigning food systems. Two key strategies — circularity (optimizing natural resource use) and the protein transition (reducing animal-based food) — are widely proposed for their environmental benefits. Using a food system modeling approach, we found that minimizing environmental impacts significantly reduces livestock numbers. However, livestock remains essential at lower levels for upcycling low-cost biomass (e.g., grass, by-products, and food waste) and ensuring nutrient-adequate diets. Regional differences are substantial, making location-based solutions critical for achieving the optimal balance between animal and plant-based foods. Adopting these circularity and protein transition strategies can greatly reduce agricultural land use and greenhouse gas emissions. Envisioning future livestock farming systems necessitates a holistic approach to ensure planetary health and adequate diets at a food system level.

The Future of EU Agriculture and Food: Livestock Sector Perspectives

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The European Commission's Vision for Agriculture and Food, released on 19 February 2025, outlines an ambitious roadmap for a competitive, resilient, and sustainable agri-food system. This Vision recognizes the essential role of the livestock sector in Europe's agricultural landscape, reinforcing its importance in achieving food security, economic stability, and environmental sustainability. FEFAC welcomes the Commission's emphasis on reducing dependency on imported feed proteins and enhancing the EU's feed autonomy. Achieving this goal requires a multifaceted approach, including setting realistic targets for homegrown protein sources, investing in novel feed ingredients, and fostering circular feed solutions. Expanding the use of processed animal proteins, insect-based proteins, algae, and synthetic amino acids will contribute to more balanced and environmentally friendly livestock diets while optimizing resource efficiency. Ensuring the long-term sustainability and competitiveness of the livestock and feed sector demands regulatory simplification, strategic investment, and a robust circular economy framework. Streamlining approval processes for innovative feed additives and promoting targeted sustainability incentives within the Common Agricultural Policy (CAP) will facilitate the adoption of greener practices. Additionally, an EU-wide biomass strategy is crucial to supporting circular economy principles and improving nutrient efficiency. To secure a fair standard of living for farmers, it is imperative to level the playing field across bio-economy sectors and ensure that biomass resources are efficiently allocated between food, feed, and energy. The EU must also enhance its manufacturing capacity for essential feed additives, reducing reliance on third-country suppliers and strengthening strategic supply chains. As a member of the European Board for Agriculture & Food (EBAF), FEFAC President Pedro Cordero will continue advocating for policies that bolster the European compound feed sector's role in sustainable livestock production. By embracing innovation, circular feed systems, and resilient supply chains, the EU can create a future-oriented agri-food system that balances economic viability with environmental responsibility.

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Theatre 4

Towards the design of a Safe and Just Operating Space for European livestock systems

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Transitioning livestock production systems towards an acceptable level of sustainability requires to identify, elaborate, assess and quantify conditions that lead to more sustainable systems. Within the context of the Horizon Europe funded STEP UP project, our aim is to embrace the concept of Safe and Just Operating Space (SJOS) to further assess scenarios related to livestock systems. The SJOS model integrates biophysical earth system tipping points, hence corresponding to thresholds at which small changes may lead to amplifying effects, with social considerations of distributional equity and justice. SJOS provides a powerful conceptual frame that combines dimensions that specify, on the one hand, limits regarding the planetary biophysical boundaries (safe operating space) and, on the other hand, limits that specify socio-economic needs that must be respected (just operating space). Initially developed for global implementation, it was later adapted for regional and national contexts and is now being explored for sector-specific applications, including agriculture. Here, we present the first steps in designing an SJOS for EU livestock. The framework here aims at integrating sustainability principles into livestock management by addressing environmental, economic, and social aspects while ensuring alignment with the EU policy objectives. We include dimensions, variables and indicators for both the Safe and Just spaces, with perspectives on threshold values and quantitative assessment of the chosen indicators.

How animal genetics can help to shape the future of Livestock Farming Systems

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The advancement of animal genetics plays a crucial role in enhancing sustainability across diverse livestock systems. This presentation examines the trajectory of genetic progress by integrating three key sustainability pathways identified in the Animal Task Force (ATF) Strategic Research and Innovation Agenda (SRIA): efficiency, diversity, and circularity. These pathways align with the three pillars of sustainability—economic, environmental, and social—offering a holistic perspective on the future of livestock breeding. Efficiency in animal genetics contributes to reducing the environmental footprint of livestock production while improving productivity and economic viability. Breeding advancements in both conventional and novel traits will be discussed, highlighting their role in mitigating climate change impacts. Circularity within livestock systems can be enhanced through genetic selection, supporting resource efficiency and waste reduction. The importance of genetic diversity is emphasized as a means to ensure resilience in breeding populations and adaptability to local farming systems. Additionally, the implications of classical and genomic inbreeding on long-term sustainability will be explored. By addressing these interconnected themes, this presentation provides insights into the role of animal genetics in shaping a more sustainable and resilient livestock sector. The discussion will highlight species-specific breeding goals and practical applications, emphasizing how genetic innovations can drive future sustainability in global livestock production.

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Theatre 6

How nutrition can help to shape the future of Livestock Farming Systems

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The extensive research in nutrition in European livestock farming systems has greatly contributed to enhance their sustainability; however, the new societal demands, the effects of climate change and the unstable geopolitical context require co-developing new vision and approaches with relevant stakeholders. While feeding (feeds production and digestion) has a major contribution to the environmental impact of livestock systems, opportunities arise to develop science-based solutions to align with the new pathways of progress to sustainability: efficiency, circularity and diversity. This work will present the main efforts developed by different European projects (Re-Livestock, Step-Up, Pathways, HoloRuminant) and other international initiatives towards those pathways. Efficiency: Optimization of the livestock production systems through improvement of the feed conversion ratio is critical but needs to be extended to the concept of environmental efficiency. This requires a stronger collaboration across agronomy in feed production, animal nutrition and environmental assessment to fully capture the improvements. The collaboration with the Global Feed LCA Institute is a good example of joined efforts. Feed additives to reduce enteric methane emissions is a reality nowadays but still faces important challenges to extend their use: effective use of excess rumen hydrogen when methanogenesis is inhibited, accurate accounting in inventories for the reduction in emissions, full understanding of the registration and regulation frameworks and careful consideration of the communication strategies to avoid misinterpretation. Circularity: the ability of livestock animals to make use of biomass (i.e. food industry by-products as feed resources) has been largely demonstrated. However, the food processing industry is constantly evolving and generates new materials that need to be evaluated. Further efforts are being made to accurately quantify the reductions in GHG emissions associated to the use of by-products. Diversity needs to be considered at different levels. A critical aspect is to account for the future climate scenarios in different geographic regions and systems to deploy strategic vision of future feeding resources and practices that specifically fit into those climate projections (i.e. grasslands management).

Future of Livestock Farming Systems – Health

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One of the main challenges for the European livestock industry is animal health. Animal diseases and antimicrobial resistance can threaten human health. Animal Task Force (ATF) is prioritizing responsible livestock in sustainable agri-food systems while ensuring high health and welfare standards for livestock and providing better human health and well-being. Animal health is closely related to human health and the links have contributed to putting forward the concept of One Health. This presentation will address some of the subjects related to animal health and welfare with a focus on the production of pigs. Pig production is one of the main users of antibiotics, and this enhances the risk of mitigating antimicrobial resistance. After the EU-ban (in year 2022) on the use of medical zinc oxide, the usage of antibiotics to treat diarrhea in pigs post weaning has increased. This calls for alternatives to the use of antibiotics, and research in the management of immunity and microbiota to enhance pigs' health is therefore on the ATF list of research and innovation topics. There is also potential in feeding and breeding strategies that support pig robustness towards infections and diseases. In pig gut health, improvements are expected from learning more on the interaction between the gut microbiome, nutrition and the host (pig) immunity, and this knowledge will force innovation in the development of e.g. plant bioactive compounds and other dietary additives to manipulate the microflora. In this context there is also great potential in studying the bidirectional "microbiome-intestine-brain" axis in relation to animal health, welfare and behavior. During the presentation some additional ATF suggested priorities in the research and innovation list will be addressed such as animal health in a systemic approach. Overall, ATF focuses on themes bringing added value at the European level compared with national approaches, and this concerns amongst other subjects of common interest such as animal health and welfare, which is highly important in the transition towards more sustainable livestock systems and agri-food chains.

Session 14

Theatre 8

Transforming livestock farming for sustainable development

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Like other economic sectors, livestock farming is affected by the multiple global crises which mankind faces: Climate, biodiversity, food security and societal demands on how animals are treated in agriculture require a holistic response of the livestock sector, taking into account the need of several actor groups. The climate crisis not only directly affects livestock and livestock keepers (heat stress, animal health, productivity etc.), it also endangers current feed supply chains. Comprehensive mitigation and adaptation strategies need to be developed and implemented. While destocking is argued for by several societal groups in order to mitigate the contribution of livestock farming to climate change and overall environmental impact, potential exists within the sector to reduce the climate and other environmental impacts of livestock farming. However, complex interactions between elements of livestock farming systems and external influencing factors need to be taken into account. Current feed supply chains are directly linked to human food security. The proportion of feed components which could be directly consumed by humans needs to be reduced. While concepts are available in order to overcome this challenge, their implementation would affect the structure and economics of livestock production. Biodiversity is impacted by livestock farming, as genetic diversity, species richness and diversity of ecosystems are affected particularly by intensive, highly productive livestock systems. Adaptation strategies need to take into account the relationship between the climate and the biodiversity crisis, among others. These challenges, together with the societal demand for high animal welfare status, call for a transformation of the livestock sector. Transformative efforts need to be undertaken not only by farmers and other actors of livestock production, but society as a whole.

The farmers' perspective on the future of livestock farming systems

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CEJA represents a network of 33 young farmers' organisations from across Europe, focusing on ensuring good living/working conditions for the younger generation setting up in the sector. As outlined in the Vision for Agriculture and Food, a key challenge for the agricultural sector is generational renewal. In 2020, the average age of EU farmers was 57 and only 6.5% of them were below the age of 35 (Eurostat, 2022). This trend has significant consequences for the dynamism of rural territories, the capacity to produce food, the preservation of regional identities & landscapes, the implementation of sustainability objectives. Young people willing to set-up an activity in agriculture face significant barriers to entrepreneurship, including limited access to land, complexified access to credit & investment, inadequacy of educational & training programmes. They are also exposed to greater risks & uncertainties from climate change, emerging pests & diseases, & low incomes in a context of highly volatile & unpredictable markets. Adding up to these, working & living in rural areas can be isolating. Livestock farming is an indispensable component of European agriculture – whether it is in terms of fertilisation & circularity, valorisation of resources, food security, biodiversity, rural livelihoods, cultural heritage, dietary needs, climate mitigation, meeting demand for animal products, etc. European young farmers expect a vision for the future of livestock farming that: i) Embraces the technical complexity surrounding the positive & negative externalities of animal farming, in all its sectors, which can translate into contradicting sustainability objectives in some cases & create trade-offs complex to address at an individual farm level. ii) Considers the diversity of challenges across sectors, systems and territories, striking a balance between economic, environmental, social objectives & enablers. Livestock farming is a broad concept that entails many sub-sectors, production systems, pedoclimatic contexts, supply chains & infrastructures. iii) Gives real prospects to the younger generation, eager to uptake challenges, take care of animals, & be a big component of the future of livestock farming in Europe. This vision needs to be in line with the parallel reflections on an Action Plan for Generational Renewal.

Session 14

Theatre 10

Livestock farming systems in next generations: can we imagine the future? Discussion and closing remarks

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Since 2013, the ATF-EAAP Special Session during the EAAP Annual Meeting aims to bring together animal science with practice of animal production and connect researchers, policy makers, industry representatives and societal organisations. Every year, a different topic is addressed during this half-day session. Once again, and for the 5th time, the EAAP Livestock Farming Systems Study Commission and the ATF Special Session have joined. This year's theme is "Livestock farming systems in next generations: can we imagine the future?". Following the theatre presentations, a general round table discussion with participants and presenters (including SCAR Foresight representative) will provide more information on the theme, and input for future discussion. The discussion will continue in November in Brussels during the ATF seminar.

European Animal Husbandry's Synergies and Conflicts within Sustainable Food System Transformation

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European policy has oversimplified animal husbandry systems, which resulted in negative social and environmental sustainability impacts. Yet, animal husbandry systems are integral for sustainable food systems contributing to food security, livelihoods, while conserving environmental and social systems. We conducted multivariate analysis to investigate the potential synergies and conflicts of animal husbandry within food system transformation. Variables from the EUROSTAT databank indicative of aspects related to food system transformation were explored using principal component analysis. Data available at the regional (NUTS-2) level were included. Eleven variables related to regional animal husbandry systems, economic viability, equity, environment and social sustainability, as well as health and nutrition resulted in five principal components describing European regions. The components highlight potential trade-offs between e.g. economic viability and equity, as well as human health outcomes being dependent on the dominant animal husbandry system within a region. For example, permanent grassland coincides with decreased number of deaths attributed to diabetes (i.e. health and nutrition impacts), whereas regions with a positive increase in livestock units over the past decade correspond with a higher rate of deaths attributed to diabetes. In addition, there exist regions that are characterized by a decreased proportion of low input farms and an increased rate of a household's inability to afford a meal containing meat every second day. These results highlight the need for promoting a nuanced understanding and subsequent policy of animal husbandry for sustainable food system transformation.

Biochar modulates microbial nitrogen cycling populations during manure storage

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Manure is a significant contributor to ammonia (NH₃) and greenhouse gas (GHG) emissions, necessitating sustainable mitigation strategies. This study investigates the impact of biochar on microbial communities involved in nitrogen cycling, specifically ammonia-oxidizing bacteria (AOB) and denitrification-associated functional guilds (nirK, nirS, nosZI, nosZII). Additionally, Next-Generation Sequencing (NGS) of 16S rRNA genes was employed to assess microbial community shifts in response to biochar amendment. A 105-day field experiment was conducted using dairy manure stored in twelve large containers, with biochar applied to six containers while the remaining six served as controls. Periodic manure sampling and microbial analysis revealed that biochar significantly altered nitrogen-transforming microbial populations. NGS of 16S rRNA genes revealed shifts in overall microbial community composition, with biochar increasing microbial diversity and favoring taxa associated with nitrogen retention. These microbial changes are associated with N₂O and NH₃ emissions, demonstrating that biochar modulates nitrogen cycling pathways in stored manure.

Mitigating Ammonia and Greenhouse Gas Emissions from Manure Stockpiles: The Impact of Biochar on Emission Reduction

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Manure stockpiles are significant source of ammonia (NH₃) and greenhouse gas (GHG) emissions, contributing to environmental pollution and agricultural sustainability challenges. Strategies to mitigate these emissions are essential for reducing nitrogen losses, improving air quality, and minimizing the climate impact of livestock production. This study evaluates the effects of biochar on NH₃ and GHG emissions from dairy manure during a 105-day storage experiment under farm conditions. Twelve large containers were used to simulate manure piles, with biochar applied as a surface layer to six containers, while the remaining six served as controls. Results revealed that biochar significantly reduced N₂O and NH₃ emissions, suggesting improved nitrogen retention within the manure. The mitigation of NH₃ volatilization is particularly important, as it reduces nitrogen losses from the manure during storage. In contrast, CO₂ and CH₄ emissions remained largely unaffected, indicating that biochar did not substantially alter overall carbon cycling dynamics within the manure piles. Beyond gas emissions, biochar also influenced manure chemical properties, including pH, organic matter content, and nitrogen availability, which may have contributed to its emission-reducing effects. These chemical changes suggest that biochar alters microbial activity and nutrient dynamics, potentially enhancing manure stability and nutrient retention over time. Overall, these findings highlight biochar's potential as a scalable strategy for reducing nitrogen-related emissions from stored manure. Incorporating biochar into manure management practices could improve environmental sustainability by minimizing nitrogen losses, mitigating emissions, and enhancing the nutrient quality of manure for agricultural applications.

Session 14

Poster 14

Healthy Plants Make Healthy Animals: Applying the One Health Concept to Livestock Holobionts

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Microbiomes provide a wide range of functions that are essential to their associated hosts. If we consider the host and its associated microbiome as a single unit or holobiont, we can begin to appreciate the full genetic and functional diversity of farmed animals and herds. Holobionts interact in shared environments with the dispersal of microbes and their genetic potential occurring through feed, air, water, soil, and across species and individuals. A knowledge-based strategy for characterizing, preserving, and controlling holobiont diversity is critical for developing sustainable agricultural management strategies. The International Phytobiomes Alliance's Animal Microbiome Working Group is focusing on this area through a One Health lens, which emphasizes the interconnectedness of plants, animals, humans, and the environment. We see microbes, from pathogenic to beneficial, as key connectors of health among systems, and are particularly interested in understanding how soil and plant microbiomes impact animal microbiomes and health. Within this context, we are exploring the following questions: What are the main factors that shape livestock microbiomes? Do feed/grazing, and/or nearby environmental plant microbiomes impact the composition and function of animal microbiomes? How can we assess, secure, and store livestock holobiont genetic diversity? How can we identify environmental microbiome links with animal holobionts? How can we harness this knowledge to improve animal farming practices?

Mitigating Ammonia Emissions from Dairy Manure: Evaluating the Impact of Biochar Under Real Farm Conditions

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Ammonia (NH₃) and greenhouse gas (GHG) emissions from dairy manure contribute significantly to environmental pollution and nitrogen losses in agricultural systems. This study evaluates the effectiveness of biochar amendment in reducing NH₃ and GHG emissions under real farm conditions during the winter season. The experiment was conducted at a dairy farm in Cyprus using two identical groups of 24 Holstein cows. Manure from 12 cows was treated with 2.5% w/w (dry weight) biochar, while untreated manure served as the control. To simulate farm conditions, fresh manure was collected daily over a 10-day period and placed in twelve polystyrene containers (six biochar-treated, six control). The manure was replaced every 24 hours to reflect continuous manure production. Gas emissions were measured using automated eosAC chambers, while daily manure samples were analyzed for chemical and microbial composition. Results showed that NH₃ emissions were substantially reduced in the biochar-treated manure compared to the control, suggesting improved nitrogen retention. The impact of biochar on CO₂, CH₄, and N₂O emissions is currently being assessed, along with changes in manure chemistry and microbial communities. These findings will provide valuable insights into the mechanisms driving manure emissions and the potential of biochar as a practical mitigation strategy for dairy farms.

Dairy abandonment in Brazil and the typology of farms and rural producers

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Dairy abandonment in Brazil and the typology of farms and rural producers Abstract: The abandonment of rural production has been the focus of several studies. In Brazil, an important world food producer, there has been a reduction in the number of dairy farms. This can have economic, social and environmental consequences, especially for family dairy farmers, who represent the majority of dairy farmers in Brazil. In this study, we sought to analyze the social characteristics of dairy farmers and the typology of dairy farms most associated with the abandonment of milk production in Brazil. Semi-structured questionnaires were administered in the field to 125 dairy farmers located in the state of Paraná, Brazil. The data were subjected to descriptive analysis, exploratory factor analysis (EFA) and Spearman's correlation (rho). The EFA generated a construct representing the farmer's intention to exit milk production (EXIT construct). The correlation analysis of this construct with the social variables of the rural producers and the structural and productive variables of the farms showed that older producers, with lower production volumes and smaller properties are more likely to abandon the activity. Therefore, it is suggested that public and private policies should be defined that offer technical support to improve the quality of work and increase the volume of milk production, to mitigate the abandonment of dairy farming in Brazil. **Keywords:** Competitividade; Agricultura familiar; Sucessão familiar; Gestão da produção; Êxodo rural.

Understanding the decision to abandon autochthonous breed farming through the Decomposed Theory of Planned Behavior (DTPB): Preliminary Results from Castilla-La Mancha (Spain)

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The decline of rural populations and the progressive abandonment of autochthonous livestock breeds in Spain, along with the associated loss of ecosystem services, necessitate a comprehensive understanding of the behavioral mechanisms underlying farmers' decisions to cease breeding these populations. This knowledge is essential for designing effective policy interventions and strategic support mechanisms. In this study, a questionnaire was developed within the framework of the Decomposed Theory of Planned Behavior (DTPB) to assess the short-term intention to continue breeding among 131 farmers raising autochthonous ruminant breeds in Castilla-La Mancha, Spain. The survey incorporated behavioral variables linked to the decision-making process and factors influencing livestock production at both short- and long-term scales, including farm structure, farmer characteristics, and family-related aspects. A Structural Equation Modeling (SEM-PLS) approach was employed to estimate a model explaining approximately 60% of the variance in the intention to discontinue purebred breeding within the next five years. The findings indicate that livestock identity and economic attitude were positively and directly associated with the intention to abandon purebred breeding. Additionally, social norms, perceived adaptive capacity, and the farmer's perception of the sector's future indirectly influenced this decision. Furthermore, product differentiation through quality certification schemes and the adoption of short supply chains were identified as critical factors promoting the continuation of purebred breeding. These results provide empirical evidence to inform policy measures aimed at mitigating the risk of autochthonous breed abandonment and ensuring the sustainability of traditional livestock systems.

How farmers communicate with society about their job and livestock's practices

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The growing disconnect between animal farming and society has been exacerbated by media portrayals, leading to widespread misconceptions about farming practices. A student's project, part of the CASDAR ENTR'ACTES research project, aims to reestablish the link between livestock farmers and the public by examining the communication efforts of livestock farmers. The main objectives are to identify the profiles and motivations of farmers who engage in communication initiatives and to assess how these efforts influence public perceptions. A qualitative approach using semi-structured interviews with 15 farmers across France explored their communication methods and challenges. The sample covered diverse animal production—dairy, poultry, and pig farming—across various regions. The study's core hypothesis suggests that the type of farming, its practices, and the local context influence communication strategies. The findings reveal that farmers who engage in public communication share a strong commitment to transparency and education, aiming to demystify their work and address societal concerns (animal welfare, environmental impacts). They use a mix of direct channels (farm visits, events) and indirect channels (social media, websites) to reach diverse audiences, ranging from local consumers to urban residents. The motivations for communicating are diverse and often combined: to secure income or a market, to foster positive public perceptions and build trust with consumers. However, challenges such as time constraints, financial pressures, and the complexity of public controversies limit the scope and effectiveness of these initiatives. Results suggest that communication is most effective when farmers are open and sincere, discussing both the benefits and challenges of modern farming. Despite the obstacles, many farmers think it is essential to maintain good relations with their community and improve society's understanding of agriculture. This study will provide guidance for future farmers wishing to embark on this path.

Exploring the margins of livestock farming systems research: Livestock farms providing on-farm services

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The livestock sector is facing a multidimensional crisis (environmental, economic, societal) while also being challenged by increasing uncertainties related to climate change, ageing of rural population, and lack of attractiveness to renew the agricultural workforce. This situation calls for a major review of the current livestock model, which is characterized by strong specialization. In this context, the diversification of on-farm activities presents an alternative way to develop livestock farming systems. In the EU, diversified farms count for 5% of all farms, while in France they represent 30% of farms. Among these diversified farms, new forms of farms providing services on-farm are emerging (7.5% of farms): these combine livestock production with non-agricultural service activities, such as leisure, culture, tourism, education, and energy production. The development of these service activities could be a major strategy for transitioning to sustainable livestock farming systems i.e. more livable and viable for workers, and socially acceptable by society. However, the combination of livestock farming and service activities are rarely considered in terms of the changes it induces on the farm. Assuming that the integration of farming and service activities within the livestock farming system can take different forms depending on the objectives assigned to livestock production in relation to the service activity, the following questions arise: How does herd and land management change? Can livestock serve different functions depending on the service activity? How do farmers cope with tensions over the use of resources (land, equipment, etc.)? How do farmers organize work between the two activities? These questions rise concerns regarding the resilience of these emerging forms of diversified livestock systems. Therefore, developing methods and knowledge to better understand the function of livestock and the functioning of diversified farm is crucial for supporting these emerging livestock systems providing on-farm services.

Session 15

Theatre 1

Longevity - a traditional concept with a future?

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This contribution reviews different approaches to improve longevity, from the definition of the trait to its inclusion in breeding objectives, as well as aspects related to breeding and selection programmes at population and herd levels. Longevity is influenced by many factors, genetics, environment, but also different management practices on the farm and external factors. Younger cows may not only be in better health, but are also more likely to produce genetically superior offspring. Farm economy, consumer concerns and sustainability requirements favour cows with a long productive life. Changing production conditions and new ways of generating phenotypes are influencing the composition of future breeding goals. Comprehensive phenotyping capabilities allow the direct capture of novel traits that contribute to culling or survival in the herd, such as veterinary diagnoses. Technological developments and in-depth phenotyping are improving and generating new direct and indirect traits. Traits such as heat tolerance or resilience are gaining interest and importance alongside feed efficiency and methane production. The future role of the longevity trait in breeding will need to be reassessed as breeding objectives broaden. Approaches and results related to the inclusion of more direct traits in breeding objectives are highlighted and discussed. The relationship between longevity, health and other traits of interest are also considered. Longevity and animal health plays an important role in social and environmental sustainability but is also very important for the profitability at herd level. Results on the impact of longevity for different levels of production intensity on a comprehensive view of environmental indicators are presented for Austrian conditions. Mostly referring to data from Austrian cattle populations, this presentation also reviews the history of genetic evaluation for (functional) longevity, aspects of its inclusion in breeding objectives and breeding programmes, and non genetic aspects that are relevant for economic, environmental and social acceptance in breeding and herd management.

Critical transitions for dairy cows and calves: challenges and opportunities for dairy cow management

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Transition periods in a cow's life, including drying off, calving, start of lactation, birth and weaning, are associated with increased health risks, and compromised welfare and longevity. The ability of cows to adapt to these transitions defines their health. Adaptive capacity of animals can be enhanced through selective breeding, early-life interventions, or by providing the right environment. A long list of nutritional and management strategies exists to ease adaptation of cows to drying off or lactation or weaning in calves. More recently, the focus seems to shift towards reducing the magnitude, frequency or timing of transitions to improve animal health and welfare. For example, the frequency of calving events can be reduced by deliberately extending the lactation, herewith lowering the frequency of periods with increased health risks. Alternatively, shortening or omitting the dry period reduces the number and magnitude of peripartum transitions for the cow, with less milk and a better energy balance after calving. In calves, prolonged cow-calf contact is gaining interest to support natural behaviour, though optimal weaning time, weaning strategy and potential impacts on cow health remain uncertain. Shifts in animal management during transition periods are driven not only by new insights into animal health and welfare, but also by global challenges, like reducing dairy sector emissions or climate change. These challenges may influence the adaptive capacity of animals during transition periods too, impacting also health and welfare. Furthermore, the optimal transition management might vary between animals, leading to the development of customised management approaches using individual cow characteristics. Future dairy farming may integrate multiple strategies to enhance adaptation, health and welfare, weighing also impacts on economic returns, labour, work pleasure and environmental sustainability.

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Dietary supplementation with *Pinus taeda* hydrolyzed lignin in the peripartum period of dairy cows

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Previous studies have shown multiple benefits of dietary supplementation with hydrolyzed lignin extract from *Pinus taeda* (PTHL), supporting further investigation into its potential use in dairy cow diet. This study evaluated the effects of PTHL supplementation on oxidative stress biomarkers, immune responses, and milk and colostrum production during the peripartum period in dairy cows. Twenty Brown Swiss cows from a farm in Puglia, Italy, were parity-balanced assigned to a control group (C) or a PTHL-supplemented group (T) (35 g/day) from 60 days prepartum to 60 days postpartum. Blood samples were collected at -60, -30, -7, -3, and 0 days relative to calving, then at +3, +7, +30, +60 days during lactation. Colostrum was collected daily for five days postpartum, and milk biweekly up to 60 days. Preliminary data were tested for normal distribution and variance homogeneity using the Shapiro-Wilk test. Subsequently, data were analyzed using analysis of variance via the General Linear Model procedure, considering treatment and time as explanatory variables and their binary interaction. A Tukey test for repeated measures was conducted to assess differences between means over time, considering the animal as random effect. Preliminary results indicated that milk chemical composition, fatty acid profile, and oxidative status were not significantly affected by treatment or time ($P > 0.05$). However, the treatment had significant effects on colostrum oxidative status, and specifically on the absorbance values read at different wavelengths (A450, A495, A532) specific for lipid oxidation products. A450 values were higher in C group than in T group on postpartum Day 1 ($P < 0.05$), while A495 values were also higher in C group on postpartum Days 1 and 2 ($P < 0.05$). Additionally, A532 values were higher in C group than in the T group at postpartum Day 2 ($P < 0.05$). Although preliminary, these findings suggest that PTHL supplementation may improve the oxidative status of colostrum in dairy cows during the peripartum period.

Impact of Swiss Dairy Housing Systems and Feeding Management Practices on Cow Longevity

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Dairy cow longevity, defined as the length of a cow's productive lifespan, is a critical factor influencing economic viability, environmental sustainability, and animal welfare in the dairy sector. This study aimed to investigate whether differences in housing systems and feeding management decisions contribute to variations in longevity among Swiss dairy farms. A total of 142 Swiss dairy farms were selected based on their exceptional longevity profiles. Farms were categorized into clearly above average long and clearly below average short longevity profiles, while ensuring representation across different production zones, farming systems (organic/conventional), and breed. Survey data, herdbook records, and metabolic trait indicators were analyzed to identify significant differences between contrasting longevity profiles. Results indicate that farms with long longevity profiles had a significantly higher proportion of barns compliant with the animal friendly housing systems (BTS) program, which promotes loose housing and enhanced comfort. These farms also had a higher prevalence of lime-straw and straw-recycled mattresses compared to farms with shorter longevity profiles, which used significantly more rubber mats as bedding. Feeding strategies also varied between the two groups. Farms with long longevity profiles provided significantly more maize silage during winter and less protein concentrate in summer, suggesting a nutritional approach with lower risk of ruminal excess protein. The metabolic trait analysis showed that the proportion of test day records with an elevated fat-protein ratio (>1.5), an indicator of ketosis risk, was significantly higher in farms with longer-living cows. This is in line with previous findings showing an elevated ketosis risk in older cows. On our farms, however, this has no influence on longevity. Our findings highlight the importance of integrating housing and feeding strategies to enhance cow longevity. Policies such as the BTS program promote welfare-friendly housing systems that contribute to extended productive lifespans, improving farm efficiency and sustainability. Continued research is needed on the interaction between further farm management decisions and dairy cow longevity.

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Theatre 5

Effect of dry period length on the successive lactation

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The dry period (DP) is an essential physiological phase for dairy cows. During this stage, the cows recover from the previous lactation and prepare for the next lactation. A well-managed DP ensures a good recovery of the mammary gland and a reduced risk of infections. The length of DP is associated to calving intervals: the longer the DP, the longer the calving intervals, the less calvings during productive life. Moreover, the length of DP can affect the productive performances of the next lactation and, thus, the culling decisions. The aims of this work were to evaluate the effects of DP length on the performances of the successive lactations in Italian Holstein and to analyze the relationship between parity and DP length. Parity up to 10, DP between 30-365 days, and milk, fat, and protein yields for 305-days lactations were considered: after the editing, 154,500 records were analyzed. Three classes of DP were created: short (< 50 days), conventional (50-65), and long (> 65). Phenotypic records were analyzed with a mixed linear model with farm and animal as random effects, and parity, DP class, and year as fixed. All fixed effects were highly significant ($P < 0.001$). The farm effect explained 38%, 41%, and 41% for milk, fat, and protein yield, respectively. Estimated means for milk were 8,830C for short, 9,140A for conventional, and 9,120B for long DP. Also for fat, the largest estimated mean was found for the conventional DP: 322C, 332A, and 330B for short, conventional, and long, respectively. The estimated means for protein yield were 291A for conventional, 287B for long, and 285C for short. Moreover, a low-moderate association between DP length and parity was observed ($X^2 = 3335$, $P < 0.0001$; CramerV = 0.20). Older cows had longer and less variable DP: 20% of 2-parity and 45% of 10-parity cows had long DP. Results of the present study confirm the importance of DP in affecting future dairy performances and the conventional DP length was found to be the best option. This study was supported by the PRIN project "BigFit" (Finanziato dall'Unione europea-Next Generation EU, Missione 4 Componente 1, CUP D53D23011860006).

Association between growth profiles of Holstein heifers and their longevity

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In many studies, early calving is more profitable than late calving in Holstein dairy heifers. However, with a first calving at 24 months old, the growth profile might influence subsequent performance and longevity. The association between Holstein heifers' growth profiles and their future longevity was analyzed in present study. Data originated from 3 French experimental farms and included heifers born from 2000 until 2019. Only animals firstly inseminated before 19 months of age and weighed at least 5 times from birth to 19 months of age were kept in the analyses. Growth profiles were then determined on 1217 Holstein heifers. Insemination and successive calving dates, 305 days milk yield, 305 days fat plus protein and culling date were available. Growth profile groups between birth and 365 d of age were established using a principal component analysis (PCA) on 6 variables: body weight (BW) at birth, at 70 d, at 120 d, at 180 d, at 275 d and at 365 d of age. Then, a hierarchical ascendant classification was performed on the axes 1 to 3 of the PCA. Four types of growth profiles were identified. Heifers in the slow-growth profile had a later age at first calving and a lower first calving rate compared with heifers in the high growth profile (+43 d and -3 points, respectively). These animals also presented a lower 305d milk yield and 305d milk solids yield during lactation one (-697 kg and -48 kg, respectively). However, percentage of second lactating cows did not differ among growth profiles. All profiles had similar performance regarding age at culling, length of productive life i.e; from 1st calving to culling, and lifetime milk yield. Growth profile affected performance during early life (before 1st and during 1st lactation) but not longevity. However, results from present study confirmed that achieving higher growth performances from birth to one year of age is of interest in Holstein dairy heifers, since age at first calving is reduced, and both first calving rate and first lactation productivity are improved.

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Theatre 7

Exploiting milking robot data for genetic evaluation of behavioral traits in Holstein cattle

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The introduction of automated milking systems (AMS) on modern dairy farms means that detailed data can now be collected on cow performance and behavior, opening new opportunities for genetic selection. This study investigates the genetic of AMS-derived behavioral traits in American Holstein by analyzing phenotypic variability throughout lactation and estimating variance components and genetic parameters. Data were collected from 5,645 cows across 36 AMS units over the period 2018 to 2021. Seven behavioral traits were evaluated: average milking time (AMT), total milking time (TMT), interval between milkings (INT), number of visits to AMS units (NoV), number of successful entries (NSE), percentage of successful milkings (PSM), and the preference consistency score (PCS). Two repeatability models were tested to capture the permanent environmental effect: the first considered only differences between parities, whereas the second accounted for both between and within parity. Heritability estimates varied across traits, ranging from low for PSM (0.05–0.06), NoV (0.10) and INT (0.08–0.10) to moderate for TMT (0.27–0.32) and reaching high levels for AMT (0.46–0.48). The PCS exhibited moderate heritability values between 0.09 and 0.24, depending on the model employed. Notably, the genetic correlations highlighted several interesting associations: a strong positive correlation between INT and PSM (0.64–0.71) suggests that optimizing the intervals between milkings may enhance milking success, while a pronounced negative correlation between PSM and NoV (–0.93) points to potential trade-offs between frequent visits and milking efficiency. These findings suggest that traits such as AMT and INT are robust indicators of a cow's adaptation to AMS environments, with promising implications for their integration into selection programs. Future research will use random regression models to better understand trait dynamics over the lactation period, and GWAS to identify genomic regions underlying these behaviors. This approach can contribute to the development of more sustainable and efficient dairy production systems.

Less Meat or Less CO₂? A Systemic Impact Assessment of Prolonging Dairy Cow Lifespan on Meat Supply and Its Emissions

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Extending productive lifespan of dairy cows is considered as a strategy to reduce greenhouse gas emissions in the dairy sector without compromising milk production. However, productive lifespan of dairy cows also affects the meat sector. Therefore, this study holistically assessed carbon footprint effects for dairy and beef production under longevity shifts. This study used an LCA modeling approach to assess GHG emissions of the Swiss bovine sector considering both dairy and beef production. Using a FiBL-developed tool, emissions were evaluated from cradle-to-farm gate on an annual basis. While an average Swiss suckler system was considered, dairy systems were analyzed by agro-ecological zones and production types. For each system, dominant breeds were characterized by an average and a long productive lifespan from herdbook data. The model calculates farm-specific environmental impacts by first simulating the farm's internal material flow and fate within the farm, and then applies models and equations to estimate GHGs. The allocation of emissions was done based on the respective net energy required for milk and body mass growth. Scenarios were developed to assess different system-adapted fattening and slaughter strategies of animals from the dairy sector, always adjusting suckler herd sizes to ensure a constant sector-wide meat output. Results indicate that even when meat from the dairy sector is reduced due to prolonged lifespan and loss of meat supply is offset by an increase of CO₂-intensive suckler beef, total sector-wide emissions are marginally lower compared to the current meat and milk production including the suckler beef system. Policies promoting prolonged productive lifespan will therefore always contribute to reduction of sectoral emissions. Findings also suggest shifting to dual-use cattle systems. Calves that have the genetic potential for greater growth can significantly increase meat production within the dairy sector. When this additional meat replaces the more CO₂-intensive suckler beef, overall sectoral greenhouse gas emissions can be reduced significantly without lowering total meat output. Dual-use cattle systems should be recognized as a key strategy for reducing CO₂ emissions in the ruminant sector.

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Theatre 9

Distinct standing time response patterns to seasonal heat stress in dairy cows

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Thresholds are needed to detect and identify heat stress. The response to heat stress may not differ between animals but the dynamics of the response may vary from one animal to another. In the present study, we hypothesized that, when cows are reared indoors, the standing position can be considered as an indicator of heat stress situation. From June 16th to September 15th 2024, data were collected on 158 Holstein dairy cows (parity 1 to 5), in 2 commercial farms located in a temperate climate in western France. In these farms, milking robots were used and cows were housed indoors all year long. Behavioural data (standing, time in holding areas, drinking and eating time, and walking distance) were continuously recorded using the Alherd® device. Temperature, humidity, wind speed and wet bulb globe temperature were also recorded. Information was aggregated on a 4-hours basis, and behavioral data were averaged per slot. Cows were classified according to their behavioural responses using hierarchical ascending classification. Four distinct standing time response patterns were determined, with 3 common clusters: C1 (37%) shows a linear increase, C2 (21%) remains stable until reaching a peak before decreasing, and C3 (19%) increases until a peak and then stabilizes. Although Farm 2 has fan systems, dairy cows in this farm consistently showed higher standing activity ($p < 0.05$), despite no greater environmental stress, as Farm 1 had a higher temperature by about 0.345°C. This may be due to the barn's larger area, layout, and the need to stand in alleys to benefit from fans. Our results indicated that standing activity is increasing while intensity of heat stress is increasing, suggesting that it could be an indirect indicator (proxy) to determine intensity of such a stress on individual basis. Including other indicators based on health status, mobility score, access to milking robot, still need to be performed, in order to improve this first classification.

The Milk Microbiome and its Association with Mastitis in Dairy Cattle

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Mastitis is a prevalent disease of dairy cattle that causes economic losses through decreased milk yield, discarded milk during treatment, and veterinary costs. Animal welfare is also negatively impacted due to painful clinical signs such as inflamed and swollen udders. The milk microbiome, which is the collection of microbes (bacteria, fungi, viruses, archaea) in milk and their interactions within the milk environment, is altered during mastitis. However, the extent of this impact has not yet been fully explored. We assessed the association between the milk microbiome and mastitis health status by sampling milk from healthy animals (H, n = 10) and animals with subclinical mastitis (S, n = 10), clinical mastitis (C, n = 12), and repeated clinical mastitis cases (R, n = 10) in lactating Holstein Friesian cattle. Samples underwent mastitis diagnostic culture and microbial DNA shotgun metagenomic sequencing. Microbial alpha diversity did not differ significantly between sample types. Amongst the top 10 most abundant genera, *Clostridium* was most dominant in all sample types but less abundant in healthy cases. In clinical mastitis cases where a fungal pathogen was cultured (n = 3), there was significantly greater evenness (adjusted Shannon diversity $p < 0.001$, Simpson diversity $p = 0.02$). Comparison of C with R revealed that milk from repeated cases contained lower abundances of known mastitis causing pathogens, including *Streptococcus*, *Escherichia*, and *Raoultella*, than single clinical mastitis cases. The number of taxa significantly differentiating C and R was noticeably higher than in other pairwise comparisons, with the majority of these taxa overrepresented in single clinical mastitis samples (71 taxa) compared to repeated cases (2 taxa). *Limosilactobacillus*, *Dietzia*, and *Propioniciclava* were found to be more abundant in healthy samples than in clinical or repeated clinical samples. They were also found to be more abundant in subclinical than in clinical samples, suggesting a possible protective role, preventing the disease from progressing.

Session 15

Theatre 11

Physiological Adaptations in Hemogasanalysis of Holstein Friesian and Brown Swiss Lactating Cows Exposed to a Short-Term Natural Heat Wave

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The study evaluated the adaptive hemogasanalysis responses of Brown Swiss (BS) and Holstein Friesian (HF) lactating cows during a four-day natural heat wave. Forty multiparous cows (20 BS, 20 HF), balanced for DIM, ECM yield, and BCS, were included. The barn's cooling system (fans and sprinklers) was turned off, and before deactivation, THI was below the heat stress threshold for both breeds. Respiration rate (RR) was recorded at 4 AM, 3 PM, and 8 PM daily, while blood samples from the jugular vein were analyzed for lactate, pH, pO₂, pCO₂, base excess (BE), bicarbonate (HCO₃⁻), total CO₂ (TCO₂), and hematocrit using an Abbott i-STAT portable analyzer. Vaginal temperature (VT) was continuously monitored via temperature loggers. THI and VT confirmed that cows were not experiencing heat stress before deactivation but began adapting thereafter. RR strongly correlated with VT, consistent with current knowledge. All hemogasanalysis parameters were affected by environmental temperature in both breeds, though BS showed slightly less pronounced changes than HF. Differences in hemogasanalysis trends, particularly in the early adaptation phase, confirmed distinct thermoregulatory responses. Correlations between hemogasanalysis, THI, RR, and VT reinforced their relevance in assessing thermal stress. These findings highlight breed-specific physiological responses to heat stress and support hemogasanalysis as a valuable tool for assessing blood biochemistry and acid-base balance under thermal stress conditions in dairy cows. Research supported by PRIN-2022 grant from the Italian Ministry of University and Research (Resiliency to heat stress: a system biology approach, CUP: J53D23010270006).

Using digital phenotypes for genomic evaluations in pigs

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As digital phenotyping techniques continue to showcase their potential for delivering large-scale, cost-effective, and accurate data in livestock production, expectations for their use in genomic selection for complex traits have risen. For instance, conformation traits, traditionally assessed using categorical scoring systems, can now be measured continuously by combining computer vision and machine learning. This approach could eliminate the inherent bias of subjective human assessments and provide more reliable data. This study aimed to estimate genetic parameters for complex traits in pigs recorded using visual and digital phenotyping methods and to investigate the impact of digital phenotypes in genetic evaluation models. The number of records for the visual (digital) traits was 577,056 (32,864) for the front leg, 563,567 (32,864) for the rear leg, and 605,867 (20,580) for weight measured at the end of a growth test. The pedigree included about one million animals. Heritabilities were estimated using single-trait models. Genetic correlations were obtained using two-trait animal models. Heritability estimates for the visual (digital) traits were 0.18 ± 0.00 (0.60 ± 0.02) for the front leg, 0.12 ± 0.00 (0.62 ± 0.02) for the rear leg, and 0.36 ± 0.03 (0.36 ± 0.03) for weight. Genetic correlations among visual traits ranged from -0.08 ± 0.02 to 0.25 ± 0.09 , among digital traits from -0.19 ± 0.05 to -0.74 ± 0.02 , and between visual and digital traits from 0.83 ± 0.03 to 0.97 ± 0.00 . In general, heritability and genetic correlations were lower among visual traits than digital traits. High genetic correlations between visual and digital traits suggest substantial genetic overlap, demonstrating they are genetically the same trait. Our findings indicate that digital phenotyping—especially for subjective traits—increases heritability, which may lead to more accurate genetic evaluations and, ultimately, better response to selection. This study provides a foundation for integrating digital phenotyping into swine breeding programs.

Session 16

Theatre 2

A comprehensive genetic evaluation of molecular phenotypes in pigs: gaining knowledge through metabolite ratios

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Metabolomics has emerged as a novel high-throughput approach for measuring all molecules at the basis of metabolism, providing an unprecedented picture of molecular phenotypes that contribute to describe the molecular phenome of an organism and define genetically determined metabolites. Within the molecular domain, molecules do not act in isolation but rather as part of networks. Specifically, two metabolites can be interconnected as substrates and products of an enzymatic reaction. Therefore, by exploiting this link, we can create new phenotypes (i.e. metabolite ratios) that expand the molecular phenome and provide novel insights into the molecular phenotype landscape. In this study, we obtained the metabolomic and genomic profile of ~700 Italian Large White pigs. Metabolite levels of 722 plasma metabolites were obtained using an untargeted metabolomic platform from Metabolon and genotypes were obtained with the Illumina 60k panel. Metabolite genome-wide association studies (mGWAS) were performed using the GEMMA tool. We then applied a powerful strategy that modelled metabolite relationships in the form of metabolite ratios, resulting in over more than 250,000 new molecular traits that underwent GWAS. The analysis of single metabolites identified several metabolite QTL (mQTL) regions involving 236 molecules and ~80 genes already known to be functionally linked to those metabolites, while metabolite ratios uncovered ~10 additional mQTL for additional 120 metabolites (and 450 ratios). Overall, by applying this powerful strategy, we expanded our knowledge of the molecular phenome, and its genetic control by ~300 times and uncovered new markers that can serve as proxies for more complex phenotypes. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).

Heat tolerance of growing-finishing pigs based on feed intake and body weight records

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Climate change poses challenges for livestock raised outdoors, and for those housed indoors due to imperfect climate control in barns. We estimated genetic parameters for heat tolerance in growing-finishing pigs based on residuals from their expected daily feed intake. Data were collected from a single pig line between July 2021 and September 2022 on a Canadian test farm. Feeding stations recorded daily body weight and feed intake of individual pigs. Pedigree information was traced back three generations. Environmental data included daily maximum and minimum indoor temperatures at room level, and outdoor temperature and relative humidity from a nearby weather station were used to calculate daily mean temperature-humidity index (THI). To quantify individual residuals in feed intake, we first standardized daily feed intake, ensuring a mean of zero and a standard deviation of one within each integer body weight unit across the line, to minimize life-stage effects. As indoor temperatures rose substantially with outdoor THI >60, we selected a subset of pigs (n = 974) with at least five daily records in this THI range for further analysis. Linear regression was applied to standardized feed intake records, using normalized THI (>60) as predictor. The slope of the regression line for each pig represented its heat tolerance phenotype, where a more negative slope indicates larger reduction in feed intake with rising THI, thus, lower heat tolerance. A multi-trait model was then fitted, incorporating the heat tolerance slope and other performance traits while accounting for group size and room as fixed effects and contemporary group (pen) as a random effect. Results indicated low heritability for heat tolerance (0.08 ± 0.066). The genetic correlation between heat tolerance and average daily gain was negative (-0.29 ± 0.443), while correlations with feed conversion rate, back fat thickness, and feed intake variation (as proxy for mortality) were near zero (-0.04 to -0.02). In summary, heat tolerance based on feed intake residuals showed low heritability and an unfavourable genetic correlation with average daily gain.

Session 16

Theatre 4

Tasting before eating: a Machine Learning solution for precise ingestion behaviour tracking in goats

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Modern farmers face dual challenges: meeting animal welfare regulations while optimizing productivity with management practices. The integration of sensor technology and machine learning offers potential to address both. As livestock operations evolve, manual logbooks become inefficient, making precision monitoring essential for economic efficiency, welfare, and environmental impact. While mathematical models have optimized growth, breeding, and feeding, farming's data demands ML approaches and extensive sensor and video data. This study focuses on semi-extensively reared goats, chosen for their complex behaviors, presenting a greater challenge than more passive species. It develops an integrated platform with video monitoring and environmental data collection via custom sensors. Accelerometers and microcontrollers recorded movement data at 4 Hz, transmitted via LoRa protocol for logging and processing. Lightweight collars ensure minimal intervention, with one-hour observation sessions allowing goats to roam freely in natural pastures, preserving their behavior. The study proposes a ML approach to identify specific behaviors through detailed video analysis. Video data is integrated with sensor data labelled frame-by-frame using drone and manual recordings synchronized with GPS timestamps. By employing deep learning models like XGBoost, the project aims to create a behavior recognition platform, focusing on fine-grained patterns. This framework will capture subtle actions like tasting before eating, enhancing welfare and nutritive assessment. Linear accelerations on the transversal axis provided the most information, while gyroscopic data was less useful. Results show that a Random Forest classifier achieved 92.5% accuracy (MCC 90.1%), and a Multilayer Perceptron (MLP) 90.5% (MCC 87.5%). XGBoost performed similarly (88.9%, MCC 85.3%). Preliminary analysis indicates MLP improves with longer sessions: 30-minute sessions achieved 82.5% accuracy, rising to 90.5% at 60 minutes. Different cereal growth stages affected ingestion time (16.6% to 56.1% in 2 weeks) and selection time (2.5% to 3.1%). Spring may offer wider pasture choices for comparative ingestion analysis.

Use of computed tomography to select anatomical sites for in vivo prediction of body composition in pigs and dairy goats

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Precise phenotyping of body composition and its temporal changes are essential in animal research and breeding, both for genetic selection through better characterisation of complex traits and for adapting breeding strategies to production goals and societal expectations. However, phenotyping in vivo body composition remains a challenge. Our objective was to determine anatomical sites from which in vivo whole-body composition of pigs and dairy goats can be predicted, based on computed tomography (CT) images analysis. Twenty-two crossbred Pietrain x (Large White x Landrace) gilts (152.5 ± 0.9 days old, 99.2 ± 7.5 kg body weight (BW)) and 20 dairy Alpine goats (3.0 ± 0.6 years old, 54.7 ± 6.6 kg BW) were scanned after anaesthesia using CT. Fat (FT) and lean thicknesses (LT) were calculated from $15 \text{ cm} \times 15 \text{ cm}$ areas on the back of the animals, centred on the spine using vertebrae as landmarks. Linear regressions were calculated to predict CT fat and lean volumes of the whole animal, excluding offal, from either 2 predictors (BW and FT) or 3 predictors (BW, FT and LT). The best anatomical site to predict fat volume with 2 or 3 predictors is the 13th thoracic vertebra (adjusted R^2 : 0.70, coefficient of variation of the root mean square error (CVRMSE): 6.6%) in pigs and the 1st thoracic vertebra (adjusted R^2 : 0.57, CVRMSE: 22.7%) in goats. For the prediction of lean volume, the 1st lumbar vertebra with 3 predictors (adjusted R^2 : 0.59, CVRMSE: 3.7%) or the 5th thoracic vertebra with 2 predictors (adjusted R^2 : 0.45, CVRMSE: 4.3%) are the best anatomical sites in pigs. In goats, the best anatomical sites were either the 5th lumbar vertebra with 3 predictors (adjusted R^2 : 0.30, CVRMSE: 9.1%) or the 8th thoracic vertebra with 2 predictors (adjusted R^2 : 0.22, CVRMSE: 10.0%). The identification of anatomical sites that are representative of the whole body of the animal provides for facilitating in vivo phenotyping of body composition with less constraining methods than CT. This work is part of Coccoone project, funded by Carnot France Futur Elevage.

Session 16

Theatre 6

GNSS tracking as a tool to phenotype cattle selectivity behaviour in mountainous rangelands

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Grazing animals show selective behaviour depending on biotic and abiotic factors. This selectivity results in a non-uniform spatial distribution of animals, which has different effects on the environment. Previous studies have suggested the existence of different landscape selectivity phenotypes, which may be associated with some DNA markers. The present study aims to explore the potential of GNSS tracking data to characterise selectivity phenotypes in mountainous rangelands. The study was carried out between July and October 2024 in a commercial farm located in Sierra Nevada National Park (Spain). The altitude of the pastures varied from 1700 to 2800 m.a.s.l. Ten cows were fitted with GNSS collars set to record fixes every 30 minutes. Landscape characterisation was based on distance to water sources, vegetation classes and geomorphology of the terrain. Geomorphological classification was based on GRASS software and vegetation classes were obtained using the Scene Classification plugin of QGIS software. GNSS data processing and spatial analysis were performed using R software. Selectivity was assessed using the Jacobs Selection Index (JSI). Cattle preference decreased with distance to water. A positive JSI mean was only observed for distances to water less than 50 m (0.233 ± 0.113). Regarding vegetation, most cattle preferred riparian meadows to shrublands and bare ground (0.591 ± 0.071). Positive values of JSI were found for most of the geomorphological units, except for areas with slopes higher than 64.5% (-0.815 ± 0.584). Significant differences between individuals were observed only in the preference for different geomorphological units, with some cows showing a total preference (+1) and others a total avoidance (-1) for certain categories. The results obtained in this study showed that areas close to water, with the most nutritious forage available, are a concentration point of cattle distribution, showing little differences between individuals. In contrast, geomorphology showed a higher variability between cows, making it more useful to obtain selectivity phenotypes.

Genetic parameters for calf social dominance indicators derived from automated milk feeding records in American Holstein calves

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Automated milk feeders (AMF) generate large-scale longitudinal milk feeding data in calves and hence provide a great opportunity for deriving novel traits for genetically improving calf health, welfare, and performance. For group-housed dairy calves, genetic analyses of social dominance have important implications on improving calf welfare and management practices. In this study, we derived and estimated genetic parameters for six calf social dominance indicators using a dataset consisting of 4,164,960 AMF visit records from 8,632 American Holstein calves, including 5,910 calves with genomic data for 64,767 autosomal SNPs. We also assessed the genetic relationship of the derived social dominance traits with seven feeding, growth, and health related traits. We observed that the evaluated time intervals between two successive visits to AMF did not have clear impact on the calculated phenotypic records. Among the social dominance traits, David's score (DS), normalized DS, Kondo-Hurnik index (KH), and adjusted KH by dominance probability were found to be moderately heritable with the heritability estimates ranging from 0.180 ± 0.017 to 0.210 ± 0.018 , whereas Elo-rating scoring and success index were lowly heritable or less robust to the varied weights of dyadic agonistic interactions. Furthermore, the heritable indicators had moderate and positive genetic correlations with average milk consumption per visit and average numbers of daily unrewarded visits, and weak positive correlations with birth weight. However, all indicators evaluated were lowly or not genetically correlated with the number of treatment records for bovine respiratory disease. Our findings contribute to understanding the genetic background of social behaviors in dairy calves. Several dairy calf social dominance indicators were found to be heritable with substantial additive genetic variance in American Holsteins. However, further studies need to be conducted to identify potential genetic relationships with economically and socially relevant traits in breeding programs.

Session 16

Theatre 8

The metabolome, a new phenotype: a comparative study of metabolic profiles in plasma and serum

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Untargeted metabolomics is increasingly used to characterize complex traits offering a comprehensive view of the physiological state of an animal. Thus, it could serve as a novel phenotype per se, or as additional source of information to improve prediction models. NMR is a technique to infer metabolic content from spectral signal intensities. Plasma and serum are commonly used as substrates in metabolomic, and their composition may vary due to sample processing. The study aims to evaluate if substrate type influences metabolic profiles and explores the potential of raw spectral data to improve profile discrimination. Metabolic profiles of plasma and serum from 15 Holstein (HOL) cows and 15 Avileña-Negra Ibérica (ANI) calves were compared. Two approaches were analyzed: (1) the concentration of 47 metabolites obtained from NMR spectra at 600 MHz and (2) raw spectral intensity data from the NMR for which spectra were preprocessed through frequency calibration, removal of chemical regions, baseline correction, alignment, and normalization. A total of 46,903 chemical shift (ppm) data points were obtained and used as predictor variables. PLS-DA was applied to assess differences between substrates, variable importance in projection (VIP), and correlation analyses. The PLS-DA model showed moderate differences between plasma and serum, more pronounced in HOL than in ANI. VIP values highlighted certain metabolites (9 and 11 for HOL and ANI, respectively) contributing to substrate discrimination. Correlation of correlations between metabolites and substrates were 0.37 and 0.49 for HOL and ANI, respectively. Regarding spectral analysis, PLS-DA showed greater substrate discrimination compared to approach (1), suggesting that raw spectra may capture more subtleties. Plasma and serum metabolic profiles share similarities but there are differences between substrates that are more relevant in HOL than in ANI. Caution needs to be taken when comparing metabolomic profiles from different substrates. The use of raw spectra is a valuable tool to assess differences. Work supported by GLOBALR_WEL (PID2020-113913RR-C31) and HE Re-Livestock.

breed4green - measuring methane emissions in commercial farms and establishing a comprehensive dataset for genetic studies

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In the project breed4green, the genetic potential for reducing methane emissions in Fleckvieh and Brown Swiss cattle is investigated. Therefore, a comprehensive cost-efficient data recording concept was developed to measure methane emissions on commercial farms and collect high quality phenotypes for genetic studies. Data from PLF systems on participating farms, like daily milk yield, animal-based sensor data or feeding data are collected. The GreenFeed system measures methane over a 6-week period, alongside body weight recordings. Weekly milk recordings, which form the basis for mid-infrared predicted proxies for methane, are conducted. After 6-weeks the GreenFeed and scales are moved to the next farm, with a second measurement period 6 months later. Body condition, lameness, blood ketotests, animal health records, hoof trimming data and detailed information on the feed rations are recorded throughout the study. Ensuring high data quality is crucial to avoid losing valuable data for genetic studies. Implementing a monitoring routine to regularly check data quality is essential for maintaining comprehensive and accurate datasets. Data recording on 30 farms will run until 2027. Currently, analyses are available for 9 farms. Animals remained in the GreenFeed system for methane measurement for up to 8 minutes, most of the measurements lasted between 2 and 5 minutes. A minimum of 20 measurements were required to determine methane emissions for an animal. With longer measurement times, fewer measurements were needed to obtain reliable results. The mean methane emission of the 355 cows measured so far was 429g per cow per day. Preliminary analyses, including variability between cows, trait correlations and heritability estimates for methane, show promising results.

Session 16

Theatre 10

Repeatability and heritability of dairy cow methane concentration using sniffer sensors

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Large-scale phenotyping of methane emissions in dairy cows is important for genetic selection, but existing gold-standard methods (respiration chambers and the SF₆ technique) are costly and labour intensive. In this study, 50 methane sensors (Arcoflex International Pty Ltd) were installed, one per bay, in a herringbone dairy at Ellinbank SmartFarm (Victoria, Australia). From March to November 2024, methane concentrations from 532 genotyped cows were recorded every 10 seconds at morning and afternoon milkings, with a mean of 227 visits per cow. Preliminary analysis tested 1,260 scenarios to identify the optimal time window (second 90 to second 410) that maximised repeatability and minimised Akaike Information Criterion and BIC Bayesian Information Criterion. Two visit-level measures were generated: (1) an arithmetic mean of all measurements and (2) a “peak” average, defined by data points exceeding their immediate neighbours. Univariate linear mixed models in ASReml were used to estimate heritability and repeatability. The arithmetic mean was 445 ppm per visit, while the peak average was 639 ppm. Per-visit peak average resulted in the greatest heritability estimate (0.12). Although heritability typically improved when data were aggregated weekly in other studies, here it remained low (0.05). This may be due to unaccounted-for bay differences once measurements were collapsed over time. However, repeatability improved with weekly averaging, rising to 0.38 for peak-based data, indicating that combining multiple visits stabilised short-term variability in measured methane concentration. These findings highlight that sniffer sensors can measure methane emissions with sufficient precision for genetic analysis, especially when focusing on peak-based measurements. Further work in additional herds and farm systems will clarify how best to address sensor placement effects and data aggregation strategies.

Methane reduction in Hanwoo beef cattle through precision feeding based on meat-yielding and marbling gene profiles

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This study examines the impact of genetic profiles on growth performance, feeding behavior, and greenhouse gas (GHG) emissions in Hanwoo steers, focusing on meat yield and marbling traits. These traits are crucial for improving breeding strategies given that Hanwoo beef has cultural significance in Korean cuisine. Two genetic groups were analyzed: meat-yielding (G; n = 12, BW: 560.00 ± 23.60 kg) and meat-marbling (Q; n = 12, BW: 492.50 ± 23.60 kg). Cattle were fed using RIC units within a smart farm system, and GHG emissions were monitored using GreenFeed technology. Data were analyzed using SAS PROC MIXED, with and without covariates (BW and DMI). Without covariates, the final BW was higher in the G group (669.58 ± 27.83 kg) than in the Q group (587.08 ± 27.83 kg; p = 0.0478). Eating rate (G: 90.29 ± 8.15 vs. Q: 99.44 ± 8.51 g/DM/min), visit duration (G: 42 ± 3.59 vs. Q: 44 ± 3.75 min/d), and frequency (G: 11 ± 1.12 vs. Q: 12 ± 1.70 /d) did not differ significantly. CH₄ production was higher in the G group (157.03 ± 12.67 g/d) compared to the Q group (113.48 ± 12.67 g/d; p = 0.0236) while CO₂ production showed no significant difference. The emission factor (EFa) was also greater in the G group (57.32 ± 4.62) than in the Q group (41.42 ± 4.62; p = 0.0237). Even with covariates, CH₄ emissions remained significantly higher in the G group regardless of influencing factors: a) Min/meal and eating rate: G: 153.09 g/d, Q: 112.95 g/d (p = 0.0416), b) Visit duration and frequency: G: 156.80 g/d, Q: 108.91 g/d (p = 0.0172); c) Final BW and DMI: G: 161.71 g/d, Q: 108.81 g/d (p = 0.0152). CO₂ production remained similar (G: 9040.91 g/d, Q: 8988.88 g/d; p = 0.9374). These findings suggest that meat-yielding steers exhibit superior growth performance and contribute to increased methane emissions. This highlights the importance of implementing targeted mitigation strategies in precision feeding programs to reduce environmental impact without compromising growth outcomes.

Session 16

Theatre 12

On-farm assessment of methane emissions in dairy sheep: GreenFeed and Laser Methane Detector, a comparison of tools

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In the dairy sector, monitoring enteric methane emissions in ruminants is crucial for reducing livestock farming's environmental impact and identifying the most efficient animals. The gold standard, respiration chambers, and the silver standard, GreenFeed® (GF), are accurate but impractical for on-farm use. The Laser Methane Detector (LMD) is a portable, low-maintenance, and user-friendly device that could provide a more feasible alternative for measuring methane emissions. In 2024, an experiment was conducted to evaluate the reliability of the LMD and its correlation with measurements collected by GF. Methane emissions from 49 Comisana lactating sheep were measured for five consecutive minutes using two LMDs on different animals. Measurements were taken twice a day (2 hours and 6 hours after feeding) over three consecutive days. The same animals were also monitored using GF devices during the same period. The average emissions measured with the LMD and GF were 25.64 ± 6.18 ppm*m for LMD, and 45.46 ± 8.31 gCH₄ day⁻¹ for GF. The average weight of the sheep was 58.44 ± 10.2 kg. Preliminary analysis of the GF results highlighted that the animals could be divided into high and low emitters, based on the first (high emitters) and fourth (low emitters) quartile, with average values of 54.1 ± 3.8 and 34.3 ± 4.9 gCH₄ day⁻¹, respectively. A similar classification was obtained using the LMD, with values of 35.5 ± 5.1 and 19.5 ± 1.5 ppm*m. The results show that the LMD has an agreement index of 51.02% with GF. Further analyses considering the effect of the main variation factors, such as weight of animals, device adopted and day of measurement were also considered in order to refine the correlation coefficient.

Rapid, non-invasive prediction of intramuscular fat in lamb carcasses using a portable microwave system
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Intramuscular fat (IMF) is a key determinant of meat quality, influencing tenderness, juiciness, and flavour. This study evaluates the accuracy of a novel, portable Microwave System developed at Murdoch University for real-time, non-destructive IMF prediction in lamb carcasses using two different probe configurations: an Open-Ended Coaxial Probe (OECF) and a Vivaldi Patch Antenna (VPA). Microwave scanning commenced within one-hour post-slaughter while carcasses were still hot. Each device was positioned to contact the C-site (45 mm from the spine midline over the 12th rib). Carcasses were chilled (3–4 °C) for 24 hours, after which the M. longissimus thoracis was sampled, freeze-dried, and analysed via near-infrared spectroscopy (NIR) to determine IMF%. Carcasses ranged in weight from 14.9 to 36.2 kg, GR tissue depth from 14.9 to 36.2 mm, and IMF from 2.37 to 6.95%. When predicting IMF%, the OECF-based system demonstrated an average $R^2 = 0.49$, RMSEP = 0.643, and Bias = 0.080, while the VPA-based system achieved $R^2 = 0.47$, RMSEP = 0.641, and Bias = 0.121, with both methods exhibiting a slope deviation from 1 of 0.14. These results highlight the potential of microwave technology for rapid, objective IMF % assessment in commercial processing environments. Future research will refine machine learning models and feature selection strategies to improve prediction accuracy and facilitate large-scale industry adoption.

Genetic and phenotypic validation of Raman-Predicted fatty acids in Atlantic salmon: from crude fat to individual profile

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Atlantic salmon is a key source of dietary fatty acids (FAs) and dominates Norwegian aquaculture. However, essential FA content in salmon fillets, particularly Omega-3, has declined over recent decades due to shifts from fishmeal-based to plant-based feed. Beyond dietary factors, genetic variation also plays a role, as lipid content is heritable. To support sustainable production while maintaining optimal FA profiles, both factors must be considered in breeding programs. The standard method for FA measurement is costly and destructive, whereas Raman spectroscopy offers a rapid, non-destructive alternative. In this study, 613 fillet samples were analyzed using both methods, and Partial Least Squares Regression (PLSR) models were applied for FA prediction. This study evaluates the genetic and phenotypic predictive potential of Raman spectroscopy for FA profiling in farmed Atlantic salmon, spanning different levels of resolution—from total fat to individual FAs, including Saturated, Monounsaturated, and Polyunsaturated FAs. Results indicate: 1. Raman-based predictions were highly accurate for group-level and some individual FAs (R^2 : 0.66–0.79). 2. Predicted phenotypes were heritable and strongly genetically correlated (R_g : 0.85–0.99) with gold standard values. 3. The predicted sum of EPA and DHA (eicosapentaenoic and docosahexaenoic acids) from the optimal Raman model was significantly heritable (h^2 : 0.32 ± 0.1), closely aligning with gold standard estimates (h^2 : 0.31 ± 0.10). These findings highlight the potential of Raman spectroscopy for FA prediction, offering a valuable tool for genetic selection in Atlantic salmon breeding programs in Norway.

A quantification of abdominal shape of dairy heifers using 3D imaging

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Sufficient development of the rumen is required for dairy heifers to mature into lactating cows capable of high-roughage intake, and this development would be reflected in both the size and shape of their abdominal region. However, quantitative monitoring methods for abdominal shape have not yet been established. This study was carried out to develop a 3D imaging-based method for quantifying the abdominal shapes of dairy heifers. A total of 18 Holstein heifers, aged between 3 and 18 months, were used in the study. Body weight (BW) was measured using an electronic scale. After restraining the animals, point cloud data were acquired from three perspectives (left, right, and top) using 3D cameras, and the depth images were reconstructed. Sixteen transverse cross-sectional areas were calculated by equally dividing the region from the hip bone to the elbow along the cranial direction. Each cross-section was further subdivided into 9 segments spanning from the ventral to the dorsal side, and the width of each segment was measured. For each cross-section, a ventral-to-dorsal ratio was determined by dividing the sum of the widths of the 1st to 3rd segments by that of the 7th to 9th segments. Pearson's correlation coefficients were calculated, with statistical significance set at $p < 0.05$. The analysis revealed a significant positive correlation between the total cross-sectional area and BW ($r = 0.977$). The maximum area was observed in 3 heifers in the 1st-3rd cross-sections (caudal side), 3 in the 4th-6th, 12 in the 7th-9th (near the last rib), and none in the 10th-12th or 13th-16th (cranial side). The mean ventral-to-dorsal ratios for the 4th-6th, 7th-9th, and 10th-12th cross-sections were 1.62 ± 0.083 , 1.70 ± 0.088 , and 1.66 ± 0.107 respectively. No significant correlation was observed between the ventral-to-dorsal ratio and BW in any cross-section. Heifers exhibiting a higher ventral-to-dorsal ratio in the 4th-6th cross-sections showed depression in the paralumbar fossa and bloating in the lower abdomen. The highest standard deviation for the mean ventral-to-dorsal ratio was observed in the 10th-12th cross-sections, a region that is surrounded by ribs. These findings indicate that proposed method is effective to quantify the abdominal shape independent of BW variation.

Session 16

Poster 16

DonKeyWorld: a comprehensive study on *Equus asinus* from biodiversity management to milk nutraceutical and technological propertiesC. Cipolat-Gotet¹, M. Ablondi¹, V. Asti², E. Ciani², V. Landi³, S. Chessa⁴, S. Mastrangelo⁵, A. Criscione⁶, S. Bordonaro⁶, M. Martini⁷, F. Salari⁷, F. Tiezzi⁸

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Donkey milk is gaining interest due to its nutraceutical properties. Thus, the goal of the DonKeyWorld project is to generate knowledge on the Italian donkey sector. One of the three objectives is to perform a whole characterization nutraceutical and technological properties of donkey milk. These parameters are quantified using deep phenotyping, including fine milk composition, nutraceutical properties and technological properties. This objective also includes the testing of infrared spectroscopy to provide new insights into the use of this technology on donkey milk to potentially introduce innovative, low-cost, and rapid tools for monitoring its quality over the chain. Within DonKeyWorld, we aim to sample milk of 450 individuals from four main Italian breeds: Romagnolo, Amiata, Martina Franca, and Ragusano collecting more than 60 milk phenotypes. The sampling is ongoing, and we have sampled and analyzed 206 individual milk samples. Standard milk quality parameters are in agreement with the literature, although there seem breed-specific differences. Analyses are in progress to evaluate differences among breeds, parity and phase of lactation. This research was supported by the project "DonKeyWorld", funded by the Italian Ministry for Universities and Research [project code P20228Z3RJ (PRIN)]

Incorporating plantain into a ryegrass-white clover mixed sward for an environmentally sustainable dairy system: Three years farm system study

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This objective of this study was to investigate the effect of different proportions of plantain in a perennial ryegrass–white clover (RGWC) mixed sward on dairy farm productivity and total oxidisable nitrogen (TOxN; nitrate and nitrite) leaching over three production years (2021/22 to 2023/24). A total of 108 Friesian × Jersey dairy cows were blocked into nine herds of 12 cows and randomly allocated into one of three replicated herbage treatments with increasing plantain seed rate: (i) RGWC with no plantain (PL0); (ii) RGWC+3 kg/ha (PL3) or (iii) RGWC+6 kg/ha (PL6). Milk and herbage production were measured for each farmlet, and TOxN leaching was measured using 736 ceramic cups installed across 16 paddocks (eight 0.3-ha paddocks each in PL0 and PL3). Averaged across years, plantain content in the sward DM was 17.3% in PL3 and 24.0% in PL6. There was an interaction effect between year and treatment on annual herbage production ($P=0.013$) and imported silage ($P=0.008$). Annual herbage production (t DM/ha) was similar across treatments in 2021/22 (average 14.4) but was lower in PL6 (15.0) than in PL0 (16.5) and PL3 (15.8 t DM/ha) in 2022/23. In 2023/24, herbage production was lower in PL6 (15.8) than in PL3 (16.8), but similar between PL0 (16.4) and PL3, and between PL0 and PL6. Imported silage (t DM/ha) was similar across treatments in 2021/22 (average 0.24) and 2023/24 (average 1.97) but was higher in PL6 (2.2) than in PL0 (1.6) and PL3 (1.5) in 2022/23. Annual milk solids production was similar across the treatments (average 437 kg/cow). Annual TOxN leaching was 26.6% lower ($P=0.031$) in PL3 (13.0 kg N/ha) than PL0 (17.7 kg N/ha) with no interaction between year and treatment. These results indicate that incorporating plantain in a RGWC mixed sward can reduce farm nitrogen loss to the environment while maintaining productivity, particularly at a low level of inclusion.

Session 17

Theatre 2

Beyond Respiration Chambers: A Field-Deployable Device for Continuous Methane Emission Measurement in Cattle

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Introduction: Methane emissions from cattle are a major greenhouse gas (GHG) source, making continuous monitoring critical. Traditional respiration chambers offer precision but are costly, restrictive, and may distort real-world data [1]. ZELP's field-deployable device presents a scalable, cost-effective alternative. To assess its performance, the device was tested within respiration chambers for direct comparison. **Materials and Methods:** The device integrates Near Infrared optical and catalytic gas sensors, thermistors for temperature, and accelerometers for motion tracking. These are mounted on a ventilated neoprene face sock near the nostrils to capture exhaled gases before dilution. A two-week trial at Hall Farm, Reading, England (October 2024) tested three Holstein Friesian heifers inside respiration chambers. Each heifer was monitored via CCTV and hourly visual inspections. Collected data included respiratory rates, breath temperatures, gas concentrations, and behavioral observations. Weight was recorded pre- and post-trial. A machine learning model, trained on sensor readings, estimated methane and carbon dioxide emissions. Due to confidentiality, further model details cannot be disclosed. **Results:** The device closely mirrored respiration chamber data, with a moderate positive correlation ($r = 0.612$, $p < 0.05$) between methane readings, supporting reliability in real-world settings. Respiration rates remained normal (26–50 breaths per minute), and behavioral assessments showed no distress, confirming no impact on animal welfare. **Conclusions:** ZELP's device provides a scalable, accurate alternative to respiration chambers for continuous methane monitoring. While correlation is moderate, findings support field deployment where chamber-based methods are impractical. Future research will refine accuracy and expand deployment across diverse conditions. **References:** [1] Llonch, P., Troy, S.M., Duthie, C.-A., Somarriba, M., Rooke, J., Haskell, M.J., Roehe, R., & Turner, S.P. (2018). *Animal Production Science*, 58(6), 1011. [2] Troy, S.M., Duthie, C.-A., Ross, D.W., Hyslop, J.J., Roehe, R., Waterhouse, A., & Rooke, J.A. (2016). *Animal Feed Science and Technology*, 211, 227–240.

Farm- and cow-level factors associated with nitrogen utilization in Norwegian Red dairy cows

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Maintaining milk production while reducing nitrogen (N) pollution is a key challenge in dairy farming. Improving the nitrogen utilization efficiency of dairy cows is essential to address this challenge. This study estimated nitrogen utilization metrics (N-traits) and explored their associations with cow- and herd-level factors using data from 1,475 Norwegian Red (NRF) cows from 14 commercial herds. Data on daily feed intake and milk yield, feed and milk compositions, and other cow information were used to investigate the N-traits, including nitrogen use efficiency for lactation (NUE: milk N output/N intake), nitrogen loss (NL: N intake minus milk N), fecal nitrogen (FN), and milk urea nitrogen (MUN). NUE and NL were calculated as 7-day averages using the test-day records and data from the preceding six days. FN and MUN were determined from spot samplings. NUE ranged from 12.4% to 59.6% (average 31.5%), while NL varied from 116 to 808 g/day (average 364 g/day). NUE was negatively correlated with NL (-0.46), indicating that cows converting more N into milk excreted less N in urine and feces. Milk yield was positively correlated with NUE (0.45), NL (0.49), and N intake (0.72). However, NUE was negatively correlated with N intake (-0.31), while NL showed a strong positive correlation with N intake (0.95). MUN showed a weak negative association with NUE (-0.11) and milk yield (-0.10) but a weak positive correlation with NL (0.13) and N intake (0.09). FN exhibited moderate correlations with NUE (0.42) and NL (-0.40). NUE and NL varied significantly ($P < 0.05$) across herds (Coefficient of variation: 15.8 and 21.4 %, respectively). NUE declined while NL increased with advance in lactation stage but both traits increased with parity. Season, dietary protein content, and roughage-to-concentrate ratio also significantly ($P < 0.05$) influenced NUE and NL. In conclusion, NRF cows demonstrated substantial variation in nitrogen utilization and excretion. Phenotypic correlations among the N-traits, as well as with milk yield and N intake, were generally moderate. Key factors affecting the N-traits included lactation stage, parity, herd, dietary protein content, roughage-to-concentrate ratio, and season.

Session 17

Theatre 4

Calculating balance of cumulative non-fossil methane emissions over consecutive or non-consecutive livestock production periods

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Methane emissions from livestock production are usually assessed as annual methane emissions or recalculated to carbon dioxide equivalents. However, these assessments cannot be used to directly determine the actual amount of methane in the atmosphere in a given year, because they do not account for continuous methane oxidation and short-term methane accumulation. Consequently, they may hinder the identification of livestock (sub)sectors or (sub)populations with warming and cooling effects and limit optimal mitigation efforts. Here we demonstrate a novel method to calculate the balance of (non-fossil) methane emissions (BME) from annual (AME) or cumulative methane emissions (CME) over livestock production periods. The BME considers a methane emission lifetime (MEL) of 12 years and allows for updates based on newer findings of MEL. In brief, we calculate the CME value by summing the AME values from the observation year and the past 11 years. Next, we calculate the BME value by subtracting two consecutive or non-consecutive CME values. A negative BME means that more methane is being oxidised than emitted, resulting in net cooling, while a positive BME indicates additional methane and net warming. Furthermore, we define BME with a chain base (BME-C), which allows comparing two CME in consecutive years i and $i+1$ and BME with a fixed base (BME-F), which allows comparing CME in year i (where i is between $-\infty$ and ∞) with fixed CME in year j . In practice, BME-C could be used to determine the actual amount of methane in the atmosphere in a given year and BME-F could be used to compare the amount of methane between periods that are close or further apart. For assessing BME, recalculations to carbon dioxide (warming) equivalents are not necessary and are therefore easier to interpret for non-climatologists. BME can be applied across livestock farming systems, aiding animal scientists, advisory services, regional development institutions or farmers themselves to assess and monitor short-term and long-term methane emission levels of any livestock population. As BME enables assessing contributions to net warming or cooling, it supports more optimal implementation of methane mitigation measures.

Implementing methane mitigation strategies on Danish dairy farms without compromising N-efficiency N. I. Nielsen¹

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The Danish parliament decided in 2021 that Danish dairy farmers should decrease the enteric methane emission from their cows by 6% in 2025. Furthermore, a newly established Green Deal in the Danish Parliament obliges Danish dairy farmers to pay a CO₂-tax from 2030 on GHG-emissions from cows and their manure. In order to achieve a reduction in enteric methane a law was implemented from 1st of January 2025 where farmers either should feed their cows 48 grams of fatty acid per kg DM for the whole year or they should feed Bovaer for 80 days in a dose of 60 mg 3NOP/kg DM. Bovaer has been used in trials on commercial farms from 2021 until 2024. Results shows no significant effect on feed intake and ECM-yield and methane measures show 30 to 44% reduction. More than 95% of the farmers have chosen to feed Bovaer in 2025. It is expected that all conventional cows will be fed Bovaer or another methane reducing feed additive all year round in 2029. Furthermore, SilvAir is expected to be a mitigation option in the future. On farm trials have shown that the rations need careful adjustments in order to avoid reductions in N-efficiency and increased ammonia emissions. However, also SilvAir have shown high reduction potential for enteric methane under on farm conditions.

Estimating the Carbon Footprint of Milk Production on Polish Farms: Developing a Decision Support Application for Climate Control in the Dairy Sector

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The carbon footprint of milk produced on domestic dairy farms was analyzed as part of a collaborative research network among Agricultural Universities in Poland. The study aims to support the environmentally sustainable development of the Polish dairy sector. The Annual Nutrient Cycling Assessment (ANCA) tool was employed to calculate CO₂-equivalents (CO₂-eq) per kilogram of fat- and protein-corrected milk (FPCM). The study encompassed 46 dairy farms across Greater Poland, Warmia-Masuria, Lesser Poland, and Lublin regions, ranging from large-scale farms with 1,300 cows to smaller operations with 6–100 cows. Average milk production per cow was 9,241 kg, from 3,520 kg in a small organic farm to 15,000 kilograms in intensive production systems. The carbon footprint of milk produced on the audited farms showed significant variability, averaging 1,679 g CO₂-eq/kg FPCM, with values ranging from 1,031 to 4,258 g CO₂-eq/kg FPCM. The most important contributor to emissions was rumen fermentation (39% on average), followed by emissions from imported farm inputs (29% on average). Future efforts will include continued monitoring and the development of an emission calculator to Polish conditions. This tool aims to provide dairy producers with practical solutions for tracking and mitigating the carbon footprint of milk production in Poland.

Environment as challenge for intensive dairy farming

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The EU Green Deal regulates a stricter Natura 2000-plan, 90% GHG emission reduction in 2040 compared to 1990, pesticides use halved by 2030 and set-aside of 4% acreage for biodiversity. Farmer protests in 2024 led to halt the set-aside and pesticides plans for time being. A max of 50 mg N in upper ground water has been a main goal since 1990ties. In intensive livestock areas, choice is between extensification, improving efficiency of inputs, and/or technical innovation. Practices of adapting the animal to the environment are genetics and feeding. In the Netherlands, on 150 farms the goal is to lower crude protein (CP) to 155 gram per kg dry matter total ration (nationally it was 165 gram per kg dm). Plan is to upgrade this to all dairy farms through a covenant between all stakeholders and Ministry. Data indicates that 1 gram less CP results in less N in manure and 1% less NH₃ emission. Denmark introduced a CO₂ tax. Dairy farmers are urged to reduce CH₄ emissions by using either more fat or Bovaer in the ration. The cost is currently around euro 0.3/cow.d and tax rate euro 16/ton CO₂eq in 2030 and euro 40 in 2035 (subsidies available). Another approach is to adapt the environment to the animals. Reducing NH₃ emissions with innovative techniques are being evaluated in case control studies in combination with commercial farms. A list of floor types, cow-toilet (-35%) and Lely Sphere (-75%) are available with the established emission factors. In addition, we study a simultaneously filtering of CH₄ and NH₃ from the air in housing facilities. The low level of CH₄ concentrations in open barns complicates this; in CCC farming project, 5 to 100 ppm on 60 farms across Europe. A review study was submitted to JDS considering collecting air at different areas in the barn (close to mouth cows) or mixed with air from a closed manure storage, different ventilation techniques and optimizing filtering techniques like biobeds. Acidification of manure reduces both gasses. Renure (REcovered Nitrogen from manURE) offers new opportunities to value manure and use N from manure instead of artificial fertilizer. A policy shift is envisaged from means regulations to target regulations, providing the farmer with his own capacity to reach the prescribed targets.

Session 17

Theatre 8

Low carbon projects developed in the LIFE Carbon Farming project

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The EU LIFE Carbon farming project aims to reduce greenhouse gas emissions by 15% on 700 livestock farms in 6 EU countries. The objective is to achieve a total of 350.000 t CO₂ eq. avoided in 2027 through low carbon practices. The results presented give an overview of the strategies chosen by farmers to achieve this goal. The different categories of action levers aim to improve herd management, herd feed, land use, energy use and carbon sequestration. The database includes 40 farms in Belgium (BE), 324 in France (FR), 16 in Germany (DE), 26 in Ireland (IRL), 33 in Italy (IT) and 125 in Spain (SP) for a total of 564 farms. In FR and BE, production systems are: dairy (D), dairy with crops (D+C), dairy with beef (DB), beef (B), beef with crops (BC) and dairy and beef with crops (DBC). In DE, only D and DC are presented. In IRL, SP and IT, farms belong to D or B. Thirty-one levers are offered to farmers, who can choose more than one. Carbon gains from lower emissions and carbon sequestration are estimated considering the expected impact of low-carbon practices. The choice of levers depends on the country and the production system. In BE, 39 out of 40 farms have chosen to improve herd management. The most popular lever aims to decrease the age at first calving (73%). In FR, extending the rotation period of temporary grassland was selected in 51% of farms. In IRL the increasing use of protected urea is widely favoured (83%), while in DE it is the planting of hedges (81%). In IT, farmers choose to improve animal welfare (80%). In SP, the use of food additives is envisaged by 70% of farms D, while B farms aim to reduce the percentage of protein in the ration. The total expected carbon gains are 281624 t CO₂ equ. The contribution of each partner to this result amounts to 13% for BE, 78% for FR, 1.5% for DE, 0.5% for IRL and finally 7% for SP. The estimate in IT must be finalised.

Nitrogen: the driver of whole-farm sustainability in New York dairy farms

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Nitrogen (N) use efficiency and greenhouse gas (GHG) emissions are two main environmental key performance indicators (KPIs) for dairy farms in the northeastern US. At the whole-farm level, N use efficiency can be monitored using a whole-farm nutrient mass balance (NMB) assessment. The whole-farm N balance is derived by calculating the difference in the amount of N imported into and exported out of the farm boundary in a given calendar year. The balance can be reported as two KPIs: (1) per unit of land to indicate the ability of the farm to recycle N, and (2) per unit of product to indicate how efficiently nutrients are being converted into milk. Similarly, whole-farm GHG emissions can be monitored and reported as GHG emission intensities per unit of land and per unit of milk produced. As N is more difficult to retain from one year to the next, a large portion of the N balance will be lost to the environment. Some of these losses will be as nitrous oxide (N₂O), a potent GHG. This suggests that higher N balances could indicate higher N₂O emissions and total GHG emissions at the whole-farm level. Here we present data from 90 unique northeastern US dairy farms, collected between 2019 and 2023, and ranging from 26 to 6000 head of lactating cows. We calculated whole-farm N balances on a per hectare and per kg milk basis as well as whole-farm N₂O, CH₄ and CO₂ emission intensities, and identified relationships between N balances and GHG sustainability KPIs. Results show that as N balances improved, GHG sustainability KPIs also improved. Results also identified feed and fertilizer practices as common drivers for both N use efficiency and GHG emissions.

Session 17

Theatre 10

Towards Net Zero in dairy production: Initial carbon footprint results

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To mitigate its impact on greenhouse gas, the Dairy Farmers of Canada have committed to achieve carbon neutrality by 2050. Aligned with this goal, 20 dairy farms in Québec, Canada, are taking part in a Living-Lab initiative. The farms are located in four regions of the province and encompass a wide variety of farming models. Some farms in the northeastern regions experience colder temperatures. Farms in the southwestern regions tend to have more diversified operations, with crop sales playing a greater role, though dairy production remains their primary focus. A baseline carbon assessment was conducted using 2022 as the reference year. Using the Holos (developed by Agriculture and Agri-Food Canada) calculator the whole farm emissions were on average ($\pm$ SD) 1390.2 $\pm$ 697.5 kg of CO₂ equivalent (eq) per year. The values ranged from 432.6 to 2988.7 kg of CO₂ eq per year. On average 79.1 $\pm$ 21.7% of the emissions were associated with milk, 9.3 $\pm$ 2.7% associated with meat and 11.7 $\pm$ 14.5% associated with cash-crops production. The role of cash-crops in farm emissions varied by region. In one of the southwestern regions, cash-crops accounted for an average of 18.8% of total farm emissions, reflecting a greater emphasis on diversified production systems. In contrast, in one of the northeastern regions, sold crops represented only 1.5% of total emissions, demonstrating the stronger reliance on dairy as the primary output. On average, the distribution of emissions by sector was as follows: 41.3 $\pm$ 10.2% of total emissions were attributed to enteric CH₄ from animals, 19.5 $\pm$ 7.1% to manure (with 76.2 $\pm$ 20.3% linked to manure CH₄ and 23.8 $\pm$ 20.3% to manure N₂O), 25.7 $\pm$ 12.2% to fields N₂O and 13.6 $\pm$ 5.2% to inputs CO₂. On average, the farms sequestered 73.0 $\pm$ 110.3 kg of CO₂ eq. per year in their soils, though three farms recorded a net carbon loss rather than sequestration. For each farm, best management practices (BMPs) aimed at reducing emissions and enhancing carbon sequestration were identified. The implementation of these BMPs is set for 2025, with a follow-up carbon assessment planned for 2026. By implementing on-farm carbon reduction strategies, this project contributes directly to Canada's climate commitments while supporting the long-term sustainability of the dairy industry.

Establishing the referential costs of carbon action plans for livestock farms in Europe

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One of the goals of LIFE Carbon farming is the uptake of carbon farming practices by the incentive of a financial scheme. To do so, the cost and benefits of carbon action plans must be evaluated. The project is implementing action plans for 700 mixed crop livestock farms across Europe. Each farm does an environmental evaluation. An action plan is built to identify the different levers relevant for their farm. Technical, environmental, and economic impacts are evaluated. For the latter indicator, direct costs and investments are considered. The first analysis concerned 353 farms from 3 countries (84% France, 11% Belgium, 5% Germany) and was done with CAP'2ER tool. The average farm of the sample had an area of 166 ha, and produced 555,044 litres and 22,442 kg LW (live weight). The average carbon gain is 671 t CO₂ eq/farm on a 5 year plan of which 34t from sequestration practices. The economic impact is positive on average with a gain of €11,942/farm or €3/t CO₂ eq. The top 3 first practices chosen by farmers are: evolution of the temporary grassland rotation, optimizing mineral fertilization and improving age at first calving. However, if we analyze the sample focusing on investment, results are quite different. The financial gain is €43/t CO₂ eq for farms without investment, whereas the cost is €35/t for farms with investment. These initial results show in general an economic gain for the farms but with important differences depending on levers choices or investments. This finding raises questions about difficulties in the uptake of those practices despite the apparent economic benefit. A further aim of this project is to survey farmers about indirect impacts of those action plans focusing on work and skills.

Session 17

Theatre 12

The Road toward sustainable farming: from science to policy

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Many studies have been conducted to help policymakers make more informed decisions on sustainable development. At the same time, scientific research is only one source of information. In addition, there are promises made by politicians to voters, lobbies, non-governmental organizations, etc., which significantly influence the decisions made. The situation is particularly difficult in agriculture, which is a complex and highly regulated sector that affects almost all aspects of well-being. Various approaches have been used to systematize information and ensure constructive dialogue between stakeholders, one of which is MACC (marginal abatement cost curve). In Latvia, the development of MACC was widely used in climate and environmental policy, which supported climate and air policy decision-making. In total, 6 studies have created MACCs that analyse various agricultural GHG and ammonia reduction measures and their impact on the farmer's economy and climate and air policy goals, as well as the CAP as a whole. The use of MACC has helped shape Latvia's agricultural, as well as environmental and climate policy by providing analytical information. A special role is played by GHG and ammonia reduction measures in livestock farming, which significantly affect Latvia's international obligations. At the same time, scientific research creates a basis for discussion and points to the potential, which is significantly influenced by the interests of farmers, lobbies and policymakers, that the measures should be transformed into direct support or investment support payments for farmers. We need ever-new ways to balance our expectations from agricultural production, which should be economically viable, support ecosystem services, meet food needs, and preserve rural lifestyles. This means being able to develop the tools necessary for decision-making.

Mitigate GHG emissions on mixed livestock systems

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The Climate Neutral Farms (ClieNFarms) project aims to reduce greenhouse gas emissions and increasing carbon storage in the agricultural sector. This project is based on a network of 20 demonstration case studies (I3S) where systemic innovative solutions were tested and evaluated using up-to-date modeling approaches and multicriteria assessment tools. We will focus on the dairy and beef case studies in Ireland, France, Netherlands, Spain, New-Zealand, Germany, Switzerland, Ukraine and Poland. Each farm carried out an initial assessment to define its environmental impact and, in particular, its carbon footprint. From this point, the farmers defined their action plan in co-construction. They rely on the catalogue of solutions drawn up by the project, as well as on the discussions held at their region's creative arena, which brought together various players in the production sector. The levers chosen by the farmer are adapted to their own system and production objectives. The aim is then to measure the environmental and economic impact of implementing these practices. This will involve a final assessment to quantify these changes, in particular the carbon footprint, but also other environmental indicators such as biodiversity, nitrogen management, water quality and soil health. The data collected will also enable a comparison of the diversity of carbon footprint calculation tools that exist in Europe. This tools comparison will highlight the different emission factors or sensitivities of the different tools. Finally, one of the aims of this project is also to disseminate these mitigation solutions via demonstration events to encourage peer-to-peer learning, or via microlearning to reach an audience of advisers and students. Involving the supply chains in this project, in particular via dairy companies, is also a way of broadening dissemination. Once the prior economic impact has been defined, suitable incentives can be set up by players downstream of production. The ambition of this upstream-downstream agricultural project is finally to introduce practical mitigation solutions on farms, which will be widely disseminated and whose impact and measurement methodology will be assessed.

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Poster 14

Ammonia measurements in a naturally ventilated dairy barn with a low-cost sensor system

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Introduction The European Union aims to reduce emissions with a negative environmental impact, such as ammonia. In order to attain these goals, emission reducing techniques are necessary. The effectiveness of those techniques needs to be quantified using reliable emission measurements, which remain expensive up to this day. Concentration measurements using low-cost devices could provide a solution to verify emission reduction techniques in a more cost-efficient way. **Materials and methods** In this study, a commercially operated, naturally ventilated dairy barn was equipped with a low-cost monitoring system, OTICE (Online Tool for monitoring Indoor barn Climate and Emissions), which consisted of 10 wireless, low-cost gas sensor nodes (VOCsSens, Belgium). In addition, a high-end emission measuring system using PE sampling tubes and a gas analyser (Axetris LGD F200-A, Switzerland) was installed as a reference. Six nodes were positioned at one of the five sampling ports of a sampling line centrally positioned inside the barn, while two nodes were positioned at the sampling ports of two background sampling lines. A thorough analysis of the ammonia concentrations measured by the sensor nodes will be shown, assessing the OTICE system as well as comparing with the reference system. The research is ongoing and results will be presented at the conference. **Acknowledgements** The authors acknowledge the financial support through the partners of the Dairymix project, funded under the Joint Call of the Cofund ERA-Nets SusCrop (Grant N° 771134), FACCE ERA-GAS (Grant N° 696356), ICT-AGRI-GOOD (Grant N° 862665), SusAn (Grant N° 696231). In addition the authors acknowledge the financial support of the department of environment of the Flemish government.

Enteric methane dilution effects achieved with the genetic enhancement of Girolando cattle raised in tropical whether

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This study aimed to i) conduct a descriptive analysis of the genetic progress in milk production achieved by the Girolando breed genetic improvement program in Brazil and ii) assess the impact of these programs on the intensity of enteric methane (CH₄) emissions. The data from 22 years of official lactation records from the genetic improvement programs were analyzed, comprising 236,659 lactations from 129,968 Girolando cows. Enteric CH₄ emissions were estimated using a model specifically developed for dairy cattle in the tropical regions of Latin America and the Caribbean. A linear regression model was applied to evaluate the dilution effect of enteric methane emissions resulting from genetic gains in milk production. Over the study period, milk production (kg milk/305-day lactation) increased by 87%, with an average daily CH₄ production of 309.77 g CH₄/day. The average of CH₄ intensity over the 22-year program was 16.65 g CH₄/kg milk. A dilution effect on emission intensity was observed with an annual decline in CH₄ emission intensity of 0.99%/year, as described by the equation: methane Intensity (g/kg milk) $y = 808 - 0.391 \times X$ (year), $R^2_{adj} = 0.93$. These findings demonstrate that adopting improved Girolando genetics can significantly reduce enteric CH₄ emission intensity, thereby contributing to enhanced environmental sustainability in livestock production systems across Latin America and the Caribbean.

Assessment of greenhouse gas emissions from beef pilot farms in Latvia

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Beef production become important part of total livestock production in Latvia and raised almost from zero to 60526 animals in the last thirty years. Farming structure of beef production system differ among countries and depend of available resources and customers or world markets preferences. Nowadays, the structure of beef farms in Latvia can divide in two basic forms extensive when feed only on grazing base on summer and hay/silage in winter. Another system also based on grazing and rough feed and grain. This research aims to choose and monitor pilot beef farms that representing different farming systems to collect reference data based on actual farm characteristics for the estimation of emission for greenhouse gas (GHG) inventory. Latest IPCC Guidelines recommend collecting data from farms with different productivity systems. Data from pilot farms were collected, additional feed samples were analysed and weight control data were provided. Information about animal age, weight according national beef production recording system and animal movements were taken from Latvia Agricultural data register. Animal weights and feed characterization data are essential for accuracy of GHG, because these types of input data vary among different farming systems, affecting the ability to realize transparent and accurate GHG emission reporting in line with the data quality objectives. Furthermore, it is important to take in account that for beef feeding in Latvia are used biodiversity areas. Understanding of the emission profile for the different beef production systems in Latvia will help farmers to implement appropriate GHG mitigation strategies.

Water Quality: Manganese in drinking water for dairy cattle

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Climate change affects livestock systems through direct impacts on the physiology, behavior, production and welfare of animals and indirectly through the availability, composition and quality of food. Water is essential for the life of animals, since it intervenes in various metabolic processes. In dairy farms, the use of quality water is essential to maximize the milk production of animals. Manganese are very common pollutant that can occur naturally inground water and cause severe staining and a metallic taste, resulting in reduced water intake and milk production. The aim of this study is to see if water quality (excess manganese) affects production, reproduction and health in dairy cows. The study was carried out on a dairy farm in Alentejo-Portugal. The objective was to verify whether there were any effects of manganese toxicity. Two experimental groups were established, balanced for: Age, Number of calvings, Milk butyric content, Milk protein content. All the experimental groups were in the same conditions: Farm/stable, feed, ambient temperature and humidity, same number of drinking troughs, same trough space, same feed distribution time, same farm health management. Variation factor – manganese concentration: Treated group <50 µg/L (DGAV, 2014), Untreated group >1500 µg/L. Blood, urine and milk samples were taken over a 4-month period. The preliminary results show that the group of animals without treated water (excess manganese) has a 22% reduction in the consumption of kg of feed and 26% in water consumption; – 879 litres/lactation; -2.8L/cow/day; + 63 days of drought; + 2.4 inseminations; + 57 days of calving interval; + 33% embryonic mortality rate; +19% use of oestrus synchronization protocols. When we compare the experimental groups, we see that the difference in production is statistically significant for milk production. ATS and GGT values are higher than normal, which means that we may be in a situation of manganese toxicity.

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Poster 18

In vitro evaluation of the effect of a microbial product applied on the slurry on ammonia emissions

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Nitrogen content of the slurry produced in the farms causes the emission of harmful gases that constitute an environmental risk. To mitigate the harmful effects, slurry pits can be treated with products able to stimulate fermentation and promote the development of positive flora. The objective of this experiment was to evaluate in vitro the effect of a mix of bacteria and enzymes (Manure Pro (MP), Lallemand SAS) on ammonia emissions of slurry samples from a post-weaning piglet facility. Two doses of MP were compared to a control sample (CON): 5 g/m³ and 10 g/m³. The CON was compared to each of the doses in independent experiments (9 and 7 days for 5 and 10 g/m³, respectively), and two replicates per treatment were prepared. A system was set up to record the ammonia emissions. Briefly: 1) an air pump, an air flow meter and 0.22 µm sterile air filter, dosing continuously 0.5 L/min; 2) a Drechsel impinger containing 100 mL distilled water; 3) the humidified air is led into a 500 mL reactor containing 120 mL of slurry; 4) the outlet gas flow from the reactor is directed into 80 mL of 0.1 mol/L H₂SO₄ which traps the ammonia and converts it to ammonium ions. Daily samples were taken for total ammonia nitrogen (N-NH₄⁺) analysis. One-way ANOVA was performed with treatment as main effect. When 5 g/m³ were applied, the CON slurry depicted higher ammonia emission than the slurry with MP (P=0.023). The differences reached 69% of reduction at day 4 of the experiment (P=0.014 and P=0.096 for day and accumulated emission, respectively). Total ammonia emission when applying a dose of 10 g/m³ was also lower in the MP samples (P=0.053). We found lower ammonia emissions in the slurry with MP at day 4 (P=0.038), a reduced ammonia accumulated in the slurry with MP at day 6 (P=0.05), and a trend to a lower ammonia accumulated in the slurry with MP at day 5 (P=0.095). Our results show the positive impact of the application of MP on the reduction of ammonia emissions of slurry. Given the observed daily trend of the emissions along the experiment, it is recommended to test the effect of the re-application of the product 4-6 days after the first application.

Effect of supplementing rumen-protected methionine to low-protein diets on the performance of dairy cows

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The low nitrogen use efficiency (NUE) is one of the increasing environmental concerns. The NUE can be improved by reducing the dietary protein content of dairy cows, which can lead to a lack of limiting amino acid intake. The objective of this experiment was to study if the rumen-protected methionine (RPM) would improve milk production also with grass silage diet when the metabolisable protein (MP) concentration is low. The experiment was conducted with 33 dairy cows in a four-period (period length 14 d) crossover design with 2 × 2 factorial diets: low (MP 91 g/kg DM) or high protein supplementation (MP 99 g/kg DM) with or without RPM (RPM+ and RPM-). The trial included grass silage fed as PMR with concentrate proportion of 450 g/kg DM. Two TMRs, comprising silages, barley, a mineral mixture and two different levels of rapeseed meal were used to adjust the treatment MP level. Methionine (6.4 g/day) was delivered via AMS during milkings. The data were analysed using a mixed model including period, block, protein level, methionine and protein level × methionine interaction as fixed variables, with cow as a random effect. RPM did not have main effects on milk (RPM+ 32.9 kg and RPM- 32.9 kg; $P = 0.861$) or energy corrected milk (ECM) yield (36.5 kg and 36.5 kg; $P = 0.888$). No effects of RPM were observed in DM ($P > 0.1$) or nutrient intakes ($P > 0.05$). However, a significant interaction between MP level and RPM in ECM ($P < 0.05$) was observed, indicating higher ECM yield for RPM when fed with high protein supplementation level. As no biological explanation for this effect is available, it is most likely due to random error of the experiment. High MP level in the diet increased milk yield (32.3 and 33.5 kg), ECM yield (36.0 and 37.0 kg) and DM intake (23.3 and 24.9 kg). The production responses for MP feeding (61 g milk protein/kg crude protein) observed in this study were markedly lower compared with earlier studies. One reason to this can be the high MP concentration with high supplementation diet, as protein supplementation responses are low with high MP level diets. It can be concluded that methionine is not a limiting factor in grass silage diets even with low level of diet MP concentration.

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Poster 20

Comparison of ammonia and greenhouse gas concentrations in tied-in and loose housing systems for dairy cows in Slovenia

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Cattle represent a source of methane and cattle manure of ammonia and a smaller part of the methane besides other GHG gases. In 2023, there were 4,805 dairy farms in Slovenia. Most of them still house dairy cows in tied-in barns, while the number of farms with freestall barns is increasing each year. The aim of the study was to determine the effects of the housing system on the concentration of NH₃, CH₄, CO₂ and N₂O on five Slovenian farms. As part of the EIP project we carried out monthly measurements of NH₃ and GHG concentrations at different places in dairy cattle barns with different housing systems from August till November 2024. Each measurement lasted 5 minutes. For the measurements we used the portable gas analyser GASMET GT5000 Terra. In addition to GHG concentrations, microclimate parameters (temperature, relative humidity and air flow) were also measured using a TESTO 435 multimeter. Based on the 663 measurements, we found that there are differences in the measured GHG concentrations among farms with different housing systems, different methods of removal and storage of animal secretions and in terms of measurement height. We found that, on average, the measured NH₃ concentrations were lower in loose (1.33±1.02 ppm), compared to tied-in housing systems (1.88±1.34 ppm). Similarly, GHG concentrations were higher in tied-in housing systems (752.77±219.28 ppm CO₂; 30.41±20.98 ppm CH₄; 0.29±0.11 ppm N₂O) compared to loose housing systems (580.61±121.68 ppm CO₂; 26.78±25.49 ppm CH₄; 0.26±0.05 ppm N₂O). In addition, we found that the height of measurements had a statistically significant effect on measured concentrations of CO₂ and CH₄ ($p < 0.001$) and NH₃ ($p < 0.05$), while it did not have a statistically significant effect on measured concentrations of N₂O ($p = 0.341$). It should be realized that the measured concentrations do not reflect the total amount of emissions produced in the different type of barns. The amount of emission is determined by the air flow in combination with the measured concentration. As known, the air flow is much higher in loose housing systems, diluting the measured concentrations, than in tied-in barns.

Nitrogen source affects intake, production, and nutrient efficiency in early lactation Holstein cows

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Essential AA supplementation has long been proposed as a strategy to improve milk production and N efficiency. We aimed to evaluate the impact of long-term (12 weeks) feeding isonitrogenous diets with different rumen degradable protein (RDP) to rumen undegradable, digestible protein (dRUP) ratios (D/U) on milk production and N efficiency. We hypothesized that reducing RDP supply and balancing for key essential AA (Arg, His, Ile, Leu, Lys, and Met) would increase AA output in milk, and in turn, N use efficiency. Cows were blocked by pregnancy and calving date, and randomly assigned to diets with high (3.2 %, HD/U) or low (1.8 %, LD/U) RDP/dRUP for the first 12 weeks of lactation. Both diets were formulated to contain 17 % CP, 22.8 % forage NDF, and 25.8 % starch. Dry matter intake was measured daily, milk yield was measured at each of the 3 daily milkings, and sampled 6 times per week for components analysis by FT-MIR. Data were analyzed with a mixed linear model, with lactation, block, week, and diet were the fixed effects, and cow within block and lactation the random one. Decreasing dietary RDP reduced DMI (2.85 kg/d; $p < 0.05$) on weeks 2 to 9, except for week 5 along with week 10, when DMI tended to decrease ($0.1 > p > 0.05$). Despite the differences in DMI, milk production was not affected by treatment. Furthermore, fat yield tended to increase by 181 g/d across the 12-week study ($p = 0.057$) for LD/U cows, driven by a numerical increase in de novo, a trend (44 g/d, $p = 0.089$) in mixed, and a significant increase (16 g/d, $p = 0.024$) in preformed fatty acid yield. In line with DMI, N intake was decreased by LD/U, while milk protein content was not affected ($p = 0.3$) and protein yield tended to be slightly decreased (20 g/d, $p = 0.1$). In turn, N use efficiency tended to increase (35 vs. 32 %, $p = 0.063$) across the trial in LD/U cows. These findings suggest that reducing RDP while balancing for key essential AA may increase butterfat production and enhance N use efficiency.

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Poster 22

Comparing methods of estimating sniffer-based background gas emissions on dairy farms

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Sniffers installed on dairy farms provide continuous measures of gas concentration which includes gases from the breath and belching of cows, and background gas concentrations. Estimation of background gas concentrations may be helpful to reduce noise in methane (CH₄) phenotypes defined from gas measurements of individual cows. In this study, we compared two methods to estimate the background CH₄ values. We used CH₄ values (recorded every five seconds) in one automatic milking system (AMS) unit recorded on the Dairy Campus in Leeuwarden, the Netherlands, from January to October 2024. For this study, we used CH₄ measurements recorded during idle periods between milkings (time between one cow-exit and another cow-entry in the AMS). Records were processed for estimating the background CH₄ values using the following two methods. In Method 1, CH₄ values on idle periods were categorized in different plateau numbers conditioned on differences between subsequent CH₄ values (a threshold was set to 15 ppm), threshold for min (0 ppm) and max CH₄ values (250 ppm), length of the plateau was set to 6 subsequent values. The start and end indices of the plateau regions were determined, and plateau regions were numbered. Stable plateaus were found that are in the range of 7 to 50 ppm. Finally, the background CH₄ values for preceding milking events were defined as the 1st quantile of each stable plateau was defined. In Method 2, we attempt to remove raw CH₄ values from the non-milking times based on criteria such as: moving average ranging from 5 to 95 percentile, outliers (t value <2.5), duration of the non-milking event (180s) and removal of records in the initial (60s) and final parts (30s) of the non-milking events. The filtered background values would be analyzed using a linear mixed model, where a single background value could be predicted for each milking event using the solutions from the linear model. Method 2 could provide varying levels of background values for different milking events, whereas method 1 provided similar ranges of background values across the months. Further study would focus on quantitative genetic analysis of methane traits after background correction.

Automated milk feeders – opportunities and challenges for calves and people

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Commonly used dairy calf rations may cause periods of hunger, delayed growth, and detrimental effects on organ development. Social factors for farmers and farm staff (life outside work) have been identified as important obstacles to reaching biologically normal milk allowances (BNMA). Automated milk feeders (AMF) may facilitate the farms' reaching BNMA but few use them. Our study aimed to understand how AMF use affects work and social lives of dairy farmers and staff as well as the milk feeding conditions essential for calf well-being. We conducted semi-structured interviews with owners or staff on 11 conventional Swedish AMF dairy farms with <70 to >250 cows feeding four week old calves between 5 and 11 L of milk or milk replacer. Interviews were recorded, transcribed and analysed thematically. Main reasons mentioned for choosing AMF were reduced time for feeding and washing, improved hygiene, ensuring similar milk feeding routines, reduced stress for the calves, difficulties in finding skilled staff and more flexible work hours. Participants perceived better-conditioned calves and less milk feeding and washing time to well cover AMF costs, indicating sufficient cost-benefit ratio. They reported that AMF significantly improved the farms' ability to provide their calves with more milk in small, frequent portions, more freedom of choice and smooth weaning, associating these factors with increased robustness of the calves. They believed the system improved their overview of the calves and allowed time for reflection. They recommended small groups of calves and stressed the importance of the calves' start in life and providing adequate attention to newly introduced calves and hygiene procedures. Although they generally reported a sense of vulnerability in case of failure and dissatisfaction with service organisation, the problems were surpassed by their overall satisfaction with the system. We suggest that well-managed AMF systems may enhance calf welfare and social sustainability in dairy calf care, leading to more robust calves with a reduced need for antimicrobials.

Session 18

Theatre 2

Cow-calf contact is an option for improving animal welfare on dairy farms: But it is not a certainty

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Over the past decade, there has been increasing interest in cow-calf contact (CCC) systems that allow cows to have prolonged contact with their own calves or to nurse other calves as foster cow. There are no standardised blueprints for implementing CCC on dairy farms. Farmers develop a system that meets the specific needs of their farm, resulting in a wide variety of systems. This makes it difficult to compare farms and often results are contradictory. Meanwhile, the number of German farmers practising CCC is increasing and one of the reasons for starting CCC on their farm is the expectation of better calf health and overall animal welfare. In the project "Calf rearing on cows: natural, healthy and feasible" we are visiting 38 CCC farms twice during the indoor and outdoor season to assess animal welfare using the German guidelines for self-monitoring of animal welfare on farms (KTBL, 2021). The large variability of the systems is also reflected in the cohort: the number of cows per farm ranges from 12 to 1,400 cows (median: 50) as well as the milk yield per cow and year (3,500 – 10,399 kg, mean $\pm$ SD: 6,280 $\pm$ 1,503 kg). Pure rearing with the dam is practised on 7 farms, while all the others use a combination between the dam and the foster cows or combine it with bucket feeding. The latter is often used to rear male calves. Severe deficiencies in the welfare of the calves were very rarely observed. However, on one farm up to 44% of the calves examined showed signs of malnutrition. A shortcoming on many farms was the provision of water to the calves. The number of drinking troughs was often insufficient or placed out of reach of small calves. Regarding the health of the suckler cows, it was found that four times more suckler cows were clinically lame compared to the dams. This suggests that farmers prefer to use cows that do not fit into the main dairy herd as foster cows – a fact that raises serious concerns about the welfare of these animals. Overall, the results of the first farm visit showed that CCC has the potential to improve animal welfare on dairy farms but it is not a self-running system.

The economic and technical impacts of management of natural suckling in dairy farming

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Early separation of young dairy animals from their dam is an increasing concern to consumer expectations and some farmers, mainly due to animal welfare issues. As a result, natural suckling —where young ruminants suckle from their dam or a foster adult—is increasingly adopted in France. However, technical and economic data are needed to help farmers to assess both monetary and non-monetary impacts of this management. This study aims to describe mother-young managements and evaluate their technical and economic impacts on dairy cow, goat, and sheep farms in the Auvergne-Rhône-Alpes region (France). Interviews on 42 farms revealed eight main types of rearing systems: 3 for dairy cows, 3 for goats, 2 for sheep. A techno-economic audit was then carried out for each main rearing type, along with a comparative audit of a conventional rearing system, using accounting data from 2021 to 2023. The farms studied were diversified, pasture-based, 90 % operated under quality labels, and 88% sold their dairy products directly to consumers. Farmers were mainly highly educated with no agricultural background, suggesting that these practices attract new farmers and meet societal expectations. These farmers were mainly motivated by ethical and animal welfare concerns. This practice reduces neonatal diarrhoea rates and improve growth, while increasing feed self-sufficiency by reducing reliance on purchased inputs such as starter feed and milk replacers. This practice improves the nature of the work, making it more observation-based, and reduces workload peaks during birthing periods and daily tasks by eliminating the need to feed young animals at milking times. Production cost per 1 000 liters of milk were on average 51% of the conventional rearing systems cost for dairy cows, 78% for goats and 68% for sheep. On average, current income was 93% of conventional systems for dairy cow, 59% for goats and 33% for sheep. Despite these differences, all farmers report high satisfaction with this rearing practice. The results need to be completed with a larger-scale study.

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Theatre 4

Development of a sociality index in suckling holstein calves to control welfare and production

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Successful group management, to reduce stress and disease incidence and optimize production, requires an understanding of the complexity of social structures. Gaining insight into the social network structure could thus serve as a basis for optimizing social herd management, thereby improving both welfare and productivity. To this end, we developed a quantitative index for sociality in Holstein calves, aiming at: 1. Evaluating the persistence of social network dynamics with age. 2. Assessing the impact of group reorganization on network structure. 3. Estimating the correlation between the sociality index and various behavioral and physiological metrics. Holstein male calves housed in groups of six, eight or twelve individuals, with an average living space of 13 m² per calf, served to develop the index. Proximity-sensing data loggers were used to monitor calves' interactions. To convert social network data into a quantitative index, we used the ANTs package in R software and focused on two metrics: The Simple Ratio Index (SRI) and eigenvector centrality (Eigen). Network analysis at a young age suggested a relatively stable social structure, with a slight deviation towards 'social isolation', which didn't persist later in life. Statistical tests confirmed that the sociality index reliably reflected changes in network structure due to 'personal' changes, caused by group reorganization. An intriguing finding was the relatively high negative correlation between the social index and feed efficiency, suggesting that social activity may come at the cost of reduced productivity. Negative correlations were found also between sociality index and the genes Fas and L-Selectin, involved in neutrophil lifespan and functionality, respectively, harboring potential risk for oxidative stress and inflammatory responses. In summary, while animal welfare principles support group housing, it may also have negative implications for health and productivity. Future examination of a broader panel of metrics is necessary to further elucidate this assumption.

Mind the gap? Spatial behaviour of virtually compared to physically fenced heifers

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Virtual fencing (VF) is a tool to improve and facilitate livestock grazing systems. The visual cue of a conventional fence is replaced by an audio cue emitted by a GPS-based VF-collar when the animal approaches the VF. The audio cue is followed by a short-time electric pulse when the animal continues to move outward. Many studies have refuted welfare concerns, but a study focusing on behavioural responses near the VF line has been lacking. Considering that an effect of VF on animal behaviour should be more prominent near the VF line than near the centroid of a respective paddock, we divided paddocks into a centre (at least 10 metres away from the VF; CZ) and a perimeter zone (PZ). Minute-wise behavioural data (retrieved from Nofence VF collars) of 32 Fleckvieh heifers (two groups of eight heifers virtually fenced; VF – two groups of eight heifers physically fenced; PF) from a rotational grazing trial in 2021 in Germany was analysed based on 2.5*2.5 m² grid cells, with each grid cell either belonging to the CZ or the PZ of the respective paddock. Generalised linear mixed effects models (R package ‘glmmTMB’) were calculated. The target variables lying time, active time and slope (topography) were evaluated on the fixed effects of fencing system (two levels) and zone (two levels) and the interaction of both. We found a significant effect ($p < .0001$) of the interaction of fencing system*zone on active and lying time. VF heifers preferred to lie in the CZ (no significant differences between zones during active time), whereas PF heifers preferred to lie in the PZ and spent more active time in the PZ. However, mean slope per grid cell was significantly lower in the CZ of VF and the PZ of PF. While the analyses of heifer behaviour suggest a significant interaction between fencing system and zone, the confounding effect of slope requires caution in attributing behavioural differences solely to the fencing system, highlighting the need for further research to disentangle these complex relationships.

Session 18

Theatre 6

Personality traits of dairy cows: Associations with home pen behavior and performance

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Personality traits cause dairy cattle to behave differently in challenging situations, thereby impacting their health and well-being. However, little is known about whether personality traits also cause individual differences in home pen behavior and production levels. Therefore, we conducted a novel object test followed by a forced human approach test to infer personality traits. Tested cows belonged to the lactating dairy herd of the research farm at Kiel University. Additionally data regarding milk production, body weight, feed intake (feed bins, Hokofarm Ric2) was available for 271 cows and information regarding home pen behavior (ultra-wide-band positioning system, metraTec GmbH) was available for 36 cows. Behavior and production level were recorded over a 14-day period prior to behavioral tests. Influences of personality onto home pen behavior and production levels were separately estimated using linear models. All models included the personality traits exploration, boldness, sociability and trust, as well as pairwise interactions between these traits. Furthermore, models accounted for differences in age (home pen behavior analysis) or lactation (milk production, body weight, feed intake). Three parameters describing home pen behavior were affected by interactions between personality traits. Highly explorative and highly trusting cows had significantly ($p < 0.05$) more contacts than less explorative cows. Highly explorative, less bold cows had more lying bouts than less explorative cows and the standard deviation of walking bout duration was negatively impacted by boldness in cows that are less explorative. Among production characteristics, feed intake was affected by varying levels of exploration, sociability and trust. These impacts also depended on the extent to which the interacting traits boldness, sociability and trust were expressed. The variation in trust also influenced body weight and milk fat content. Sociability negatively impacted milk yield, while enhancing milk protein content. Furthermore, boldness and trust altered milk fatty acid profiles when cows additionally expressed a certain level of exploration.

Allowing dairy cows to diversify their diet: effects on feed intake and welfare

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Contrary to natural conditions, dairy cows are frequently fed indoors with total mixed rations (TMR). This monotonous diet does not allow them to express their natural feeding behaviour, i.e. to select different food items. The aim of this study was to investigate the potential benefits of allowing each dairy cow to diversify her own diet on feeding behavior and welfare. Twenty-four Holstein dairy cows were fed ad libitum with a nutritionally balanced TMR during a 4-weeks pre-experimental period, and then, were divided into 2 groups (mean milk production of 41 ± 0.8 kg/d for 111 ± 2.7 DIM). In Period 1 (P1, 29d), the Control group was fed with the TMR only, whereas the Choice group had an ad libitum access to a good hay (regrowth of fescue) and a coarse hay (1st cut of permanent pasture) in addition to the TMR. Then, in Period 2 (P2, 27d), all cows were fed ad libitum with the TMR only. Feed intake and milk yield were recorded daily. Cortisol concentration was measured as a physiological indicator of welfare in plasma samples at the end of the pre-experimental period and three times each period, and in hair samples at the end of each period. During P1, the total dry matter intake was similar in both groups but cows in the Choice group reduced their intake of TMR by 2.1 kg DM/d ($P < 0.05$) and chose to diversify their diet by eating 2.5 kg DM/d of good hay. They did not eat the coarse hay. Milk yield of cows in Choice group slightly decreased (-0.9 kg/d, $P < 0.05$). During P2, cows in both groups had similar TMR intake and milk yield. Hair and plasma cortisol concentrations did not differ between groups and periods. When given the opportunity, dairy cows chose to diversify their diet even if they had an ad libitum access to a nutritionally balanced diet and preferred the high quality hay. This feeding strategy had no effect on the welfare of the dairy cows as assessed by hair and plasma cortisol concentrations.

Session 18

Theatre 8

Drinking behaviour of dairy cows prior to calving

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Cows exhibit behavioural changes before giving birth and reduce drinking time in the 3 hours prior calving. However, the effect of calving time on drinking behaviour remains to be investigated. The aim of this study was to evaluate the effect of parity, calving time, and observation period on drinking behaviour of dairy cows in the final hours prior calving. A total of 17 primiparous and 18 multiparous pregnant Holstein-Friesian cows were housed in 3 calving pens with a maximum of 8 cows. Cows were moved into the calving pens at three weeks before expected calving date. Cows were continuously monitored under 24 h video surveillance. The video footage was split into 6-hour time according to the period of the day: overnight (0:00 to 5:59 h), morning (6:00 to 11:59 h), afternoon (12:00 to 17:59 h), and evening (18:00 to 23:59 h). Duration and frequency of drinking behaviours over 9:45 h (SD 00:30) prior to calving were calculated yielding one value for each animal per "observation period", expressed as %. Calving time was also classified according to the period of the day that cows gave birth. A generalised linear mixed model was used to assess the effect of parity, calving time, and observation period on the duration and frequency of drinking behaviours, with Gamma distribution. In total, 5 cows gave birth overnight, 8 cows in the morning, 13 in the afternoon, and 9 in the evening period. Parity, calving time, and observation period did not affect the duration of drinking behaviour ($p > 0.05$). Parity and observation period did not affect the frequency of drinking behaviour ($p > 0.05$); however, cows that gave birth during the evening ($2.2 \pm 2.83\%$) and the afternoon ($1.2 \pm 1.97\%$) tended to show higher frequency of drinking behaviour compared to those calving at night ($1.1 \pm 1.09\%$) and in the morning ($0.6 \pm 1.16\%$; $p = 0.07$). Although drinking behaviour occurs at a very low frequency, our findings suggest it is still affected by the calving time, which could be associated with other factors, such as feeding time and environment temperature.

Use of Infrared Thermography to Monitor Ocular Temperature in Dairy Cattle

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Infrared thermography is emerging as a promising non-invasive technology that provides valuable information about the surface temperature of animals without direct contact. The main objective of this work was to monitor eye temperature using infrared thermography to identify its fluctuations during the day in order to validate this indicator. As a secondary objective, we endeavoured to correlate this indicator with THI. This study was carried out at the research facilities of the University of Trás-os-Montes e Alto Douro with a total of 22 lactating dairy cows. The animals were housed in a free stall barn, with access to water and silage ad libitum. The study took place a period of four days and thermographic images were taken every 2 hours between 7am and 3pm, using an infrared thermography camera FLIR E4 (FLIR Systems AB, Sweden) (60 x 80 pixels, spectral range 7.5–13 µm, 0.98 emissivity) at 0.5 m from the animal. Maximum, minimum and average temperatures were measured in an oval area traced around the eyeball. Ambient temperatures and relative humidity using IPMA data and transformed into the index THI. The data was analysed using JMP 12 to determine correlations between eye temperatures and the THI. A comparison of eye temperature averages between days, hours and animals was carried out using an ANOVA test. Low correlations were found between the minimum, average and maximum temperatures and the THI ($R = 0.23, 0.25, 0.18$; $p < 0.05$). No differences were found between animals for all eye temperatures ($p > 0.05$). Maximum eye temperatures showed the least fluctuations between days and times of day ($p > 0.05$). In this study we were able to observe a relationship between outside temperature and relative humidity and eye temperature, although this was very low. The maximum ocular temperature seems to be the one that fluctuates the least during the day, and may be the most reliable for carrying out stress-related tests. This work was supported by the projects UI/00772 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

Session 18

Theatre 10

Linking animal welfare indicators and serum resilience biomarkers using milk infrared spectroscopy in the Parmigiano Reggiano cheese production area

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In Italy, animal welfare (AW) is assessed according to the official protocol developed by the Italian National Reference Centre for AW (CReNBA), making routine verification and validation of welfare standards a priority in the dairy industry. In parallel, official milk recording schemes, along with the well-established mid-infrared spectroscopy (MIR) technology, have proven to be effective tools for detecting the potential metabolic distress in dairy cows. This study aimed at evaluating the association between CReNBA evaluation and milk MIR predictions of blood biomarkers related to stress response (including hepatic function, oxidative stress, inflammation, and innate immunity) within the Parmigiano Reggiano production area. The CReNBA assessment is divided into three macro areas: management (MGT), structure and equipment (SAE), and animal-based measures (ABM). These were divided into three classes for the analysis based on their score (0–100): low ($\leq 25^{\text{th}}$), medium ($> 25^{\text{th}}$ to $\leq 75^{\text{th}}$), and high ($> 75^{\text{th}}$). The final dataset included 1,297,505 test-day records spanning two years, collected from 966 multi-breed farms and 147,923 cows. Prediction equations were developed using a gradient boosting machine algorithm (R^2 from 0.45 to 0.82) on an independent calibration database of 1,367 lactating cows. A linear mixed model was fitted considering the MIR biomarkers predictions as function of days in milk, month, year, parity, breed, herd, animal, and the effects of MGT, SAE, and ABM. Results showed that SAE was the CReNBA score having more impact on the predicted resilience biomarkers ($P < 0.05$). Specifically, cows in the highest SAE class, those with high milk production, experienced a slightly higher metabolic overload, with lower level of paraoxonase and albumin, and higher for total reactive oxygen metabolites, advanced oxidation protein products and globulins. This study will enhance our understanding of the relationships between models used to assess overall farm AW and individual health parameters, assessed in the whole population.

Serum pepsinogen level and simple criteria for a targeted selective treatment approach in heifers at housing
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Serum pepsinogen level is currently the best indicator for assessing gastrointestinal nematode infections at the end of the first grazing season in heifers, but its evolution in late autumn and winter, a period of larval hypobiosis, is poorly documented, leading to risks of misdiagnosis and inappropriate treatments. Once the diagnosis is made, treatment is most often administered systematically to the entire group, increasing the risk of developing parasite resistance. This project aims to better understand the kinetic pattern of serum pepsinogen level and to determine whether criteria allow for selective treatment at housing by assessing weight gain post-treatment. To achieve this, blood samples were collected from 152 heifers across 10 farms to measure serum pepsinogen levels: monthly from late August until housing, then every 14 days for 2 months and again monthly until turnout. Half of the individuals in each herd were dewormed at housing, allowing for a comparison of growth rates as a measure of treatment impact. Pepsinogen levels of untreated heifers peaked in October at 1.45 ± 0.59 U Tyr, then decreased to 1.04 ± 0.16 U Tyr 1.5 month after housing, before rising again to 1.36 ± 0.41 U Tyr until turnout. The decline was more pronounced in herds with pepsinogen levels above 1.5 U Tyr at housing. Out of 4 farms > 1.5 U Tyr on pasture (including 2 > 2.0 U Tyr), only 3 still exceeded this threshold at housing, none did between 15 and 21 days later. The model indicated that treated heifers showed significantly greater growth than untreated ones ($p=0.004$) and heifers not considered at risk by the farmer also grew better than those deemed to need treatment ($p=0.008$). This study highlights the importance of pepsinogen assay and sampling period for deciding on deworming treatment at the group-level. Further research with higher infection levels is needed to confirm these observations in particular for assessing the selective treatment approach.

Session 18

Theatre 12

Novel neurobiomarkers for welfare assessment in intensively reared dairy cattle

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Identifying reliable biomarkers reflecting physical and mental health is crucial for improving welfare monitoring. Brain-Derived Neurotrophic Factor (BDNF) and kynurenine pathway metabolites have emerged as potential well-being indicators in dairy cattle. Our preliminary study reported alterations in these biomarkers in tie-stall systems, highlighting the need for validation. This study aimed to establish plasma reference values for BDNF, kynurenine (KYN), serotonin (5-HT), tryptophan (TRP), and the KYN/TRP (KT) ratio, and analyze their relationship to breed, housing (tie-stall vs. free-stall), welfare score, and productivity. A total of 377 dairy cows (Italian Friesian, FRS; Valdostana Pezzata Rossa, VPR; and Montbéliarde×Friesian crossbreds, MTT) from 42 farms in Northern Italy were enrolled. Plasma concentrations were determined by ELISA or LC-MS/MS, and welfare was assessed using ClassyFarm. Data were analyzed using variance analysis or regression models. BDNF was significantly lower in MTT than in FRS ($p<0.001$) and VPR ($p<0.01$). TRP was higher in FRS, while no breed-related differences emerged for 5-HT, KYN, or KT ratio. Tie-stall cows exhibited a higher KT ratio and lower TRP ($p<0.001$), suggesting increased TRP conversion into KYN. Although individual BDNF levels were higher in tie-stall cows, mean concentrations did not differ from free-stall cattle. BDNF levels decreased with lactation cycles ($p<0.05$), but no significant correlation was found between welfare scores and biomarkers. Reference values for BDNF, KYN, TRP, 5-HT, and KT ratio provide a foundation for their use as welfare indicators. Higher KT ratios and lower TRP in tie-stall cows support findings linking the kynurenine pathway to anxiety and depression-like behaviors. Greater BDNF variability in tie-stall cows may reflect neuroadaptive responses to chronic stress, while its decline with lactation cycles suggests cumulative physiological stress. These findings support using neurobiological biomarkers to improve welfare assessments in intensive dairy farming.

Correlation between panting score of suckler cows and heat stress

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The Hamburg Earth System Model MPI-ESM has so far been used to model scenarios for global warming and it is expected that temperatures will be around 4.8 °C higher by 2100. In addition, phases with long periods of heat have been increasingly observed in the last 5 years, which makes it more difficult for grazing animals in particular to adapt to these conditions. These statements must be taken into account and evaluated for suitable measures to reduce the stress factors in relation to heat stress. For the assessment of possible signs of heat stress, the cattle's respiratory rate, heartbeat and rectal temperature are very frequently recorded internationally as indicators. The frequency of respiration was measured and recorded. The panting score (PS) could be determined from the data, which would then enable a classification of possible heat stress in the animals. The 5 time zones of the day of 06:00, 09:00, 12:00, 15:00 and 18:00 were again used to describe the change in the respiratory rate of the suckler cows. Due to the grazing activities of the herd, the recordings were always made with a time interval of ± 30 minutes. The frequency of respiration can be categorized and used for evaluation. A mean value of 32 ± 6 breaths per minute was determined in the resting state of the suckler cows (fluctuations 18 to 44). Due to the data structure and based on the evaluations from the literature, 3 temperature ranges were determined: [1] up to a maximum of 24 °C, [2] 24 to a maximum of 30 °C and [3] more than 30 °C at midday. On the cool days, the panting score changed only insignificantly over the course of the day. On days with medium and also high midday temperatures, it was very clear that the highest panting score was recorded at 15:00. Towards the evening, a tendency towards a drop in breathing rates could be detected.

Session 18

Poster 14

Effect of alpine grazing on plasma BDNF, Kynurenine/Tryptophan ratio and 24-hydroxycholesterol in dairy cows

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Assessing welfare considering animals' ethological and psychological needs is challenging. New standardized biomarkers are needed to measure dairy cows' physical and mental health. In a previous study, we reported Brain-Derived Neurotrophic Factor (BDNF), Kynurenine Pathway (KP), and plasma lipid-profiling alterations in dairy cattle in permanent tie-stall housing. Valdostana Pezzata Rossa (VPR) is an Italian dairy breed characterized by tie-stall housing in winter and alpine pasturing in spring-summer. To evaluate whether alterations induced by tie-stall housing on BDNF expression, tryptophan (TRP) metabolites, and lipid profiles are chronic or reversible, 27 VPR cattle were monitored during alpine grazing. Plasma was collected before (T1) and after (T2) 17 weeks on alpine pasture. BDNF and neurofilament light chain (NF-L) plasma concentrations were determined by enzyme immunoassay (ELISA/ELLA), while TRP, Kynurenine (KYN), serotonin (5-HT), fatty acids, and cholesterol oxidation products (COPs) were assessed by chromatographic techniques coupled with MS/MS. Metabolites were compared by variance analysis. Our results showed a significant decrease in BDNF ($p < 0.01$) and KYN/TRP (KT) ratio ($p < 0.01$) during alpine pasture (T2) compared with T1, while NF-L plasma concentrations showed no significant modulation. Differences in COPs plasma concentrations were also observed. Specifically, 24-hydroxycholesterol (24-HC), a brain cholesterol metabolite, was significantly lower in animals on alpine pasture. High 24-HC, KT ratio, and proBDNF plasma levels are often associated with central nervous system inflammation and depression-like states. The decreased BDNF, KT ratio, and 24-HC plasma levels in VPR cattle suggest alpine pasture may improve their neuropsychological performance and well-being.

Agroecological Approaches in Poultry Production: Challenges and Opportunities for Advancing Animal Welfare and Health

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The integration of agroecological principles into poultry production presents a promising pathway toward more sustainable, resilient, and welfare-oriented livestock systems. Agroecology emphasizes ecological balance, biodiversity, and the co-creation of knowledge with local communities, offering a framework for addressing the challenges associated with conventional intensive poultry systems. Across diverse agroecological zones (from Europe, Africa, Asia and South America) smallholder poultry farming plays a vital socio-economic role, particularly for women, by enhancing food security, income generation, and cultural resilience. Despite these benefits, key constraints persist, including high chick mortality, disease prevalence (notably Newcastle disease and parasitism), feed limitations, and inadequate housing and veterinary support. Scientific evidence highlights the potential of locally adapted poultry breeds and low-input systems to enhance animal robustness, welfare, and adaptive traits in harsh environments. Breeding programs aligned with agroecological goals prioritize functional traits such as health, reproduction, and environmental adaptability, over high productivity alone. For example, the development of dual-purpose poultry breeds and the integration of chickens into agroforestry systems reflect efforts to balance productivity with ecosystem services such as pest control, nutrient cycling, and improved animal welfare. Challenges remain in aligning farmer knowledge with scientific research, improving access to markets, formulating sustainable feed options and scaling business models that support agroecological principles. Nevertheless, participatory training, policy support, and innovative value chains can facilitate the transition to agroecological poultry systems. This transition not only promotes animal welfare and health but also enhances resilience, equity, and sustainability in rural livelihoods.

Session 19

Theatre 2

Pecking blocks do not affect eggshell quality or feed intake of hens in enriched colonies

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Pecking blocks (PB) are a form of edible enrichment designed to reduce feather pecking behaviour in laying hens, but they also provide an opportunity for hens to supplement their diets with additional minerals. Previously, we found that individual hens who use PB have better eggshell quality at end of lay. We aimed to determine the impact of PB on egg quality parameters as well as feed intake (FI) at the group level. We used a 2x2x3 factorial design in which treatment (PB (TRT) or no PB (CONT)), genetic strain (796 Dekalb White (DW) and 796 Bovans Brown (BB)) and enriched colony (EC) style (30-bird (N=12), 32-bird (N=16) and 60-bird (N=12)) were the main effects. TRT were provided PB continuously from 18-70 weeks of age (WoA). The weight (WT), shell breaking strength (BS), and shell thickness (TH) of ~20% of eggs from each EC were measured on two consecutive days each month beginning at 24 WoA. Feed intake of each EC was measured over 3 days at 34, 49 and 69 WoA and expressed on a per-bird basis. GLMMs with repeated measures compared the fixed effects of treatment, strain, and EC type on egg quality and FI. TRT and CONT did not affect BS ($P=0.384$), TH ($P=0.486$) or WT ($P=0.694$). As expected, BS generally decreased with hen age ($P<0.001$), and DW eggs overall had a higher BS than BB eggs ($P<0.001$). TH was highest at 24 WoA, and there was an interaction between strain and age ($P<0.001$), in which DW shells were thicker than BB at 24, 28 and 32 WoA, and BB shells were thicker than DW at 64 and 68 WoA. As expected, WT increased with age, and there was an interaction between strain and age ($P<0.001$) in which BB eggs were heavier than DW at 24 WoA only. FI increased with age, and there was an interaction between strain and age ($P=0.011$) in which BB FI was greater than DW at 34 and 69 WoA. There was an interaction between treatment and EC style on FI ($P=0.029$) in which the 60-bird TRT consumed the greatest amount of feed, but this was not a consistent effect across the three EC styles. In conclusion, PB did not meaningfully affect egg quality parameters or feed intake at the group level.

Resilience of autochthonous Spanish breeds of laying hens to heat stress

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Global warming adversely affects poultry production. Autochthonous breeds are particularly valuable in a climate change context because of adaptation to the local environment. Differences in daily egg production between ten local breeds of laying hens was evaluated for a total of 295 days between December 2023 and September 2024 in 60 hens per breed between 9 and 18 months of age. Average daily egg production was highest in Andaluza Franciscana (0.54), Castellana Negra (0.53) and Cara Blanca (0.52), and lowest in Villafranquina (0.45) and Prat Roja (0.43) ($P < 0.01$). The impact of environmental temperature on egg production was estimated by a linear regression in each of five periods of 50 days and in one period of 45 days; the average temperature in these periods was 6, 9, 14, 21, 28, and 22 °C, respectively. In period 1 (mid-December through the end of January), production increased 0.014 egg/°C ($P < 0.01$). A positive relationship can (partly) be explained because growth coincides with rising temperatures. No significant relationship was observed in periods 2 and 3 (the end of January through mid-May). In periods 4 and 5 (mid-May through mid-August), higher temperatures reduced egg production at -0.008 ($P < 0.05$) and -0.012 egg/°C ($P < 0.01$); in period 5, Andaluza Azul (-0.022 egg/°C) and Andaluza Perdiz (-0.021 egg/°C) were most affected. In period 6 (mid-August through the end of September), higher temperatures again resulted in higher egg production (0.006 egg/°C; $P < 0.05$). Our results show differences in resilience to heat stress in autochthonous breeds. Crossbreeding of commercial with local poultry breeds may combine the benefits of high production with resilience to the local environment. Sustainability of production systems in the context of climate change requires novel breed choice and genetic selection objectives for improved heat tolerance.

Session 19

Theatre 4

Impact of Management Practices on Antibiotic Resistance in Broiler Farms: A Preliminary Analysis

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To reduce the incidence of antibiotic resistance in broiler farms, this preliminary analysis aimed to evaluate management practices in relation to the appearance of antibiotic-resistant bacteria. Nine distinct commercial broiler farms were assessed and extensively surveyed for their respective management practices, which encompassed specific aspects of farm, barn, fattening, feeding, cleaning, and health management. Samples were collected from water pipes, dust spots, and manure during the final week of fattening and analyzed for *Staphylococcus aureus*, MRSA, and ESBL-producing microorganisms. Target species were further identified by MALDI-TOF MS, and their antibiotic resistance profiles were characterized. The results revealed a high variability in management practices among the participating farms. Notably, feeding, cleaning, and health systems exhibited high heterogeneity, but fattening management can be clustered into two groups: conventional and ecological management. *Staphylococcus aureus* was detected in all dust and manure ($n=9$) samples, but only in three water samples (3/9). MRSA was absent in all samples ($n=27$). Dust samples and water samples (4/9) exhibited the highest rate of ESBL-producing target species, whereas in manure, only one sample was detected positive (1/9). Identified ESBL-producing species included *Enterobacter hormaechei*, *Escherichia coli*, *Stenotrophomonas maltophilia* and species with resistance to 3./4. generation cephalosporins (*Pseudomonas aeruginosa*). Correlations between the management systems and the occurrence of ESBL-producing species/species with resistance to 3./4. generation cephalosporins in dust appeared by increasing the food DM (-0.8; $p=0.01$) or decreasing the relative humidity (0.8; $p=0.03$). In conclusion, the findings indicate a substantial risk of the emergence and dissemination of resistant microorganisms via water lines and dust in broiler farm environments. This work (Model- and Demonstration Project for animal welfare) is financially supported by the Federal Ministry of Food and Agriculture, granted by the Federal Office for Agriculture and Food.

Diagnosing poultry infectious diseases: an overview on options and applications

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Infectious diseases pose a significant risk to poultry health and welfare as well as profitability in production. Reliable diagnosis of pathogens is crucial for therapy and prophylaxis. Clinical signs in poultry flocks usually include non-specific symptoms such as apathy, ruffled feathers and inappetence, which can occur in the context of various diseases. In contrast, some diseases produce pathognomonic symptoms that indicate pathological processes in certain organ systems. The use of new technologies, including on-farm biosensors and big data, should enable diseases with less obvious symptoms to be detected at an earlier stage. Necropsy and histological examination findings can lead to presumptive diagnoses of bacterial, parasitic and viral pathogens, which can then be confirmed or ruled out by pathogen detection. Bacteriological cultivation methods are relevant alongside staining techniques and biochemical detection methods. In addition, matrix-assisted laser desorption/ionisation with time-of-flight analysis of released ions for mass spectrometry (MALDI-TOF MS) is used, based on ribosomal protein profiles of bacterial isolates. Parasites such as ectoparasites or helminths can be observed directly on the bird, in faeces or at necropsy. For protozoa, microscopic examination of samples is indicated in routine diagnostics. Virus isolation is often used for the diagnosis of viral diseases, but this is not always practicable. Therefore, in many cases molecular biological methods are applied to detect and identify viruses. In general, molecular diagnostic methods, especially PCR, are used to allocate specific DNA and/or RNA to the causative in addition to methods described above. Pathogen-specific antibodies or antigens can also be identified using immunological tests. The combination of histological methods, molecular biological and immunological procedures (as in situ hybridisation or immunohistochemistry) allows to draw conclusions about tissue changes and pathogen localisation. In this overview, diagnostic methods and tools in poultry medicine will be discussed. Different case studies will be presented focussing on relevant diagnostic options for pathogen detection.

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Limiting ascarid infection of organic laying hens with vinegar: an on-farm trial

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A key characteristic of organic livestock rearing is the outdoor access. However, outdoor access increases the susceptibility for ascarid (*Ascaridia galli*, *Heterakis gallinarum*) infestation. For laying hens, consequences of worms include abnormal behaviour, low performance and high mortality rates. Laying hens can be treated with anthelmintics (e.g. flubendazole or fenbendazole). However, anthelmintic treatments have several disadvantages: (i) residues can be detected in eggs up to 10 days after treatment, (ii) resistance to anthelmintics could develop in laying hens as it is common in other species and (iii) anthelmintics or their metabolites in faeces can harm coprophagous organisms. Therefore, preventive deworming is not acceptable in organic farming. To minimise anthelmintic use, a combination of practices can be applied: (i) regular faecal egg counts to support targeted treatment schemes, (ii) hygiene management to reduce infection pressure and (iii) alternative products to reduce worm infestation. Among alternative products, acidification of water with vinegar is a common practice. To better understand the impact of vinegar, we studied ascarid egg count and laying hen welfare on 33 organic farms, with a total of 40 flocks, 20 each with or without vinegar in water. We observed no effect of treatment on ascarid egg count at 40 weeks of age and at slaughter (between 66 and 87 weeks of age). However, a linear mixed model revealed that ascarid egg count at slaughter was positively and significantly related to (i) the use of gravel (and not wood chips) in the uncovered outdoor area before the pasture and (ii) the breed. Similarly, we observed little to no effect of vinegar on hen welfare but the type of litter and the frequency of litter change improved animal welfare. Finally, we studied tibial bone health. While bone breaking strength was not related to the use of vinegar, we observed a slight decrease of ash. Therefore, vinegar may impact calcium mineralisation. To conclude, while vinegar did not limit ascarid infestation, our study confirms the usefulness of repeated faecal egg counts for targeted anthelmintic treatment and contributes to identifying management factors that play a role in ascarid infestation.

Evaluating the Impact of Untreated, Extruded, and Super-dosed Enzyme-Treated *Chlorella vulgaris* on Broiler Welfare

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This study evaluates the effects of incorporating microalga *Chlorella vulgaris* (MCV) into broiler diets—untreated, enzyme-supplemented, or extruded—on animal welfare parameters. A total of 120 male Ross 308 broilers (14–35 days old) were assigned to four dietary treatments (10 replicates each) and fed ad libitum: CTR (maize and soybean meal), CV (15% MCV), CVZ (CV + 0.3% pancreatin), and CVX (15% extruded MCV). Growth performance, beak and excreta scoring, organ measures, digesta viscosity, and blood samples were determined. All data were analysed using ANOVA (SAS software). MCV inclusion did not adversely affect growth performance, and the mortality rate was 0%. The CVZ group showed lower digesta viscosity relative to CTR and CV. Compared to CTR, all MCV groups presented worse beak scores without a decrease in feed intake and a temporary decline in excreta scoring during the first week, suggesting dietary adaptation. Pancreas and spleen weights remained unaffected. In comparison with CTR, heart weight decreased in CV animals, whereas liver weight decreased in all MCV groups, suggesting protective cardiovascular and hepatic effects. Blood analysis revealed higher glucose levels in MCV-fed groups and reduced AST and ALP, suggesting hepatic benefits. Urea levels were lower in all MCV groups, suggesting improved protein metabolism. C-reactive protein was increased in CV and CVZ groups compared to CTR, while white blood cell counts had an increasing trend with MCV, indicating a possible immune response. MCV appears to be a viable feed ingredient, showing possible health benefits without a negative welfare impact. Further studies should incorporate comprehensive behavioural assessments, stress biomarkers, and immunological analyses.

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Genotype-feeding interactions and the effects of insect protein on performance, health and welfare traits in organic laying hens

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Sustainable organic poultry farming implies to improve resource efficiency based on alternative protein feed components, while simultaneously improving animal health and welfare. In this regard, feeding of insect meal from the black soldier fly larvae (*Hermetia illucens*; HI) has been suggested, but genotype-specific effects on primary and functional traits are unclear. This study aimed to establish a cross-classified feeding experiment with equal genotype distribution. One group received 15% defatted HI, while the control (CON) received a standard organic ration to assess genotype-feeding interactions (GFI) on health, welfare, and performance. 360 hens were included from the 3 genotypes Lohmann Dual, ÖTZ Coffee (dual-purpose lines) and Lohmann Sandy (laying hybrid). Production traits for egg weight (EW), laying performance (LP) and feed conversion ratio (FCR) were recorded in weekly intervals. Health and welfare traits for diarrhea (DI), tail feather (TF) and belly feather (BF) damage were monitored three times using validated scoring schemes. Caecum length (CL) and liver weight (LW) were measured post-mortem. Effects of the genotype, the feeding group and of GFI on all traits were inferred by applying generalized linear mixed models. EW significantly increased in Coffee with HI feeding, but not in the other genotypes. FCR was improved in Sandy and Dual HI groups compared to the respective CON groups, but not in Coffee, explaining the significant GFI. CL was significantly shorter in Sandy HI compared to Sandy CON, but no significant feeding effects were observed in Dual and Coffee. Also, for health and welfare LW, DI, TF and DF, significant GFI were observed. Results from this cross-classified feeding experiment indicate that HI effects on performance and health are genotype-dependent, implying that insect meal as an alternative protein feed component does not affect all hen genotypes in a same direction.

Genetic selection for feather pecking and galacto-oligosaccharide supplementation shape the cecal microbiota of mature laying hens

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During severe feather pecking (FP) behaviour a hen damages the feathers of another, leading to compromised welfare. Genetic selection for FP and FP itself are associated with changes in the gut microbiota. Prebiotic galacto-oligosaccharides (GOS) promote beneficial bacteria, change microbiota-gut-brain communication, and may alter behaviour. This study investigated if GOS supplementation modifies the cecal microbiota during stress in hens of two genetic lines. White Leghorn hens (N=191) selected for high (HFP) or low (LFP) FP activity were housed together in 12 pens. From 29 to 32 weeks of age, hens received GOS (Nutrabiotic®, 74% GOS; Dairy Crest Ltd., Davidstow, United Kingdom) in water or plain water and underwent social stress via mixing with unfamiliar hens or remained undisturbed in a 2×2 factorial design. Next, hens were housed individually for up to 48 hours to collect cecal droppings. DNA was extracted from 16 samples per line and treatment combination (n=96). After 16S rRNA sequencing, taxonomy was assigned via DADA2 in QIIME2 using the Genome Taxonomy Database. Alpha diversity (Chao1 species richness, Shannon's diversity, Pielou's evenness) was analyzed in a generalized mixed model (SAS) for the effects of line, supplementation, stress, and their interactions. Beta diversity (Bray-Curtis) was evaluated using PERMANOVA. Microbiota differed between HFP and LFP hens in evenness (p=0.033) and richness (p=0.049) but not overall diversity (p=0.547). Supplementation with GOS altered diversity (p=0.003) and Chao1 richness (p=0.0001) but not evenness (p=0.306). Beta diversity differed by genetic line (p=0.001) and GOS supplementation (p=0.001). The findings confirm differences in the microbiota of HFP and LFP hens. GOS influenced species richness and overall community composition. Further analysis will identify the taxa driving these changes and their potential association with FP behaviour.

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Bacteriophage vB_SalS-KY05 Shows Potential in Reducing Salmonella Contamination in Poultry

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Salmonella serovars Typhimurium (S. T) and Enteritidis (S. E) are major zoonotic pathogens responsible for global foodborne contamination, primarily linked to poultry eggs and meat. Addressing this critical public food safety issue and the challenge of multidrug-resistant bacteria, researchers have turned their attention back to a powerful yet long-overlooked weapon—bacteriophages (BPs). BPs offer key advantages, such as host specificity and low toxicity to animals, making them a promising natural biocontrol tool. However, the interaction between BPs and their bacterial hosts is complex, particularly in live animal models. To assess the applicability of our novel bacteriophage vB_SalS-KY05, isolated from a local poultry farm in Taiwan, we conducted both in vitro characterization and an in vivo study using SPF chicks artificially infected with Salmonella. Additionally, we optimized the production process by transferring the bacteriophage to an alternative host to enhance productivity, administering it via drinking water. After a 7-day treatment, the low-dose phage group (L-phage, 10⁴ PFU/mL) significantly reduced the Salmonella load in the spleen (p < 0.05, Kruskal-Wallis test followed by Dunn's multiple comparisons), exhibiting an effect comparable to the 0.02% colistin group. Interestingly, we observed that bacteriophage dosage plays a crucial role, as the high-dose phage group (H-phage, 10⁸ PFU/mL) unexpectedly promoted Salmonella growth. Further analysis revealed elevated IL-1β and IL-6 concentrations in the plasma, as determined by ELISA, suggesting that inflammation contributed to bacteriophage-induced pathogen clearance. In conclusion, vB_SalS-KY05 demonstrated strong in vitro inhibition of both S. T and S. E and showed promising efficacy in a chicken model. However, our findings highlight the necessity of a thorough evaluation of BPs from isolation to application, ensuring their optimal use in poultry farming. This study lays the foundation for utilizing bacteriophages as a sustainable strategy to combat Salmonella contamination at its source.

Enhancing Growth and Minimizing Nitrogen Excretion in Broilers with Multi-Component Protease Supplementation in Low-Crude Protein Diets

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Supplementing synthetic amino acids in low-crude protein (CP) broiler diets is a strategy to reduce feed costs and nitrogen (N) excretion. However, excessive CP reductions can compromise growth performance. This study evaluated the effect of a fermentation extract (FE) on growth performance and N excretion in broilers fed low-CP diets. A total of 720 male Ross 308 broilers were assigned to one of five dietary treatments: 1) Positive Control (PC), a standard diet with 23%, 21.5%, and 19.5% CP in the starter, grower, and finisher phases; 2) Negative Control 1 (NC1) with 1.5-point CP reduction (21.5%, 20.0%, 18.0%) from PC; 3) Negative Control 2 (NC2) with 3.0-point CP reduction (20.0%, 18.5%, 16.5%) from PC; 4) NC1+FE, NC1 supplemented with FE; 5) NC2+FE, NC2 supplemented with FE. Diets were supplemented with methionine, lysine, threonine, tryptophan, valine, arginine, and isoleucine as needed to meet digestible amino acid requirements. Sodium bicarbonate and potassium chloride maintained dietary electrolyte balance. Phytase was included in all diets. Each treatment had 12 replicate pens of 12 chicks. Performance data were recorded on days 10, 24, and 42, and fecal N content was measured on day 42. On day 42, broilers fed PC, NC1+FE, and NC2+FE had significantly higher body weights (BW: 3,271 g, 3,263 g, and 3,257 g, respectively) compared to NC2 (BW: 3,143 g). ADG was significantly higher in PC, NC1+FE, and NC2+FE (76.9 g, 76.7 g, and 76.5 g, respectively) compared to NC2 (73.8 g). Mortality-adjusted feed conversion ratios (FCR) were better in NC1+FE (1.487) than NC2 (1.556), while NC1+FE was similar to PC (1.501), NC1 (1.528), and NC2+FE (1.499). The European Performance Index (EPI) for NC1+FE and NC2+FE was comparable to PC ($P=0.001$), indicating performance recovery despite reduced CP levels. Fecal N was lower in NC1+FE (1.79%) and NC2+FE (1.79%) compared to PC (1.95%; $P=0.011$). In conclusion, supplementing low-CP diets with FE effectively mitigated the adverse effects of CP reduction, restoring growth performance and reducing N excretion.

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Poster 12

Metabolic and Physiological Implications of Keel Bone Fractures in Laying Hens: A Study on Daily Energy Expenditure

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Keel bone fractures (KBF) are a significant welfare issue in the poultry industry, affecting up to 90% of laying hens by the end-of-lay. Despite their prevalence, research on the long-term metabolic and physiological impacts of KBF, particularly post-healing, is limited. To address this, we examined the effects of KBF on daily energy expenditure (DEE) in adult laying hens using metabolic measures like doubly labelled water. From 120 Lohmann Brown laying hens housed in 12 multi-tier systems, we selected 8 hens with and 8 hens without healed KBF at 92 weeks of age (confirmed via X-ray). DEE was measured by injecting isotopically enriched water (¹⁸O and ²H) into the pectoralis muscle of each hen, with dosage based on body weight. Birds were placed in individual cages for 1 hour to allow isotopes to equilibrate with body water, followed by blood sample collection for initial isotopic enrichment measurement. A second blood sample was taken 48 hours later to measure final enrichment using a liquid isotopic water analyzer. Results showed no statistically significant differences in mean DEE between hens with healed KBF and those without fractures. However, variations within groups and allometric relationships between body mass and DEE suggested potential metabolic implications of KBF. While the average DEE (1067.5 ± 46.66 kJ/day) differed from existing measures of peak-lay hens, the DEE of hens with KBF (1024 ± 134 kJ/day) did not significantly differ from those without fractures (1083 ± 189 kJ/day). Nonetheless, the allometric relationships of body weight and DEE were significantly different, with slopes nearly opposite between hens with and without KBF ($F_{1,12} = 5.979$, $p = 0.0309$). These findings highlight the need for further research to determine the specific effects of KBF on metabolism, production parameters, and welfare in laying hens.

Antioxidant Response After 2 Weeks of Supplementation With Different Primary Antioxidants

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Antioxidant supplementation is crucial for enhancing the antioxidant status of animals, helping to neutralize reactive oxygen species (ROS) and reduce oxidative stress. This study aimed to validate the efficacy of two primary antioxidant sources, a coated freeze-dried melon juice (MELOFEED; M-SOD) and selenized yeast (ALKOSEL; Se-Y), in stimulating antioxidant defenses and to demonstrate the stability of M-SOD when added to feed prior to extrusion. In a 15-day trial, 50 Ross 308 day-old chicks were fed crumble extruded pet food. The chicks were divided into 5 groups of 10 birds each (one pen per group): CONTROL (no supplementation), M-SOD on top, 130°C Extruded M-SOD with gastro-resistant coating, non-protected melon juice on top, and Se-Y added on top of the feed (0.2 ppm). At d15, blood samples were collected from the venous occipital sinus to measure the activity of two major antioxidant enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPx). Results indicated that SOD activity in whole blood was higher for M-SOD supplementation, regardless of whether it was added on top or before extrusion ($p < 0.1$). Interestingly, non-protected melon juice did not increase SOD activity, confirming the importance of the gastro-protection of M-SOD allowing the SOD enzyme to reach the ileum to interact with specific receptors and induce genes coding for the production of endogenous antioxidant enzymes in all tissues. Se-Y supplementation resulted in higher GPx activity compared to the other groups, highlighting the importance of providing adequate selenium levels for optimal GPx activity. This trial confirmed the mode of action of M-SOD, demonstrating its ability to increase endogenous blood SOD activity while maintaining stability and efficacy under typical pet food extrusion conditions. Proper coating is essential to ensure M-SOD's resistance to stomach acidity, preventing denaturation and loss of effectiveness. Se-Y enhanced GPx activity, highlighting that each type of antioxidant may stimulate natural defenses in a specific way. Combining different sources of antioxidants offers a promising approach to alleviate the detrimental effects of oxidative stress on birds.

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Poster 14

Metabolomic analysis reveals the molecular mechanism related to leg abnormality in broilers

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Fast body weight gain in broilers overloads their metabolic system resulting in leg abnormalities, which seriously affects animal welfare and industry economics. In this study, broilers with normal and deformed leg bones were examined. Serum biochemical indices showed that the serum calcium to phosphorus ratio was extremely decreased in leg deformed group. In addition, abnormal serum lipid levels suggested a disruption in lipid metabolism. Based on widely targeted metabolomic analysis of serum and cartilage tissues, a total of nine differential metabolites (DMs) significantly associated with leg abnormalities and serum calcium and phosphorus levels were screened, including carnitine C16:0, carnitine C18:1, 3-hydroxymethyl-L-tyrosine, cis-4-hydroxy-D-proline, cis-L-3-hydroxyproline, trans-4-hydroxy-L-proline, and so on. Pathway analysis revealed that fatty acid degradation and arachidonic acid metabolism were enriched. Analysis of DMs in these two pathways showed that prostaglandin D2, prostaglandin J2, prostaglandin A2, 15-keto prostaglandin F2 α , and Δ 12-prostaglandin J2 significantly differed between the groups with normal and deformed legs. It was hypothesized that these important metabolic pathways and metabolites were involved in the metabolic regulation of leg abnormalities.

Susceptibility of a lactic acid probiotic bacteria with coccidiostats

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Probiotics for farmed animals are gaining exponential interest as a feed additive to enhance gut health and improve zootechnical performance. Maintaining the integrity of the live microorganisms is fundamental to guarantee that the probiotics will deliver their benefits. However, the combination of a microbial additive with other additives, such as chemical compounds, could harm the microbial cells viability. Further to the minimum inhibitory concentration method typically used, assessing the probiotic strain viability under conditions closer to the practical exposure of both additives is of field interest. In this context, this study aimed to develop an in vitro model to test the compatibility of coccidiostats with the probiotic strain *Pediococcus acidilactici* (CNCM I-4622) whose beneficial effects are notably driven by its ability to produce L+ lactic acid. Five different coccidiostats were mixed in a crumble starter feed to test stability at three time points (immediately after mixing, after 2 weeks and 1 month) using counts of the bacteria in MRS media. Controls of the probiotic without coccidiostat were also included as a reference parameter. Then, a liquid feed was produced at 41°C to mimic the intestinal tract of birds to measure the kinetics of acidification of the soup. *Pediococcus* counts were also taken after 18 h and 24 h to evaluate the viability of the probiotic strain in the intestinal-like chyme. The lactic acid produced helps maintaining a healthy gut environment by inhibiting harmful bacteria and promoting beneficial microorganisms. Results showed that the tested probiotic can survive in the dry feed (± 0.5 Log CFU of the target) after 1 month of storage with coccidiostats. Moreover, it massively produces lactic acid (pH evolution from 6.0 to 4.3) with all the selected coccidiostats, aligned with the growth of the strain ($+4$ log) already 18 h after preparation of the liquid feed. Overall, the growth and the acidification recorded in this in vitro model support a good compatibility of the probiotic strain with the five tested coccidiostats.

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Poster 16

Pecking block use is associated with a lower incidence of keel fractures in laying hens in enriched colonies

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Keel fractures (KF) are a welfare concern in the laying hen industry. KF are influenced by many factors and occur in all housing types. Pecking blocks (PB) are an edible enrichment designed to reduce feather pecking, but they also provide an opportunity for hens to supplement their diets with minerals that may be beneficial for bone integrity. We investigated whether providing PB impacted KF incidence (KFI) and severity (KFS). We used a 2x2x3 factorial design in which treatment (PB (TRT) or no PB (CONT)), genetic strain (796 Dekalb White (DW) and 796 Bovans Brown (BB)), and enriched colony (EC) style (30-bird (N=12), 32-bird (N=16), and 60-bird (N=12)) were the main effects. TRT were provided PB continuously from 18-70 weeks of age (WoA). Keels of ~20% of hens in each EC were radiographed every 8 weeks and scored yes or no (Y/N) for KFI and scored for KFS on a tagged visual analogue scale (ranging continuously from 0-5). PB disappearance (PBD) was measured via monthly weighing and evaluated on a per-bird basis. The proportion of KFI in each EC was analyzed using a GLMM with a binary distribution to compare fixed effects of treatment, strain, and EC type. A GLMM with repeated measures compared the same fixed effects on KFS scores. The correlation between the total PBD and KFI in each TRT EC was determined with Pearson's correlation coefficient. There was no effect of treatment on KFI ($P=0.962$) or KFS ($P=0.119$). There was an interaction between strain and EC type on KFI ($P=0.040$), in which 60-bird DW had a higher KFI than all other strain and EC combinations. KFI and KFS both increased over time, and there was an interaction between strain and hen age on both KFI ($P<0.001$) and KFS ($P=0.029$). BB had higher KFI and KFS at 20 WoA, and DW had higher KFI from 36-68 WoA and higher KFS at 68 WoA. PBD varied considerably between EC, and there was a moderate negative relationship between PBD and KFI ($r=-0.553$) ($P=0.011$). In conclusion, there was no treatment effect of PB on the incidence of KF at the group level. However, a higher use of PB was associated with a lower KFI.

Salmonella Risk Assessment Protocol for Pastured Poultry: Sampling approach for Evaluating Environmental Exposure and Mobile Coop Management

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In Italy, pastured poultry systems with fewer than 250 birds are exempt from mandatory sanitary analysis under current regulations, which classify them as low-risk due to the low number and short commercial range. This study evaluated a HACCP plan for managing Salmonella risks in a mobile coop system where poultry freely foraged within electric net enclosures as a part of an agroforestry setup. The mobile coops were weekly rotated to enhance soil fertility and prevent nutrient overload, minimizing reliance on chemical inputs. The HACCP plan focused on critical control points (CCPs), utilizing a multi-tiered sampling protocol to monitor Salmonella risks on pastured poultry farm. Shoe cover sampling was conducted to assess contamination on operational pasture grounds, future grazing areas, and coop floors. For internal coop surfaces, such as perches, nests, and feeding areas, absorbent sponges were used to identify hotspots. Additionally, water samples were collected from both the coop waterers and, if available, from natural puddles in the pasture to evaluate waterborne risks. The HACCP assessment identified distinct risk profiles for each area. While internal coop showed low risks of traversal contamination due to overall low density and minimal fecal matter accumulation, pasture areas exhibited both vertical risks from wildlife and environmental vectors and horizontal risks from bird-to-bird transmission. Future pasture areas presented potential vertical contamination risks from rodents and wild birds. The sampling protocol effectively detected these risks, enabling targeted biosecurity measures and pasture rotation schedules. The findings demonstrate that, despite unique challenges, a HACCP approach to manage Salmonella risks in pastured poultry systems is feasible. Effective risk assessment and control measures can enhance poultry health and soil management, supporting a sustainable and resilient model for small-scale farms, especially if new regulations extend sanitary analysis requirements to these systems.

The effect of Tanacetum vulgare on the production performance, health and welfare of a mountain laying hens' farm: a case study

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The aim of the present study was to analyse the effect of Tanacetum vulgare methanolic extract (ME) on production performance, health, welfare in free-range laying hens on a mountain farm. For this experiment, ÖTZ Bresse-Gauloise laying hens, 34 weeks old, reared in a free-range system, were randomly distributed into three groups (n=40). The control (C) and the anthelmintic (A) groups received a basal diet, while the experimental group (T) received the same diet with 0.1% of ME. Daily feed intake (DFI), animal weight, egg production and quality and individual faecal egg count (FEC) and plumage condition (scale 1-4) were assessed. Group A showed overall improved feed conversion ratio and higher egg production (p<0.05). Egg quality was significantly higher for egg yolk colour, in group T (p<0.05). Throughout the experiment, faecal analysis consistently detected Ascaridia galli (A. galli) and Coccidia in all three groups. Group T showed significant lower A. galli infection compared to group A and overall lower Coccidia infection. Total welfare scoring did not differ between the groups, but group A showed lower neck scorings, indicating reduced welfare. In conclusion, ME supplementation enhanced yolk colour, reduced parasitic load and had better comb scores, suggesting potential beneficial effect on the overall performance, health and welfare of laying hens.

Exploring Genetic Determinants of Avian Influenza Resistance in Chickens

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Poultry farming is vital for food security, yet avian influenza virus (AIV) remains a major threat, causing economic losses and zoonotic risks. Due to AIV rapid evolution and vaccine limitations, genetic resistance offers a promising mitigation strategy. This study investigates the genetic basis of AIV resistance in chickens using genome-wide association studies (GWAS) (n=474), RNA sequencing (RNAseq) (n=45), and Reduced Representation Bisulfite Sequencing (RRBS) (n=52). We analysed commercial broilers (BC) and Sonali (SC) chickens from Bangladesh, and hybrid indigenous (HC) chickens from Vietnam, all naturally exposed to AIV, with infections confirmed via qRT-PCR targeting the AIV M gene. Genomic DNA from blood underwent skim-genome sequencing and imputation to 28 million variants. RNAseq was performed on spleen tissues, while blood DNA underwent RRBS. GWAS and Regional Heritability Mapping (RHM) were conducted using GEMMA and REACTA, accounting for principal components and environmental factors. After quality control, 452813, 798554, and 810701 SNPs were analysed from BC, SC, and HC, respectively. RHM identified 39 and 28 significant genomic regions in BC and HC, with >80% overlapping with GWAS-significant loci. These regions harboured genes involved in innate immunity, inflammation, and antiviral responses. While no shared loci were found between populations, both showed enrichment in cytokine signalling, neutrophil chemotaxis, gut immunity, and antiviral defence pathways. RNAseq from spleen samples identified 151 differentially expressed genes, four within significant RHM regions. These findings highlight the complexity of AIV resistance and key immune pathways involved. Future integration of larger GWAS datasets and epigenomic profiling will refine resistance mechanisms, informing genetic selection strategies to enhance poultry health and mitigate zoonotic risks.

Session 19

Poster 20

Modulation of early life gut microbiota through inclusion of earthworms and vermicompost in broiler diets

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Providing earthworms (EW) during early life can mitigate dietary challenges induced by soluble non-starch polysaccharides (NSP). However, it is unknown how this influences the gut microbiota composition. This study investigated the effects of EW and vermicompost (VC) supplementation on gut microbiota diversity in broilers fed either a standard corn-soy diet as a positive control (CON+) or an NSP-rich diet as a negative control (CON-). A total of 120 male Cobb-500 birds were used over two 8-day periods (P). In P1, birds were allocated into four groups: two groups received the CON+ diet (n = 30), one group received CON+ plus 1% EW (CON+EW, n = 30), and one group received CON+ with 1% VC (CON+VC, n = 30). At the end of P1, half of the birds in each group were euthanized and ileal digesta was collected for microbiota analysis. In P2, one CON+ group continued the same diet while the remaining groups were subjected to a dietary challenge with an NSP-supplemented negative control diet (CON-, n = 15), or with CON- supplemented with EW (CON-EW, n = 15) or VC (CON-VC, n = 15). At the end of P2, ileal digesta was analysed for microbial composition using V3-V4 regions of 16S rRNA gene sequencing. In P1, the CON+VC group exhibited a significantly higher Chao1 index compared to both the CON+ and CON+EW groups (P < 0.05). In P2, although overall α -diversity metrics remained unchanged across groups (P > 0.05), the CON+ group showed a trend toward increased Chao1 (P = 0.078). β -Diversity analysis revealed significant differences between dietary groups in P2 (P = 0.001). Differential abundance analysis identified that *Enterococcus* was prominent in the CON+ group during P1, whereas *Lactobacillus* was significantly enriched in the CON+EW group. In P2, the CON-EW group continued to exhibit increased *Lactobacillus* abundance, while *Escherichia-Shigella* was overrepresented in the CON- group. In conclusion, dietary supplementation with earthworms positively modulated gut microbiota composition and predicted metabolic functions in broilers facing NSP-induced dietary challenges.

Evaluating the Impact of Stress on Laying Hens' Synbiotic Water Preference

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Synbiotics (SYN) are used in human and animal studies by incorporating them into drinking water or feed to improve health and well-being. With the growing popularity of SYN, it is essential to determine if animals can distinguish between supplemented and unsupplemented options, especially under stress, where acceptance may be more challenging. This study aimed to evaluate if laying hens can differentiate between SYN-supplemented drinking water and unsupplemented placebo (P) water, or if their consumption changes under stress. The experiment involved 226 White Leghorns, 37 weeks old, divided into 12 enriched floor pens with 18-19 birds per group. Hens were provided with a choice between P and SYN water to assess their relative preference. Daily liquid consumption was recorded at the group level using manual weighing. A commercial SYN was freshly mixed into the water each day at 80 mg SYN/L water. Hens were introduced to P and SYN water in color-coded water jugs during the associative learning period. A 6-week preference testing period, consisting of pre-stress, social stress, and post-stress phases, allowed hens simultaneous access to both liquid types. During the stress period, birds were subjected to repeated, unpredictable social stress by being moved to new pens and mixed with unfamiliar birds. A generalized linear mixed model assessed changes in SYN consumption across the pre-stress, stress, and post-stress phases. Results indicated that during pre-stress, 56% of the total liquid consumed was SYN-enriched water, compared to $52 \pm 2.3\%$ ($p = 0.0010$) during stress, and $53 \pm 2.3\%$ ($p = 0.0131$) post-stress. Findings suggest hens prefer SYN-enriched water even under stress, showing their ability to differentiate between SYN and P water. This is significant under stressful conditions, as additives like SYN may help manage stress. Although this study tested only one commercial SYN product, it is the first to investigate this research question and opens new avenues for future research.

A picky eater? Advancing Feeding Strategies to Maximize Dual-Purpose Poultry Productivity

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There has been a growing body of scientific research focused on the productivity of dual-purpose poultry (DP) breeds. Information regarding the optimal rearing conditions, particularly in terms of feeding regimes are scarce. Previous research has indicated, that DP poultry can tolerate certain nutrient variability while maintaining productivity, compared to high-performing breeds, which may be more sensitive to dietary imbalance. We aimed to investigate how DP and layer chicken (LC) pullet breeds react to feeding changes during the rearing period under organic conditions. A total of 720 chicks (360 ÖTZ Coffee (DP), 360 ISA Warren (LC), 2 replicates/breed) were raised under organic conditions (EU Reg. 2018/848). During the rearing period, four feeding phases were implemented: starter1 (wks 1-3), starter2 (wks 5-7), grower1 (wks 9-12) and grower2 (wks 14-18) with a one-week transit period in between (feed mixture: 70:30, 50:50, 30:70). The nutrient content was reduced from 20.6%-16.0% crude protein, 0.39%-0.31% methionine, and 11.3-11.0 MJ AMEN per kg feed (88% DM). A greater variability in feed consumption was observed for LC than DP, however both breeds showed a consistent trend in body weight gain (BWG). Uniformity exceeded 80% in both breeds while reaching 2.2 kg (DP) vs. 1.6 kg BW (LC) at week 18 and the average daily BWG (ADG) was 17.2 g vs. 12.9 g, for DP and LC, respectively. The total feed consumption was 8.28 kg (DP) and 6.95 kg (LC), with a feed conversion ratio (FCR) of 3.83 and 4.28, respectively. Methionine expenditure was calculated to 12.62 g/kg BWG (DP) and 14.09 g/kg BWG (LC). Hence, the DP was more efficient with the offered feed. Also, LC reacted more sensitive to feed changes in terms of feed selection. In conclusion, the DP pullets were better fitted to adapt to environmental and feed fluctuations, making it a strong candidate for sustainable, organic farming systems. We acknowledge funding by the by Federal Ministry of Food and Agriculture (BMEL)

Heterophil/Lymphocyte Ratio Level Modulates Salmonella Resistance, Cecal Microbiota Composition and Functional Capacity in Infected Chicken

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The gastrointestinal microbiota plays a vital role in ensuring the maintenance of host health through interactions with the immune system. The Heterophil/Lymphocyte (H/L) ratio reflects poultry robustness and immune system status. Chickens with low H/L ratio are superior to the chickens with high H/L ratio in survival, immune response, and resistance to Salmonella infection, but the underlying mechanisms remain unclear. This study aimed to identify microorganisms associated with resistance to Salmonella enteritidis infection in chickens based on the H/L ratio. The 16S rRNA and metagenomic analysis were conducted to examine microbiome and functional capacity between the two groups, and Short Chain Fatty Acids (SCFAs) and histopathology were conducted to explore the potential difference between susceptible and resistant groups at 7 and 21 days postinfection (dpi). The microbiome exploration revealed that low H/L ratio chickens compared to high H/L ratio chickens displayed a significantly higher abundance of Proteobacteria (*Escherichia coli*) and Bacteroidetes (*Bacteroides plebeius*) at 7 and 21 dpi, respectively. Anaerostipes ($r = 0.63$) and Lachnospirillum ($r = 0.63$) were identified as bacterial genus significantly correlated with H/L ratio ($P < 0.001$). Interestingly, *Bacteroides* was significantly and positively correlated with body weight post-infection ($r = 0.72$), propionate ($r = 0.78$) and valerate ($r = 0.82$) contents, while *Salmonella* was significantly and negatively correlated with body weight post-infection ($r = -0.67$), propionate ($r = -0.61$) and valerate ($r = -0.65$) contents ($P < 0.001$). Furthermore, the comparative analysis of the functional capacity of cecal microbiota of the chickens with high and low H/L ratio revealed that the chickens with a low H/L ratio possess more enriched immune pathways, lower antibiotic resistance genes and virulence factors compared to the chickens with a high H/L ratio. These results suggest that the chickens with a low H/L ratio are more resistant to Salmonella Enteritidis, and it is possible that the commensal Proteobacteria and Bacteroidetes are involved in this resistance against Salmonella infection. These findings provide valuable resources for selecting and breeding disease-resistant chickens.

Session 20

Theatre 1

Indicators of (Declining) Quality of Life in Elderly Horses

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Aging is the primary risk factor for disease, disability, and mortality. Currently, 22–34% of the equine population is geriatric, with 69% affected by chronic diseases, most commonly osteoarthritis (48%). While chronological age is a strong predictor of health and mortality at the population level, substantial interindividual variability exists in the rate and trajectory of biological and functional decline. In horses, functional assessments, biological age markers, and objective quality-of-life indicators remain to be established. Identifying these markers could not only facilitate preventive care strategies but also help distinguish between age-related decline and pathological processes, enabling more accurate diagnoses and individualized treatment plans. This study aimed to identify morbidity and mortality risk factors in horses to enhance individualized care and improve quality of life. Over three years, 123 horses at an animal sanctuary underwent quarterly comprehensive health assessments and activity monitoring via wearable sensors. Data included body condition scores, musculoskeletal pain scores, lameness evaluations, and time budgets for eating, resting, and activity. During the study, 31 horses died—10 due to acute decompensation of their chronic condition (DAC) and 21 due to progressive deterioration leading to euthanasia (DCC). Statistical modeling revealed strong associations of body condition, pain scores, and time budget data with mortality risk. Notably, low body condition score and reduced eating time were key predictors of DAC mortality, aligning with human studies linking weight loss to frailty and increased mortality risk. Additionally, depression-like behaviors were prevalent in DAC horses, mirroring findings in human aging research. While elevated pain scores were present in all deceased horses, weight loss was specific to DAC cases, suggesting multifactorial influences beyond pain alone. These findings lay the groundwork for developing equine-specific predictive tools to support clinical decision-making, enabling tailored treatment approaches that improve welfare and quality of life for aging horses.

Post-racing Thoroughbreds in Equine Assisted Services: personality and welfare

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The retraining and welfare of post-racing Thoroughbreds (TBs) is a highly topical issue. TBs are often retrained for sport or leisure purposes and are increasingly being considered for Equine Assisted Services (EAS). However, the personality and behavioural profile of successful TBs in EAS have not yet been evaluated. The objectives of this project were: 1- to identify the perceived characteristics of TBs in EAS programmes, 2- to characterise the personality and behavioural profile of successful TBs used in EAS. The first phase, an online survey aimed at EAS practitioners, received 129 responses, of which 56.3% (71) agreed that TBs have specific characteristics making them especially useful for EAS. Respondents particularly mentioned their sensitivity, body/movement characteristics, responsiveness, intelligence, and past experience. Detailed survey information was provided about 57 TBs (median age: 14 years old, geldings: 78.57%) who were less frequently used in ridden EAS programmes than other breeds (OBs) (31.58% vs 64.85%, $p < 0.001$). The second phase, an experimental study, compared the personality of 52 TBs and 44 OBs, all used in EAS, and 55 TBs in general retraining. TBs in EAS were more sensitive to tactile stimulation ($p < 0.001$) than OBs. Even if TBs showed stronger responses on some emotivity tests (unknown surface, $p < 0.05$), their response to other tests such as suddenness test was lower than OBs ($p < 0.01$). TBs in general retraining expressed higher emotivity reactions ($p < 0.01$) and more positive behaviours towards humans ($p < 0.05$) when stabled than successful EAS horses. These results move towards understanding the key features of successful TBs used in EAS programmes. TBs can adapt to some EAS programmes and could be selected from general retraining.

Session 20

Theatre 3

The impact of barefoot racing in young Standardbred trotters on career length

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Racing barefoot is popular because it improves the speed in trotting horses. However, whether racing barefoot with young trotters should be allowed or not is under debate, and age restrictions differ between European countries. The wear and tear of the hoof from repeatedly racing barefoot as young has been argued to affect the durability of the horse, and is also seen as a possible animal welfare concern. Therefore, the aim of this study was to analyze the effect of the proportion of barefoot races as 3- and 4-year-olds on the career length in Swedish Standardbred trotters. In total, 13,923 horses born from 2002 to 2018 that raced in the years 2005-2022 were included. All horses were required to have at least 10 observations as 3- and 4-year-olds. The survival analysis was performed in Rstudio using the Cox model, where career length in days was measured from the horses' first race as 3-year-old. The model included sex, birth year group, raced as a 2-year-old or not, earnings (as 3- to 4-year-old), number of races (as 3- to 4-year-old), inbreeding coefficient, proportion of barefoot races divided into six classes, and an interaction between earnings and sex. The hazard ratio (HR) was lower than one for more recent birth years and when starting in more races as young. Racing as a 2-year-old had a small negative effect on the survival with an HR of 1.06. Higher earnings increased the HR for mares (> 1), but decreased HR (< 1) for geldings and stallions. Inbreeding percentage had a negative effect on the survival with an HR of 1.01. For proportion of barefoot races, the HR was significantly higher for the four groups where the horses raced more than 15% barefoot compared to those that raced 5% or less barefoot. The HR for horses with more than 70% races barefoot was 1.17 compared with the lowest class. This corresponded to a shortening of the median career length by 124 days from 1373 to 1249 for geldings/stallions and by 73 days from 920 to 847 for mares, compared with horses racing 5% or less barefoot. We can conclude that a higher proportion of barefoot races was associated with an increased HR, however, the effect on median career length was relatively small.

The Influence of Group Formation and Space Availability on Equine Social Behavior

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Horses are highly social animals that, under naturalistic conditions, live in stable groups and establish long-term bonds, with proximity indicating affiliation. However, domestic horses face social stressors, including frequent changes in group composition and limited space. This study investigates how regrouping and space availability influence equine social behaviour using continuous measurement of interindividual distances with ultra-wideband sensors to provide a quantitative assessment of social dynamics. The first study tracked 15 horses throughout three regrouping phases: 1) Grouping (15 days, introduction of subgroups (2-3 horses/subgroup that were familiar to each other) every 3 days), 2) Stabilization (12 days post-grouping), and 3) Consolidation (two months post-stabilization). During stabilization, distances between original subgroup members increased ($p < 0.001$; $10 \pm 9\text{m}$ to $11 \pm 10\text{m}$), while horses from different subgroups moved closer over time ($p < 0.001$; $23 \pm 12\text{m}$ to $14 \pm 11\text{m}$). Suggesting a shift from original subgroup associations toward a more cohesive, integrated structure. The second study examined how enclosure size affects proximity and interactions. In smaller enclosures, horses displayed more agonistic behaviors and maintained greater relative distances, likely as a conflict avoidance strategy. Affiliative partners maintained closer proximity ($p \leq 0.0001$) and spent 42% of their time within 3m in the paddock and 10% in the field, while other group members spent 24% and 2%, respectively. In larger spaces, affiliative partners showed greater interindividual distances than previously reported (median: 3.6m in the paddock, 95% CI: 3-6m; 14m in the field, 95% CI: 10-16m). Despite increased space, horses did not disperse to the maximum possible extent, suggesting a threshold distance for maintaining group cohesion. These findings demonstrate that both social disturbances and environmental conditions significantly influence equine social behavior. While regrouping disrupts associations and fosters new bonds over time, space availability affects social spacing and interaction patterns. Effective management strategies should consider factors to promote social cohesion and equine welfare.

Session 20

Theatre 5

Learning from the animal's perspective on transitioning from a pen to a stall

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The breeding/gestation stall was developed as a husbandry tool to improve individual animal nutrition and care and widely adopted across the global swine industry. However, the use of these stalls has been limited in many countries due to welfare concerns. Albeit on most farms, even in Europe, non-lactating sows still are crated for some period of their production cycle. To better understand the animal's experience in a stall, we studied the transition from pen to stall by naïve replacement gilts from 1 hour to 4 weeks after moving to a stall. Diurnal patterns of cortisol release are initially disrupted after moving for the first time to a stall, however, within 3 weeks of crating the original pattern was reestablished. Behaviors like standing, moving, and rooting increased while lying down decreased over 4 weeks in a stall. Increases in oral stereotypies and other abnormal behaviors were also observed during crating. Overall, gilts were busier when crated and rested less despite the confinement of the crate, perhaps related to the frustration of not being able to realize the intent of their behaviors. We also provided naïve gilts free access to a gestation stall or an open pen over 7 days of observation. Most gilts significantly preferred the open area. However, time of day, day after crating, and personality traits appeared to influence individual animal housing preferences. We measured the work done by a naïve gilt to exit the confinement of a stall. Forces applied to the back of the stall were quantified in the hour following the initial introduction to a stall. Interestingly, all the gilts worked to leave the stall but exhibited different amounts of effort. One group identified by hierarchical cluster analysis had more frequent and stronger force generation and appeared to be more motivated to exit the stall. Taken together this work, while preliminary, highlights aspects of gilts physiology and behavior triggered by crating that can be interpreted as contributing to the animal's negative psychological health. However, these studies also identify the complexity of the confinement issue and support the need to further understand differences in individual animal responses as well as an animal's capacity to adapt to confinement.

Human-animal interactions as perceived by Austrian pig farmers

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Society expects higher levels of animal welfare, not least on farms keeping fattening pigs. Most studies on animal welfare focus on the pigs themselves, collecting data on clinical, performance-related or behavioural aspects, as well as emphasizing the role of adequate housing systems. Much less attention is given to the role of farmers, despite strong evidence that the quality of the human-animal relationship significantly impacts pigs' affective state and thus welfare. To capture how farmers perceived their relation with their fattening pigs and how they interact with them, semi-structured qualitative interviews were held with 15 farmers with standard housing and with 28 farmers with welfare housing systems. The interviewed farmers kept between 150 and 2000 fattening pigs. The results show that most of the interviewed farmers are aware that their behaviour affects their relation to their pigs and thus strive to ensure calm interactions, soft vocalisations, gentle handling, and slow movements to reduce fear and stress. They also strive to maintain regularity in their activities to ensure predictability for the pigs. Yet, the human-animal relationship is characterized by the tension between emotional involvement and care to ensure the pigs have a 'good life', and the awareness that ultimately the pigs are destined for slaughter. As a result, some farmers had a more instrumental view of their pigs, perceiving them mostly as a means to earn an income, whereas others viewed the pigs as akin to 'partners' emphasizing that only together could they ensure the continuity of the family farm. The study shows that even on farms with fattening pigs where the animals spend only a relatively short time (about 3-4 months), many farmers develop an emotional connection to the pigs in their care. Openly addressing the emotional aspects of working with fattening pigs may validate farmers who care about their animals and reassure those who may be unsure whether it is 'professional' to express such emotions. Including emotional along technological and economic aspects of pig production might thus contribute to the welfare of the pigs.

Session 20

Theatre 7

Impaired Memory and Anhedonia in Slow-Growing Nursery Piglets

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The objective was to evaluate whether nursery pigs with different post-weaning growth rates exhibit differences in their responses to sweet rewards and memory. Trial 1 involved 64 pigs of 44 d-old with Fast (244 ± 9.8 g) or Slow (151 ± 9.8 g) ADG until the third nursery week. Pigs were evaluated in pairs to study their preference between water and sucrose solutions (0.5% and 1%) during 30-minute sessions to assess anhedonia. Trial 2 used 38 pigs of 42 d-old with Fast (186 ± 11.4 g) or Slow 82 ± 10.4 g) ADG until the second nursery week. They were evaluated individually to assess their memory through a novel object recognition test, which was divided into an initial exposure phase and a recognition phase, where preference for the novel object over a previously exposed sample object indicated recognition. In the anhedonia test, an interaction was found between growth and solution type during the water vs. 0.5% sucrose test ($p = 0.036$). Slow-growing pigs consumed more water (52.0 ml) than 0.5% sucrose (43.9 ml), whereas Fast-growing pigs consumed more 0.5% sucrose (64.7 ml) than water (42.9 ml). When offered a 1% sucrose solution, both groups increased their sucrose intake compared to water. However, Fast-growing pigs consumed more 1% sucrose solution than Slow-growing pigs (153.8 vs. 82.7 ml, respectively). In the novel object recognition test, during the first phase, Fast- and Slow-growing pigs showed no difference in time spent touching the sample object ($p > 0.1$). However, during the recognition phase, Fast-growing pigs spent less time touching the sample object (9.1 vs 19.1 %; $p < 0.001$) and preferred exploring the novel object over the sample object in relative terms (77 vs. 44 %; $p < 0.001$) than the Slow-growing pigs. Results show that Slow-growing pigs exhibit impaired memory and reduced sensitivity to low sucrose concentrations compared to Fast-growing pigs during the first weeks of nursery, suggesting they are likely experiencing depressive-like states, which could negatively affect their welfare and future growth.

Play in fattening pigs: Prevalence and potential as indicator of positive welfare

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Play behaviour has been suggested as an indicator of positive animal welfare. However, there are still knowledge gaps concerning occurrence of and influencing effects on different types of play at different age classes of pigs. Therefore, we analyzed play among fattening pigs on a conventional German farm. Groups with on average 10.5 (one to 13) pigs/pen were continuously video observed for 24 hours at the beginning (20 groups) and at the end (16 groups) of fattening. The evaluation considered total play and its components social (SOC), locomotor (LOC) and object (OB) play. The latter included occupation with a chain (CHAIN) and a nibbling toy with a hard pressed cylinder of *Miscanthus*, both available in all pens. The statistical analysis focused on play event duration, number of play events/(hour*pig) and play duration/(hour*pig). Effects of gender, fattening stage, group size, pen, round and time of day were considered. The prevalence of total play (1.42 +/- 2.35 events/(hour*pig), 36.59 +/- 64.17 sec/(hour*pig)) was increased in males (events: +34.1%, $p=0.0086$), at the end of fattening (events: +38.8%, $p=0.0031$; duration: +116.9%, $p<0.0001$) and in the smaller groups (≤ 11 pigs; duration: +47.1%, $p=0.0016$). These results were strongly impacted by the predominance of CHAIN (events: 67.3%, duration: 90.1%, 34.94 sec/event). Excluding CHAIN reverted the finding with 61.8% more events/(hour*pig) in early fattening ($p=0.0279$) and 65.1% more sec/(hour*pig) in the larger groups ($p=0.0413$). CHAIN partly exhibited characteristics of stereotypic behaviour. Additional difficulties in OB arose from a potential confusion with exploratory behaviour. Further challenges also in reliably identifying and in understanding SOC make us propose to focus on LOC as a potential indicator of positive welfare in pigs. We found a numerically higher LOC prevalence in younger pigs (events: +36.4%, duration: +30.8%) and in smaller groups (events: +38.1%, duration: +45.7%). Given its low prevalence (events: 18.2%, duration: 1.2%) and its extremely low event duration (1.71 +/- 1.63 sec), LOC should be evaluated continuously.

Session 20

Theatre 9

Brush use in lot-fed cattle shows continued use and positive behaviour

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The concern about the impact of intensive management systems on beef cattle welfare has been increasing. Monitoring of cattle should include health, access to resources including enrichments, and the freedom to display natural behaviour. Cattle held in a commercial feedlot were assessed for the consistency of brush use, incidence of natural behaviour, and agonistic or abnormal behaviour compared to cattle without the brush. Cattle were assigned to one of two treatment pens, (1) access to a grooming brush (EB; $n=89$) or (2) no access control (CON; $n=80$), for a period of 107 days. Repeated measures ANOVA was performed on ethogram scores collected for an hour duration over four time periods (0830, 1030, 1400, and 1600 h). A Principal Component (PC) analysis was used on pen-side demeanour scores taken by two observers. Statistical analysis on pen-side behaviour and demeanour PCs tested for impact of treatment, day, and time. Frequency of brush use and the duration of each cow-brush interaction did not decrease over the assessment period. Self-grooming and allogrooming did not differ between treatments and were similar in frequency to brush grooming, resulting in total grooming being higher in the EB treatment group ($p<0.05$). Cattle in the EB pen were scored as more content (PC 2; $p<0.05$) and sociable (PC 3; $p<0.01$), while CON cattle were scored as more anxious (PC 3; $p<0.05$). Overall, this study suggests that the provision of a brush for enrichment enhanced lot-fed cattle wellbeing and permitted prolonged engagement, making it an effective enrichment device. Further larger studies in commercial contexts are recommended.

Emotional contagion from humans to horses through odors

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In the past few years, numerous studies have shown that domestic mammals are sensitive to human emotions, highlighting their reactions to our facial and vocal expressions. Recent experiments revealed that odors may also be involved. For example, we showed that horses can discriminate human odors from fear and joy contexts, and that they seem to perceive the latter positively. In this new study involving 43 horses, we examined whether these human emotional odors could further influence horses' behavior and physiology. We conducted tests to evaluate horses' fear response (suddenness test, novel object test) and their reaction to humans (grooming test, human approach test), while wearing cotton pads carrying human odors from a fear context, from a joy context, or unused pads (control odor) applied to their nostrils. Horses exposed to odors from the fear context showed stronger fear reactions and interacted less with humans, compared to horses exposed to odors from the joy context or control odors (overall behavior: generalized linear models (GLM), $p < 0.001$). Specifically, when exposed to fear-related odors, horses interacted less with the human during the approach test (GLM, $p = 0.032$), directed more attention toward the novel object (GLM, $p = 0.027$), and exhibited stronger startle responses, as indicated by higher startle intensity ($p = 0.04$) and maximum heart rate (GLM, $p = 0.0042$) in the suddenness test. These findings suggest emotional contagion from humans to horses via olfactory cues, highlighting the importance of odors in human-horse interactions. This is particularly interesting, as many equestrian activities involve humans remaining outside the horse's field of vision while still within its olfactory range. More broadly, this underscores the importance of chemosignals in interspecific interactions and provides valuable insights into the impact of domestication on emotional communication.

Session 20

Theatre 11

Quality of relationships in reproducing does kept in a part-time collective housing system

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Part-time collective housing systems for reproducing rabbit does have raised concerns for aggression among animals and occurrence of injuries and lesions. Within this framework, the present study addressed social interactions among does in a part-time collective system based on negative (such as aggression) and positive (affiliative, resting, comfort) behaviors. Videos were recorded at -11 days before kindling – when does were first grouped in 18 collective parks containing 3 or 4 does (F3, F4) – and at +25 days after kindling – after individual housing from -2 days before until +18 d after kindling, with litter size standardized at 9, 10, and 11 kits. Behaviors were scored for each pen in the first 30 minutes of an hour. At the first grouping (-11 d), the number of interactions “seek-escape from the mate” was lower than “seek-remain with the mate” without differences between groups, as was the number of aggressive events (bites per doe: 0.620 vs. 0.500 in F3 vs. F4 parks; $P > 0.10$). During resting, the does were found mainly with a crouched body, in pairs or alone. At +25 d after kindling, observations of does resting in pairs and/or in groups were higher than at -11 days before kindling; a higher number of observations of pair-resting with crouched body was recorded in F4 compared to F3 parks (1.108 vs. 0.760; $P < 0.05$), whereas no significant effect of litter size was recorded. Based on the first results, under our conditions, reproducing does were observed seeking each other and resting together, which deserves further investigation to balance the negative and positive sides of group housing. Acknowledgement: The study has been funded by European Union – Next Generation EU, Project PRIN “No cage system for rabbits: indoor and outdoor scenarios – RABBINOUT” (Cod. 2022JAX4TZ; CUP: C53D23005340006).

The influence of selected factors on the play of horses staying in a herd on the paddock

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The available literature lacks comprehensive studies on horse play and the influence of various factors on it. Knowledge of behavior during play can help improve the well-being by giving the opportunity to express natural need for play. The aim of the study was to investigate the influence of selected factors on the occurrence and duration of individual forms of play in horses. The study included 21 horses that were observed during their stay in the herd on a sandy paddock. Behaviors was recorded during 20 hours using a digital camera, and the experimenter did not interfere with the horses' behavior. For the purposes of the study, an ethogram was created that took into account the horses' behaviors during play. Statistical analysis included the U-Mann-Whitney test compared data on gender and the Kruskal-Wallis test for data taking into account the hierarchical and age range. Spearman's rank correlation coefficients were used to determine the impact of the occurrence of play behaviors among themselves and between the duration of the behaviors and the parameters of the tested weather conditions. It was found statistically significant difference in the duration of play behavior between mares and geldings and between horses in individual hierarchical groups. The age of the horses did not significantly affect the occurrence of specific behaviors, except for chasing and wrestle. There were statistically significant negative and positive correlations between weather conditions and the duration of behavior. There was a correlation between behaviors within the types of play: play fighting, locomotor play and object play. Research has shown how diverse the play behavior of horses is and that the duration of play behavior may result from many factors. Although play may initially seem to be just a form of entertainment, it is an important part of the life of horses. It is not only an indicator of well-being, but also a factor that can improve it. A better understanding of the playful behavior of horses will allow breeders and owners of these animals to significantly improve the conditions in which they live. Funded by the Minister of Science under „the Regional Initiative of Excellence Program”

Session 20

Poster 13

The influence of the lack of access to paddocks and pastures on the physical and mental health of horses

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With the use of horses in horse riding, horses are increasingly locked in stalls, where they cannot meet their natural needs. Free movement on the pasture, contact with other horses, the possibility of eating roughage, these are important factors affecting the comfort of horses and the proper functioning of the whole organism. The aim of the study was to assess the impact of the inability to use paddocks and pastures on the physical and mental health of horses. The problem was developed by means of a questionnaire addressed to owners or users of sport horses used for jumping. Responses were received from 503 people. The survey consisted of 15 closed questions – 6 general questions and 9 addressed to a given group of horses, i.e. a group of horses going out to pastures or paddocks and horses standing only in a stall. The relationship between the paddock/pasture box variables and undesirable behaviors and physical and mental problems (yes/no answer) was examined using the Pearson chi-square test. The analyses were considered significant if $p \leq 0.05$. The data was analyzed using Statistica 13.3 (TIBCO Software Inc.) software. Own results confirm that the inability to use paddocks and pastures has a negative impact on the physical and mental health of horses. It was statistically confirmed that horses kept only in stalls were more likely to develop addictions ($\chi^2 = 18.84$, $p \leq 0.00001$), problems with the respiratory system ($\chi^2 = 5.66$, $p \leq 0.017$), peri-articular swelling ($\chi^2 = 18.25$, $p \leq 0.00002$), lameness ($\chi^2 = 12.61$, $p \leq 0.0004$) and apathy ($\chi^2 = 24.48$, $p \leq 0.00001$). It is only in the presence of weaving and cribbing in horses ($\chi^2 = 2.92$, $p \leq 0.087$) and hoof problems ($\chi^2 = 0.004$, $p \leq 0.948$) that the probability of their occurrence is similar for both outbound and non-outbound horses. In conclusion, horses that do not have the opportunity to use paddocks and pastures are more likely to develop various mental and physical health problems. This is due to the reduced level of welfare and the associated chronic stress. Funded by the Minister of Science under „the Regional Initiative of Excellence Program”.

Hematochemical Profile, behaviour and BCS of Murgesse mares free grazing on *Camelina sativa* pasture
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The Murgesse is an autochthonous Apulian horse breed characterized by a good temperament, strength, and attitude to several uses. The National Centre for Forest Biodiversity Carabinieri "Martina Franca" rears this breed and distributes young stallions to the equestrian military services present in Italy. The Murgesse horse is a superb grazer able to use poor pastures; it is highly resistant to diseases, to organic and intestinal disorders. The improvement of the nutritive value of pasture may be useful to enhance animal welfare and performances. Therefore, this study investigated the effect of free grazing on two forage systems containing a mixture of oat, barley, vetch, with or without *Camelina sativa* (20%, CAM). Eight Murgesse mares (age: 5 – 6 years; body weight: 450 ± 45 kg) were divided into two homogeneous groups (n=4) in relation to the feeding system and subjected to blood collection on days 0 and 45 in order to assess the metabolic profile. Faeces composition was also assessed. The horses didn't show any kind of stereotypies. There were no differences in faecal apparent digestibility. No significant difference between groups was observed for total protein, albumin, globulin, creatinine, total bilirubin, creatinine kinase, HDL, LDL, aspartate transaminase, alkaline phosphatase, blood urea nitrogen, cholesterol and triglycerides. At the end of the trial, horses grazing on the CAM crop showed a better visual appearance of the coat, probably due to its fatty acids profile.

Session 21

Theatre 1

Developing pasture-based management systems for sustainable sheep meat production

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Improving efficiency in grazing systems of animal production is a significant challenge for the future, to meet increased global food demand while competing for resources, adapting to climate change, and delivering ecosystem services. The sustainable intensification of pasture-based food production systems provides an opportunity to re-green ruminant production. The objective of this paper is to describe recent scientific achievements within pasture-based systems of sheep production in addition to future opportunities to further improve such systems. The production of lamb in pasture-based production systems is principally based upon the utilisation and conversion of herbage into lamb carcass. Over 95% of the annual energy requirements of both ewes and lambs within these systems can be met by pasture offered as grazing or conserved forage. Successful grazing systems require animals that can efficiently convert feed into a high value product. At present, lamb production systems are limited by the efficiencies at which they operate, such as the number of lambs weaned per ewe and the level of herbage utilised per hectare. In order to remain competitive, improvements in the efficiency of such systems are of paramount importance. To achieve these objectives in a sustainable manner, increases in pasture growth, utilisation and quality are required but will need to occur without increases in chemical nitrogen application through the incorporation of legumes and other companion forages within grazing swards.

Meta-analysis of tannins supplementation to small ruminant diets: Effects on growth performance, serum metabolites, anti-oxidant status, ruminal fermentation, meat quality, and fatty acid profile

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Tannins are secondary metabolites present in various plants as defense mechanisms. In animal nutrition, they act as both anti-nutritional factors and bioactive agents, with potential benefits in nutrient metabolism and product quality. This meta-analysis aimed to evaluate the effects of dietary tannins on nutrient intake and digestibility, blood serum metabolites, growth and carcass characteristics, and meat quality in small ruminants. Ninety-seven studies were included, and the effects of tannins were analyzed using a random-effects model to determine the weighted mean difference between the treatments and the control diet. Publication bias and heterogeneity between studies were explored, and meta-regression and subgroup analyses were performed. The results indicated that tannin significantly ($P < 0.05$) increased the intake of dry matter, crude protein, nitrogen (N), fecal N, and meat fatty acids (FA), such as C18:2 ω 6, C18:3 ω 3, C20:4 ω 6, C20:5 ω 3, and total polyunsaturated FA. However, NH₃-N, urinary N, blood urea nitrogen, cold carcass weight, subcutaneous fat thickness, drip loss, and nutrient digestibility significantly decreased ($P < 0.05$). In conclusion, tannin did not affect animal productivity, but modified N metabolism and improved FA profile of meat.

Session 21

Theatre 3

Body morphology of sheep in Greek farms

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The aim of this study was to assess the body morphology of sheep in commercial farms in Greece. Morphological traits were measured in 260 ewes across 15 farms in the region of Eastern Macedonia and Thrace, Greece. Body traits measured included rump height (RH), rump width (RW), chest width (CW), body length (BL), neck perimeter (NP), neck length (NL), udder's height from the floor (UH), left teat length (LL) and right teat length (RL). Measurements were recorded using a Lydlin rod, a large caliper, measuring tape, and a standard caliper. Data analysis included descriptive statistics, principal component analysis (PCA), and k-means clustering to classify sheep based on their morphological traits. Mean ($\pm$ SD) body measurements for RH, RW, CW, BL, NP, NL, UH, LL, and RL were 75.4 cm $\pm$ 4.24 cm, 29.1 cm $\pm$ 3.40 cm, 25.6 cm $\pm$ 3.09 cm, 76.1 cm $\pm$ 5.57 cm, 36.4 cm $\pm$ 4.01 cm, 29.2 cm $\pm$ 6.83 cm, 29.5 cm $\pm$ 5.06 cm, 2.9 cm $\pm$ 1.00 cm, and 3.0 cm $\pm$ 0.99 cm, respectively. Two groups were identified by K-means clustering, Cluster 1 (n=136) and Cluster 2 (n=104). Sheep in Cluster 1 had significantly larger body traits compared to Cluster 2, particularly regarding NL (31.9 cm vs 24.4 cm, $P < 0.001$) and CW (27.1 cm vs 23.8 cm, $P < 0.001$). Teats were significantly longer in Cluster 2 sheep (LL: 3.3 cm vs 2.5 cm, $P < 0.001$; RL: 3.4 cm vs 2.6 cm, $P < 0.001$). UH did not differ significantly ($P > 0.05$) between clusters. These findings highlight the morphological variation among sheep in Greek commercial farms, likely influenced by breeding strategies and farm practices. This work is part of a research project funded by the Region of Eastern Macedonia & Thrace (ELKE-AUTH: 76290).

Ram traits benefit from divergent selection for number of lambs weaned per mating in South African Merinos

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Overall reproductive output, expressed as number of lambs weaned per mating (NLW), is a trait of economic importance in South African sheep. We compared latencies and counts for mating behaviour, test weight (TWT) and testis diameter (TD) between Merino lines subjected to 37 years of divergent selection for NLW, resulting in a High- or H-Line selected for NLW and a Low- or L-line selected against NLW. These lines grazed in a single flock from 1986 to 2023, except at single-sire mating. Rams available for replacement were annually exposed to 5-8 ewes in oestrus. Latencies to first mount (tmount) and service (tserve), as well as counts of mounts (nmount) and services (nserve) were recorded during 20-minute mating dexterity tests. Mating success was defined as the ratio of services to mounts. Count traits (n=737) were recorded from 1992 to 2023 and latency traits (n=725) from 1993 to 2023. Fixed effects of selection line, birth year, their interaction, and the random effect of additive animal were assessed in single-trait analyses. An overall proportion of 0.829 of 484 H-Line rams showed interest in the ewes, compared to 0.687 from 243 L-Line rams ($P < 0.01$). Expressed relative to L-Line performance, behavioural latencies were reduced by respectively 40.3 and 37.2% for tmount and tserve in the H-Line ($P < 0.05$). Count traits and success in the H-Line exceeded those in the L-Line by respectively 13.9, 23.4 and 72.8% ($P < 0.01$). TWT was 13.7% heavier in H-Line rams ($P < 0.01$), while TD of H-Line rams exceeded those of their L-Line counterparts in all but 11 of 32 years recorded. Estimable h^2 -values were 0.028 ± 0.066 for tmount, 0.041 ± 0.066 for tserve, 0.113 ± 0.076 for nserve, 0.151 ± 0.079 for success, 0.258 ± 0.090 for TWT and 0.245 ± 0.092 for TD. Despite having low h^2 -values, ram behaviour benefited from selection for an increased NLW in the H-Line relative to the L-Line, with similar benefits for TWT and TD. Selection to improve NLW is thus unlikely to result in unwanted correlated responses. Further studies are needed as more data become available to add precision to the derived genetic parameters.

Session 21

Theatre 5

Effects of age and genotype on ewe productivity and on the performance of their lambs

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The objective of this study was to evaluate the effects of age on ewe productivity and on the performance of their lambs, using ewes from 3 genotypes that differ in prolificacy. A total of 424 ewes [Belclare (B), Suffolk×Belclare (SB), Suffolk type (>75%S); 2 cohorts] were joined with Charollais rams each year. Ewes for this study were aged 19 months at first joining; a random 50% of each genotype had been joined at 7 months, of which 85% lambled and 79% reared ≥ 1 lamb. Ewes exited the flock when culled for health/welfare reasons (e.g., udder issues), died or were 8.5 years old. Ewes lambled indoors (March) and were put to pasture within 3 days. Ewes rearing triplets were offered concentrate (0.5 kg/d) for 5 weeks post lambing, and their lambs had access to concentrate (max 300 g/d per lamb) until weaning (14 weeks). Between weaning and slaughter all lambs co-grazed on pasture (sole diet). The data were analysed using Proc MIXED and Proc GENMOD of SAS. There was no interaction ($P > 0.05$) between ewe age and genotype. For the B, SB and >75%S ewes, lambs reared/ewe-joined averaged 1.62, 1.70 and 1.34 ($SE \approx 0.045$) and mean age at exit from the flock was 4.6, 4.9 and 4.8 years ($SE = 0.17$), respectively. There were significant curvilinear age effects ($P < 0.01$) on: the incidence of ewes that failed to lamb (0.08, 0.10, 0.06, 0.08, 0.07, 0.12 and 0.24 for age (years) at lambing of 2, 3, 4, 5, 6, 7 and 8, respectively; $SE \approx 0.016$ up to age 6, 0.032 at age 7 and 0.061 at age 8), litter size (increased until age 7), lambs reared/ewe-joined (1.40, 1.61, 1.67, 1.67, 1.69, 1.60 and 1.18 for ages 2 to 8, respectively), body condition score (BCS) and BW. The BCS at post mating, lambing, 5 weeks post lambing and at weaning increased until ages 4, 5, 3 and 3, respectively, and then declined. Ewe BW at post mating, lambing and 5 weeks post lambing increased as ewes aged from 2 to 7, but declined at age 8. There were significant ($P < 0.01$) curvilinear effects of ewe age on lamb BW at birth and weaning, ADG from birth to weaning (253, 294, 284, 282, 310, 306 and 291 ($SE \approx 3.4$) g/d for ages 2 to 8, respectively) and age at slaughter (207, 183, 175, 179, 172, 171 and 167 ($SE \approx 2.6$) d for ages 2 to 8, respectively).

Greenhouse gas emission intensity of Irish Sheep meat over time

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Sheep farming contributes to economic, environmental and social sustainability in rural areas where other land use options are limited. It is important to determine the GHG intensity of sheep meat to identify and adopt practices that are environmentally sustainable, economically viable and socially acceptable. The main objective of the LIFE Green Sheep Project which involves 5 countries (Ireland, France, Italy, Romania, Spain) is to reduce GHG emissions of sheep meat production by 12% whilst ensuring farm level sustainability. In Ireland a suite of data is collected annually on over 100 specialist sheep farms through the Teagasc National Farm Survey which is part of the EU Farm Accountancy Data Network. These farms tend to be either lowland or hill sheep systems. In 2020 the GHG emissions per kg of carcass weight and the per hectare emissions was established on these farms. Since then the Irish Government has established a GHG reduction target of 25% for agriculture sector to be achieved by 2030 and have promoted GHG mitigation measures across the farming sector. This analysis explores the changes across specialist sheep farms between 2020 and 2024 in terms of GHG emissions intensity per kg of carcass weight and per hectare and examines what mitigation practices if any have been adopted over this period. Results indicate significant heterogeneity of GHG emissions across different sheep systems. More intensive farms produce less GHG emissions per kg of sheep meat, whereas extensive farms produce less GHG emissions per ha. Results highlight the trade-offs between farm size, resource inputs, and productivity in different sheep farming systems, with more intensive systems showing higher productivity but also greater resource use. Further development and implementation of low emission technologies is necessary to reduce the GHG intensity and total GHG emissions of sheep systems.

Session 21

Poster 7

LIFE GREEN SHEEP project: multivariate analysis of sheep milk carbon footprint in Italy

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LIFE Green Sheep project (LIFE19 CCM/FR/001245) aims at reducing the carbon footprint of milk and meat sheep by 12%, while ensuring farms' sustainability, in five European countries (France, Ireland, Italy, Spain, and Romania). One of the project's actions is to carry out a large-scale assessment of GHG emissions and carbon storage in 1284 "demonstrative" sheep farms (825 in France, 180 in Ireland, 102 in Italy, 92 in Romania and 85 in Spain) using a life cycle inventory tool. This work aims to evaluate the application of multivariate statistical analysis to identify the factors most involved in the gross greenhouse gases (GHG) emission of the 102 Italian dairy sheep farms, calculated using the CAP'2ER® tool level 1 (C2E; "Institute de l'Elevage", France) and expressed as kg CO₂eq/l of fat-protein corrected milk. A Partial Least Square Regression (PLSR) analysis was applied to a dataset consisting of the farms data, processed by CAP'2ER® (original variables), to verify if the farms' data could serve as predictors of GHG. PLSR enables the construction of predictive models when the factors are numerous and highly collinear. The Variable Importance in Projections (VIP) procedure was employed to identify the most important original variables in the prediction. The means±SE of current and predicted gross carbon footprint, Δ 2 value and root mean square error of prediction (RMSEP), used to assess the predictive ability of PLSR, were 3.60±0.18, 3.51±0.14, 0.78 and 0.4 (P value<0.0001). The original variables that appear to play the most crucial role in determining the amount of gross GHG emission are: the milk yield, the amount of energy, both consumed by the animals and used to power the farm (electricity, fuel...), the quantity of feed purchased and the amount of nitrogen used as fertiliser. Further insights are needed to better analyse the role of these factors more effectively.

Effects of Finishing System in Pampa Biome Grasslands on Crioula Lamb Meat Quality

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It is essential to add value to the products derived from the Pampa biome to support the sustainability of its native grasslands. The Crioula sheep, one of the oldest domesticated breeds in this region, plays a crucial role in both the ecosystem and the livelihoods of local farmers. This study aims to compare the meat of Crioula lambs finished in two production systems: in native grassland of Pampa biome and in feed-stall system (Control). The study used 21 Crioula castrated lambs with similar slaughter weight of 34.38 ± 1.02 kg, aged between 5 and 6 months old. In the native grassland, the animals were submitted to a continuous grazing with an herbage allowance of 14% (herbage mass/liveweight), whereas in the confinement system, the lambs were individually housed and fed ad libitum with a balanced diet consisting of ground corn, soybean meal, and calcitic limestone. All animals had unrestricted access to water and mineral salt. Lambs raised on native grassland exhibited lower cold carcass yield ($P < 0.0001$), lower subcutaneous fat thickness ($P = 0.0281$), and lower L^* (luminosity) ($P = 0.0130$) and b^* (yellow-blue intensity) ($P < 0.0001$) colour characteristics. Regarding meat fatty acid (FA) composition, lambs finished on native pasture showed higher concentrations of C22 ($P < 0.0001$), C15:1 ($P = 0.0093$), C24:1 ($P < 0.0001$), C18:3n-3 ($P < 0.0001$), C20:2 ($P < 0.0001$), CLA2 ($P = 0.0455$), and n3 ($P = 0.0001$). In contrast, lambs finished in confinement had higher concentrations of C14 ($P = 0.0112$), C16 ($P = 0.0024$), C17:1 ($P = 0.0001$), C22:6n-3 ($P = 0.0237$), total saturated FA content ($P = 0.0002$), and a higher n6:n3 ratio ($P = 0.0080$). The results indicate that finishing systems significantly influence carcass and meat quality. Lambs finished on native grassland produced meat with biochemical characteristics beneficial to human health, suggesting that this production system can add value to the meat, support farmers, and contribute to the preservation of the Pampa biome.

Session 21

Poster 9

Effect of breed and rearing system on milk fed lambs carcass quality

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In order to study the influence of the rearing system, linked to breed differences, on the carcass quality of milk fed lambs, thirty suckling lambs from three different breeds were selected: The Latxa (n=10) and Castellana (n=10) lambs were reared outdoors on their mothers' milk. Latxa (n=10) and Castellana (n=10) lambs were reared outdoors, suckling their mothers' milk throughout the lactation period. The Latxa lambs were raised in mountainous areas, and Castellana lambs were reared in cereal-producing regions. By contrast, the Assaf (n=10) lambs were reared indoors on milk replacer. The lambs were weighed one day after birth (6.64 ± 1.30 kg) and every two weeks until slaughter (13.3 ± 1.13 kg). After 24 hours of chilling, carcass measurements were taken: weight, conformation, fatness degree, kidney fat quantity, carcass length, leg length, rump width and perimeter, thorax width and depth, as well as colour (visually on m. rectus abdominis and with chromameter on m. longissimus dorsi) and pH. The production system had no significant effect on conformation, fatness degree, kidney fat, carcass length, leg length, thorax depth and width, rump width and pH ($P > 0.1$). However, Latxa lambs exhibited a higher carcass weight and rump perimeter than Assaf and Castellana lambs ($P < 0.001$). Castellana meat had lighter colour on m. rectus abdominis ($P < 0.001$) and higher values of lightness (L^*), yellowness (b^*) and hue angle (H^*) colour indexes ($P < 0.001$) in m. longissimus dorsi. These changes in colour parameters did not affect the estimated myoglobin content. Overall, despite the general similarity between carcasses, there were some differences in carcass morphology and meat colour that would contribute to characterize and differentiate between breeds and rearing systems. This could eventually have an influence on meat purchasing decisions. Project "Ganadería de montaña Pro-biodiversidad" is supported by the Biodiversity Foundation of the Ministry for Ecological Transition and the Demographic Challenge (MITECO) in the framework of the Recovery, Transformation and Resilience Plan (PRTR), funded by the European Union – NextGenerationEU.

Effect of breed and rearing system on the meat quality of suckling lambs during storage under retail conditions

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Meat purchasing decisions are influenced by extrinsic cues such as nutrition, health, and convenience. Shelf life depends on microbial growth, oxidation, storage conditions, and meat composition. Suckling lambs in mountain regions are raised by pasture-fed ewes, while those in intensive systems are fed milk replacer. This study evaluated how the rearing system, linked to breed differences, affects meat quality during retail display. Two groups of 10 suckling lambs were compared: MR (Assaf lambs reared indoors on milk replacer), and PROBIO (Latxa lambs reared outdoors on mothers' milk in mountain areas). The lambs had an average carcass weight of 7.9 ± 0.2 kg. The shelf life was evaluated in sliced loins packaged under high oxygen modified atmosphere and stored in cabinet with white fluorescent light at $4 \pm 1^\circ\text{C}$, simulating retail display conditions. Sampling points were 0, 4, 9 and 13 days after packaging, when lipid oxidation (thiobarbituric acid-reactive substance-TBARS) and microbial charge (total bacteria at 30°C , enterobacteria, *Salmonella* spp, *Escherichia coli* and lactic acid bacteria counts) were analysed. Data were subjected to ANOVA using the GLM procedure of Statgraphics Centurion software. All microbial populations increased significantly during storage ($P < 0.05$). However, PROBIO meat showed lower microbial counts from day 11 onwards ($P < 0.05$). Values of TBARS increased with time of storage ($P < 0.05$) but PROBIO resulted in lower lipid oxidation at the end of storage than MR. These effects are probably related to the composition of pasture, which could confer a higher antimicrobial and antioxidant capacity to the meat. Therefore, the meat from suckling lamb reared on mothers' milk in mountain areas, showed longer shelf life than meat from intensive systems. Project "Ganadería de Montaña Pro-biodiversidad" is supported by the Biodiversity Foundation of the Ministry for Ecological Transition and the Demographic Challenge (MITECO) in the framework of the Recovery, Transformation and Resilience Plan (PRTR), funded by the European Union – NextGenerationEU.

Session 21

Poster 11

Growth rates of dairy lambs reared to weaning using an automatic milk feeder and milk replacer

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Higher purchase prices of sheep's milk and the low price of Easter lambs (lambs slaughtered for meat before Easter at the age of 8 – 12 weeks) are the two main reasons that lead dairy sheep farmers to replace mother's milk by feeding lambs milk replacer (MR). Our aim was to investigate the effect of lamb breed, lamb sex and litter size on daily liveweight gains (DLWG) when feeding lambs milk replacer ad libitum using automatic lamb feeder (ALF) to ensure there is enough milk heated to the correct temperature for the lamb when it needs to drink. Tsigai (Ts) (n=62) and Lacaune (Lc) (n=40) lambs were moved to one of three group pens for lambs after two days on colostrum reared by the dam. They were fed with an ALF with two or three teats per pen at 13 lambs per teat. Lambs were weighed immediately after birth, at 10, 20, and 30 days of age, and at weaning at 37 days of age. Data were analysed using the PROC GLM (SAS) and effects were considered significant when $P < 0.05$. Differences (mean $\pm$ SD) were between Ts:Lc breeds in weight at birth 4.38 ± 0.66 : 3.98 ± 0.71 kg, also at weaning 13.44 ± 1.58 : 12.18 ± 1.88 kg. Ts lambs at weaning had a higher DLWG of 0.247 ± 0.037 kg than Lc lambs of 0.221 ± 0.039 kg. In Ts lambs, the weight of the animals was not influenced by sex or litter size up to 30 days of age. At weaning, animals born as a singletons had a higher weight than the triplet lambs 14.19 ± 1.15 : 12.46 ± 2.16 kg. There were no differences in the weight of Lc lambs due to sex at birth, but already at day 10, males had a higher weight than females 6.08 ± 1.09 : 5.37 ± 0.55 kg and the differences continued until weaning 12.75 ± 2.03 : 11.31 ± 1.26 kg. Males had higher DLWG from day 20 to day 30 of age 0.253 ± 0.088 : 0.196 ± 0.066 kg than females, but also for the whole period until weaning 0.232 ± 0.037 : 0.205 ± 0.037 kg. Differences in weight due to litter size were only at birth, when singletons had a higher weight than triplets 4.29 ± 0.55 : 3.38 ± 0.77 kg. For rearing lambs for sale, milk nutrition using ALF and MR appears to be a suitable solution. This study was supported by APVV -21-0134.

Evaluating cucumber and straw silage as a sustainable forage alternative: effects on digestibility, rumen fermentation and blood metabolites in sheep

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Horticultural agriculture in the Mediterranean basin generates by-products that can cause environmental problems. Their use as animal feed represents an opportunity but requires preservation and evaluation of their nutritional value. This study assessed the impact of silage made from cucumber and wheat straw as an alternative forage source for sheep. Eighteen Segureña sheep were randomly assigned to one of two forage-based diets (n=9) in a randomized block design: an oat hay control diet (CTL) or a cucumber+straw silage diet (SIL). The experiment lasted three weeks, including a two-week adaptation period and a one-week evaluation phase. Feed intake, total tract digestibility, microbial protein (MCP) synthesis, rumen fermentation, and blood metabolites were measured and analyzed by ANOVA. No differences in dry matter intake (DMI) were observed when forages were fed ad libitum. However, when intake was restricted to the maintenance level, animals on the SIL diet exhibited higher DMI than those on the CTL diet. Cucumber silage had a higher neutral detergent fiber (NDF) content (78% vs. 51%) and a lower crude protein (CP) content (6.49% vs. 8.29%) than oat hay. While ruminal ammonia-N and total volatile fatty acid concentrations did not differ, the SIL diet increased ruminal pH, acetate (P=0.004), and butyrate (P=0.023), while reducing propionate (P<0.001). Apparent digestibility of dry matter and organic matter was unaffected, but NDF digestibility was greater (P=0.001), and CP digestibility was lower (P<0.001) in the SIL diet. The lower N intake (-12%) in SIL lambs was compensated by enhanced ruminal proteolysis and N recycling, as indicated by a tree-fold increase in iso-acid levels and a 62% increase in blood urea concentration. This ensured similar ruminal NH₃-N levels and MCP synthesis between diets. Silage made from cucumber and straw can be a viable forage alternative for ruminants at maintenance levels. However, its low N content and reduced N digestibility may require additional protein supplementation for animals with higher nutritional requirements.

Session 21

Poster 13

Effect of dietary vitamin E supplementation on animal performance, carcass traits and meat quality of culling Sarda dairy ewes

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The aim of this study was to investigate the effect of dietary vitamin E supplementation of cull ewes on their carcass characteristics and meat quality. Thirty-nine Sarda dairy ewes (mean $\pm$ SEM; 3 $\pm$ 1.6 years old) candidates to be culled, were allocated to two dietary groups homogenous for body condition score (BCS) of the animals: control (CTRL; BCS= 2.97) and vitamin E (VIT-E; BCS= 2.88). Both groups had ad libitum access to a commercial concentrate (CP:14%, NDF:28%, starch: 46%, on DM) and hay (CP:7%, NDF:71.7%, on DM) for 4 weeks. The concentrate offered to the VIT-E group was supplemented with 0.5 g/kg vitamin E. The BCS and body weight (BW) data were recorded weekly, and daily feed intake of the groups was also measured weekly. At slaughter the carcass yield and measurements, and meat quality were evaluated. Dietary treatment did not affect dry matter intake (0.82 vs. 0.95 $\pm$ 0.06 kg DM/d; CTRL and VIT-E, respectively; P=0.191), BCS, live and slaughter BWs. A significant effect of time was observed both for BCS (P<0.0001) and for BW (P=0.0103). Muscle colour parameters, pH, cooking and drip losses, proximate composition, fatty acid profile and alpha-tocopherol content did not differ between the two groups. Longissimus thoracis et lumborum (LTL) muscle from VIT-E group showed higher oxidative stability than CTRL (Thiobarbituric acid-reactive substances (TBARS): 0.52 vs. 1.10 mg malonaldehyde (MDA)/kg meat). In conclusion, the supplementation of the diet with about 0.5 g/d of vitamin E for culling Sarda dairy ewes did not affect animal performance and the overall quality of meat. However, it improved the oxidative stability of LTL muscle. Thus, it can be effectively used to increase the shelf-life of sheep meat-derived products. Research funded by VALPESA project (PSR Sardegna 2014–2020).

Antimicrobial resistance in *Campylobacter* spp., *E.coli*, *Enterococcus* spp. and *Salmonella* from ovine feedlots and crop fields fertilized with composted manure

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Antimicrobial resistance (AMR) is a global problem that should be addressed under a One Health approach. This work aimed at identifying zoonotic indicator bacteria (*Campylobacter* spp., *E. coli*, *Enterococcus* spp. and *Salmonella*) in ovine commercial feedlots and their manure, as well as their AMR, according to Directive 2003/99/EC. For this purpose, five pool fecal samples and two samples of fresh and composted manure were collected from two feedlots at three timepoints over a year, four months apart. Additionally, to assess environmental AMR dissemination, soil, oatmeal plant and root samples were taken from unfertilized and fertilized soil with composted manure. Bacterial isolation and AMR determination were performed according to ISO standards using Sensititre® plates. Identification was done by PCR or 16S rRNA sequencing. No *Salmonella* was isolated from any sample. *Campylobacter* spp. was isolated in 60% (15/25) and 10% (1/10) of fecal and fresh manure samples, respectively, showing high resistance to tetracyclines (87%) and ciprofloxacin (53%), while it was not found in composted manure. *E.coli* was isolated in 92% (23/25), 40% (4/10) and 13% (1/8) of faeces, fresh and composted manure, with elevated tetracyclines (81%) and sulfamethoxazole (94%) resistance. *Enterococcus* spp. was isolated in 48% (12/25), 40% (4/10) and 38% (3/8) of faeces, fresh and composted manure, displaying elevated macrolide resistance (93%) and 17% resistance to vancomycin. Multidrug resistance was found in 29%, 81% and 10% of *Campylobacter* spp., *E. coli* and *Enterococcus* spp., respectively. Regarding soil, plants and roots, only *E. coli* and *Campylobacter* spp. were isolated in 7% of samples (5/76) irrespective of fertilizer application. No bacterial isolates were detected in plants. Under the conditions of this study, the transmission of AMR from feedlots to manure, soil and plants seems to be limited, but further studies are required. Funded by GANARAM project (PPCCDemo Agroalnext) and Aragón Government (R. group A21_23R)

Effects of Dietary Supplementation with Oyster Mushroom By-products on Lamb Performance and Meat Quality

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This experiment investigates the effects of dried oyster mushroom waste (OMW) supplementation on lamb growth performance and meat quality. Twenty four 3-month-old male Chios lambs were randomly assigned to three groups (every group consists of eight lambs): a control group (C) receiving a basal diet; P2 group that was offered a diet supplemented with 2 % dried oyster mushroom waste (OMW) of the *Pleurotus ostreatus* species; and the P4 group, supplemented with 4% OMW, respectively. Individual body weight measurements were recorded weekly for each lamb. After a 28-day feeding period, lambs were fasted for 12 h, weighed and slaughtered. Then samples of longissimus thoracis muscle were collected from each lamb for subsequent quality evaluation. The meat pH, color, water holding capacity, shear force and oxidative stability were determined. On the day of slaughter, the internal organs (spleen, kidneys, liver, lungs, and heart) were weighed, along with the hot and cold carcass weights of each lamb. The supplementation of lambs' diet with OMW did not affect carcass traits, internal organ and fat tissue weights. No significant differences were observed among the three lamb groups in meat quality characteristics, such as pH, color, water holding capacity and shear force values ($P>0.05$). The supplementation of the diet with OMW has no adverse effects on performance or meat, suggesting that this byproduct may be successfully employed in lambs' diets, in a circular economy scheme, with benefits not only for the consumers and farmers but for the environment as well. This research project was implemented within the framework of the Project "PAS-AGRO-PAS (PRIMA), Horizon 2020 Research and Innovation Programme" under Grant Agreement No 857510 with Research & Innovation Foundation of Cyprus

The influence of dietary supplementation with oyster mushroom waste on antioxidant capacity and the fatty acid (FA) profile of the lamb's meat

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This study evaluates the impact of incorporating dried oyster mushroom waste (OMW) into lamb diets on antioxidant capacity and the fatty acid (FA) profile of the meat. A total of twenty-four 3-month-old male Chios lambs were randomly allocated into 3 groups. The first group received a basic diet (C), was fed a standard diet. The second group received a diet that was enriched with 2% OMW from *Pleurotus ostreatus*. The third group was provided with a diet enriched with 4% OMW. Samples of longissimus thoracis muscle were collected for subsequent determination of fatty acid (FA) profile and oxidative stability of the meat. No significant effects were detected on the meat FA profile ($P > 0.05$). However, a notable improvement in oxidative stability was observed in the P2 and P4 groups compared to the control, as there were significant differences both during refrigerated and frozen storage ($p < 0.05$). These positive effects of OMW on the oxidative stability of the meat, without negative impacts on FA profile, suggest that this byproduct could be effectively used in lambs' diets. This supplementation offers potential advantages not only for consumers, farmers, and the environment but also contributes to the sustainable use of natural resources within a circular economy framework. This research project was implemented within the framework of the Project "PAS-AGRO-PAS (PRIMA), Horizon 2020 Research and Innovation Programme" under Grant Agreement No 857510 with Research & Innovation Foundation of Cyprus

Session 22

Theatre 1

Metabolic and hormonal control of nutrient partitioning in sheep and goats

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Ewes and goats are both small ruminants but differ to a certain extent in their feeding behavior and selectivity, milk production, and possibly their nutrient utilization and metabolism. Several studies classified goats as intermediate feeders and ewes as grass and roughage eaters, differing in their feeding preferences and selectivity, with goats more prone to browse and be more selective and ewes more devoted to grazing herbaceous swards. Other differences concern their milk production. Indeed, ewes in general are less productive with shorter lactations and produce milk with much higher fat and protein concentrations compared with goats. Moreover, in ewes, high-starch diets (20.0% starch, on DM) favor milk production in early lactation and body fat deposition in mid-lactation, whereas dairy goats seem to benefit from high-starch diets during the whole lactation. These different responses could be due to species differences in the concentration of the hormones which control energy partitioning, or in the responsiveness of tissues involved in the energy partitioning and blood glucose utilization. This review will explore the biological reason that would explain the previously reported different behavior in the use of carbohydrates (starch and highly digestible fiber) between lactating dairy goats and ewes. Overall, it appears that specialized dairy goats have a hormonal status (high GH and low insulin concentration) that favor the partition of dietary energy for milk production, whereas dairy ewes show a hormonal status (low GH and high insulin concentration) more prone to partition dietary energy for body reserves accumulation. Indeed, ewes and goats have a different control of glucose metabolism both in early and mid-lactation. Due to the higher peak insulin, insulin increment, linear insulin area under the curve, insulin resistance index, and lower insulin sensitivity indices, ewes appear to be more hyper-insulinemic than goats throughout the lactation. In contrast, goats show lower insulin levels and similar glucose clearance, which might indicate higher glucose partitioning toward mammary gland than body reserves.

Prepartum high-starch diet enhances colostrum IgG concentration in dairy goats

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This study hypothesizes that feeding a high-starch (HS) diet during the last month of gestation can enhance colostrum yield and composition as well as dam and goat kid metabolism and immune status. Thirty multiparous pregnant Majorera dairy goats were randomly assigned to a prepartum dietary treatment at wk -4 relative to expected parturition. Goats were fed either a control (n=15; 100% DM of starch requirements) or HS (n=15; 134% DM of starch requirements) diet during the last month of gestation. Blood samples were collected on wk -4, -3, -2, and -1 relative to expected parturition, immediately after parturition and on d 1, 2, 3, 5, 10, 15, 30 postpartum. Colostrum yield, chemical composition and IgG concentration and serum metabolites and plasma IgG concentration were determined. Data were analysed using MIXED and ANOVA procedure of SAS (SAS 9.4). The model included the prepartum diet, time, and the interaction between both as fixed effects. The statistical significance was set as $P \leq 0.05$. No differences were obtained for colostrum yield and chemical composition, whereas the HS group showed higher colostrum IgG concentration than the control group (i.e., 85.4 ± 8.39 and 60.5 ± 8.30 mg/mL, respectively). Serum BHB concentration in the HS group increased progressively until parturition, whereas the control group showed a sharply increased from wk -1 to parturition (i.e., 0.24 ± 0.02 and 0.29 ± 0.02 mmol/L at parturition, respectively). In goat kids, no differences were observed for plasma IgG concentration although the HS group showed an increased serum total protein concentration. The present study indicates that feeding a high-starch diet prepartum does not affect either colostrum yield or chemical composition but increases colostrum IgG concentration and promotes a smoother transition from late pregnancy to early lactation without affecting the metabolic and immune status of goat kids.

Session 22

Theatre 3

Induced lactation in dairy goat

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In a context where kid meat is undervalued, French dairy goat farmers face significant economic, logistical, and ethical challenges. An innovative solution, induced lactation (IL), allows goats to produce milk without gestation, following a dry period. This study identifies the success factors of IL for the first time. An experiment was conducted in South-Eastern France with 162 dairy goats selected from 10 farms. The goats were divided into 2 balanced groups (n=81) based on breed (Alpine, Saanen), parity (primiparous, multiparous), and productivity (low, high): a gestating group (G) and a non-gestating group (NG). NG goats were excluded from reproduction and kept in the same flock as G goats. All goats were dried off in late autumn. After kidding of G goats, farmers manually stimulated the teats of NG goats following a standard protocol for 3 weeks. No hormone treatment was applied. Serum prolactin level, udder thermal and standard images, udder hairiness, udder consistency, and body condition score (BCS) were recorded at five time points: 30 d and 10 d before kidding, 1 d before stimulation, and after 10 d and 21 d of stimulation. The number of NG goats that induced lactation and their milk yield during the 3-week stimulation were recorded. The effect of farm-level and animal-level parameters on the success of IL was tested through logistic regression. The effect of IL on udder parameters and prolactin levels was assessed using mixed ANOVA for repeated measures. After 3 weeks of stimulation, 51% of NG goats began to induce lactation. Breed, parity, productivity, daytime, and prolactin levels had no significant effect ($P > 0.05$) on IL success. On the contrary, NG goats with $BCS \geq 3$ at the end of the dry period had a higher chance of induction (odds ratio=4.7, $P=0.003$). A similar result was observed in goats with outdoor access during the stimulation period (odds ratio=3.3, $P=0.023$). NG goats that induced lactation had higher teat temperature ($P < 0.001$) and udder width ($P=0.013$) during the dry period. These parameters could be used to identify goats with a higher chance of inducing lactation. The physiological determinants of IL remain to be studied, probably later in lactation when milk production is better induced.

Quality determination of fresh, refrigerated, frozen and refrozen goat colostrum using a digital BRUX refractometer
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Colostrum is the primary source of immunoglobulins, particularly IgG, for newborn goat kids. However, limited colostrum availability or poor-quality colostrum (i.e., low IgG concentration) forces farmers to store high-quality colostrum. Given the strong correlation between IgG concentration and BRUX degrees, optical and digital refractometers have been widely used to rapidly assess the quality of fresh colostrum. However, no information is available in the literature regarding this correlation in stored colostrum. This study aimed to evaluate the use of a digital refractometer for the rapid assessment of colostrum quality in fresh, refrigerated, frozen, and refrozen colostrum. The statistical analysis was performed using the software SAS 9.4 (SAS Institute, Cary, NC, USA). The variance and the normality of the residuals were graphically assessed using the PROC UNIVARIATE of SAS. The Pearson correlation coefficient was determined using the PROC CORR of SAS. These correlations were categorized according to Schober & Schwarte, (2018) as follows: negligible ($r < 0.30$); low ($r = 0.30$ to 0.49); moderate ($r = 0.50$ to 0.69); high ($r = 0.70$ to 0.89); or very high ($r \geq 0.90$). Statistical significance was set as $P \leq 0.05$. All the results are presented as Mean $\pm$ Deviation. The findings showed that IgG concentration remained unaffected by the storage method ($P = 0.995$). However, BRUX degrees tended to be influenced by the storage method ($P = 0.086$). A strong correlation between IgG concentration and BRUX degrees was observed in fresh colostrum ($r = 0.80$), whereas no significant correlation was found in refrigerated, frozen, or refrozen colostrum ($r = 0.11$, $r = 0.33$, $r = 0.29$, respectively). In conclusion, while a digital refractometer is an effective tool for assessing the quality of fresh colostrum, it is not reliable for evaluating colostrum stored under refrigeration or freezing conditions. These findings enhance the understanding of the chemical changes occurring in stored colostrum and set the path for future research in this field.

Session 22

Theatre 5

Ceramide Dynamics in Periparturient Ewes: Metabolic Adaptations to Nutrient Restriction
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The periparturient period in ruminants is marked by maternal insulin resistance to support fetal development and the onset of milk synthesis. While ceramide (Cer) metabolism is well-characterized in dairy cows, its role in transition ewes remains unclear. This study examined plasma and milk Cer profiles in 20 ewes subject to either ad libitum (AL) feeding or two 5-d 50% nutrient restriction (NR) periods: late gestation (GNR; d-6 to -2 relative to parturition) and early lactation (LNR; d11 to 15). Plasma and milk samples were collected on d-7, -1, 1, 10, and 16 and analyzed via LC-MS/MS. Following GNR, AL ewes showed a mild accrual of plasma C22:0-dihydroCer (DHCer; fold change [FC]: 1.14, $P < 0.05$). Conversely, plasma C26:1-monohexosylCer (HexCer) and C20:0-dihexosylCer (Hex2Cer) rose in NR (FC: 1.34 and 1.27, $P < 0.05$), suggesting GNR redirected Cer metabolism toward more complex glycosphingolipids. Upon parturition, AL ewes exhibited transient increases in total plasma Cer while NR ewes showed suppression ($P < 0.05$). However, NR enhanced glycosylation, reflected in higher C22:0- and C22:1-HexCer (FC: 1.59 and 1.77, $P < 0.05$). By d10, NR ewes sustained increases in plasma Cer, HexCer, and Hex2Cer, especially C18:1-Cer and HexCer (FC: 4.30 and 3.46, $P < 0.05$), indicating prolonged metabolic effects of GNR. Following LNR, AL ewes showed increased plasma Cer, HexCer, and Hex2Cer, most notably for C18:1-Cer and HexCer, and C20:0-Cer (FC: 4.13, 3.33, and 2.38, $P < 0.05$), while NR maintained elevated glycosylation markers (C16:0- and C20:1-Hex2Cer; FC: 1.81 and 1.60, $P < 0.05$). Plasma C18:0-DHCer increased in AL but decreased in NR (FC: 1.17 and 0.82, $P < 0.05$), while C16:0-HexDHCer decreased in AL but increased in NR (FC: 0.61 and 2.01, $P < 0.05$), reinforcing a shift toward glycosylation under NR. Milk Cer showed opposing trends to plasma Cer, however, glycosylation trends were similar. At d10, milk C20:0-Cer was higher in NR (FC: 1.87, $P < 0.05$), while AL favored glycosylation (C20:1-HexCer; FC: 1.54, $P < 0.05$). By d16, milk C20:1-Hex2Cer increased in NR (FC: 1.56, $P < 0.05$), suggesting compensatory glycosphingolipid secretion post-LNR. Transition ewe plasma was enriched in C24:0, C22:0-, and C22:1-Cer (54.7, 16.2, and 6.7%), while milk was enriched in C24:0-, C24:1-, and C18:0-Cer (58.6, 17.1, and 8.8%). This aligns with the prominence of C24:0-Cer across tissues and plasma in both cows and ewes but newly highlights the metabolic significance of C18:0- to C22:1-Cer species in transition ewes. Despite uniform AL feeding from d-1 to 10, NR ewes displayed prolonged metabolic alterations, reinforcing the need for optimized periparturient nutrition to support metabolic resilience.

DART-HRMS as an advanced chemometric approach to assess the impact of dietary black grape pomace silage on the metabolomic profile of goat kefir

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The metabolomic characterization of goat products is a challenging task especially in the case of using dietary by-products to increase both the milk and nutritional value of the derivatives and the sustainability of the dairy systems. This study examines the impact of incorporating black grape pomace silage (BGPS) into a hay-based control (C) diet on the metabolic profile of goat milk and the resulting kefir. To this aim, two groups of Murciano Granadina goats were used, one group receiving the control diet, while the other being fed with the C ration partially replaced by 12% of BGPS. Kefir was produced with pasteurized milk and commercial starter cultures. After 1, 14, and 28 days of cold storage, C- and BGPS- kefir samples were analyzed for the metabolomic fingerprint via a direct analysis in real time coupled with high-resolution mass spectrometry (DART-HRMS) technique. The resulting data were interrogated by multivariate statistical analysis. The main outcomes suggest that the DART-HRMS fingerprints, acquired in negative and positive ion mode, effectively differentiated the kefir samples and captured a set of informative biomarkers more related to sugars, phenolic acids, fatty alcohols, and small volatile compounds. These preliminary findings provide insights into the metabolomic changes associated with goat kefir produced feeding dairy goat with a by-product of winery industry, a dietary strategy that seems useful to reduce feed costs and optimize waste management in the winery sector.

Session 22

Poster 7

Effect of dietary lemon essential oil supplementation on ewes' milk

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Heat stress affects the physiological functions of livestock animals, with negative effects on welfare, health and productivity; addition of bioactive plant extracts to the diet may improve milk production also enhancing animals' resilience to HS. The present study aims to investigate the effects of lemon essential oil administrated in non-encapsulated (EO) or microencapsulated (EOM) form on performance of dairy ewes. Since some bioactive components of EOs are unstable, the encapsulation was assessed for its ability to coat and protect them. The experimental trial lasted 42 days was conducted on a farm located in the Sicily region (Italy) during summer. Forty primiparous dairy ewes were divided into 5 homogeneous groups. Each group, composed of 8 animals, was fed the same conventional diet, one representing the control groups (C) and the other differing for the inclusion level (L=low, 400 mg/d; H=high, 800 mg/d) of microencapsulated or not encapsulated lemon EOs (EOM, EO). Thus, the 5 groups were C, LEOM, HEOM, LEO, HEO. Milk yield, milk composition and coagulation parameters were evaluated weekly. All data were subjected to analysis of variance using the GLM procedure of the SAS statistical software. The model included the fixed effects of the diet treatment and random residual error; all effects were tested for statistical significance (to $P < 0.05$). A significant increase in live weight ($P=0.01$) was observed in all groups from the start to the end of the trial, primarily due to the ongoing growth of the primiparous ewes. Milk yield tended to be affected ($P=0.082$) by dietary supplementation, particularly, LEOM and HEO groups showed a higher production compared with the other groups. Regarding milk quality, the effects of EOs supplementation emerged in the somatic cell count which tended to be higher in the group C compared to HEOM group (5.45 vs $5.25 \log_{10}$ n/ml, $P=0.075$). Research supported by PRIN 2022 PNRR grant– Ceo-SUSTAINSHEEP

Dietary n-6 vs. n-3 PUFA. Effect on the resilience of dairy sheep to a mammary inflammatory challenge: 1) Intake and milk production

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Resilience is an animal's ability to withstand a relatively short disturbance and quickly recover its previous production or health status. Despite limited research on the influence of dietary polyunsaturated fatty acids (PUFA) over resilience, there might be a potential positive effect of n-3 PUFA. This study aimed to evaluate the effect of diet supplementation with n-6 vs. n-3 PUFA on the productive performance of dairy sheep in response to a mammary inflammatory challenge causing mastitis. Twenty-two Assaf ewes were assigned to 2 dietary treatments: TMR supplemented with 2% soybean oil (SO, rich in n-6 PUFA; n=11) or 2% linseed oil (LO, rich in n-3 PUFA; n=11). The experiment had 3 periods: pre-challenge (4 weeks), challenge (10µg of *Escherichia coli* lipopolysaccharides (LPS) infused in each mammary gland), and post-challenge (3 weeks). Time-series data of daily DM intake (DMI) and milk yield (MY) were analysed using a 4-parameter piecewise model (R v4.2.1): V1 (pre-challenge constant), V2 (linear slope of challenge-response, 3d), V3 (recovery period, 5d after the response) and V4 (stabilization period, remaining post-challenge days). Orthogonal contrasts compared V1 vs. V4. Two animals on the LO diet were removed after 48-72h due to the need for anti-inflammatory treatment. Intake and MY did not differ by diet in the pre-challenge period (V1; $P>0.10$). However, the LO group showed a deeper decrease in DMI due to the challenge (V2; $P<0.05$) and also a higher recovery slope (V3; $P<0.05$), with both treatments recovering pre-challenge values in the stabilization period. Indeed, the SO group exceeded initial values during post-challenge ($P<0.05$ for V1 vs. V4). No significant differences were detected between groups in MY response and recovery (V2 and V3; $P>0.10$). However, in the LO group, MY during stabilization exceeded pre-challenge levels ($P<0.05$ for V1 vs. V4), which is likely due to the low MY values in the 2 removed animals. Replacing dietary soybean oil (n-6 PUFA) with linseed oil (n-3 PUFA) may affect DMI response patterns but without an overall improvement of the resilience of dairy sheep to this type of inflammatory challenge. Acknowledgment: PID2023-146979OB-I00 and PRE2021-098235 (MCIN/AEI, Spain).

Session 22

Poster 9

Targeted milk metabolomics for assessing feed efficiency in dairy sheep

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Assessing feed efficiency (FE) in dairy sheep is challenging due to the difficulty of accurately measuring feed intake. However, understanding the biological basis of FE can facilitate the selection of more efficient animals. Identifying proxy variables that can reliably predict FE would be highly valuable. Previous findings from our research group support growing evidence that protein metabolism plays a key role in FE in dairy animals. This study explores the potential of milk metabolites to effectively classify dairy sheep based on divergent FE profiles. Data from 40 Assaf ewes (62 ± 0.1 days postpartum) were collected over three weeks. Ewes were divided in two groups, high- (FE+; n=6) or low-feed efficiency (FE-; n=6), using residual feed intake and a feed efficiency index. Targeted metabolomics was performed on milk samples, quantifying amino acids, amines, metabolic intermediates, nucleosides, nucleobases, and carnitines using UPLC-MRM/MS. Data processing involved peak detection (Agilent MassHunter Profinder) and alignment (Mass Profiler Professional). Metabolites detected in at least 75% of samples were analyzed using sparse Partial Least Squares Discriminant Analysis (sPLS-DA) via mixOmics in R (v4.4.2). Variable Importance in Projection (VIP) identified key metabolites (VIP > 1.5). The Mann-Whitney U test assessed metabolite abundance differences between groups. After quality control, 106 metabolites were analyzed. The sPLS-DA model effectively separated FE groups, with the first two dimensions explaining 19% and 14% of variability. VIP selection identified 12 key metabolites (e.g., D-alpha-aminobutyric acid, diaminopimelic acid, glycine, leucine, methionine, proline, and uric acid). Applying sPLS-DA to this subset improved group separation, increasing variance explained by the first dimension to 56%. These 12 metabolites showed significant abundance differences between FE+ and FE- ($p < 0.05$). These preliminary results support the potential use of milk metabolites for classifying and selecting more feed-efficient animals. Acknowledgments: PID2020-113441RB-I00 and PID2023-146979OB-I00 (MCIN/AEI, Spain).

Impact of Birth Weight and Early Growth on Milk Yield in Ewes' First Lactation

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The aim of this study was to evaluate the effect of birth weight and daily weight gains by life day 10, 20, and 30 on milk yield and somatic cell score (SCS) during the first lactation. Slovak dairy sheep (N=26) were divided into three groups based on birth weight (BW1: 2.5-3.5 kg; BW2: 3.5-4.5 kg; BW3: ≥ 4.5 kg) and average daily gains by life day 10, 20, and 30 (NDG: 0.01-0.20 kg; MDG: ≥ 0.2 -0.25 kg; HDG: ≥ 0.25 kg). Artificial rearing was carried out from the 3rd life day using a feeding machine, providing ad libitum access to a milk feeding mixture. From life day 10, animals received supplementary concentrate feed and hay. Animals were regularly weighed using a digital scale (accuracy 0.1 kg). Individual milk yield was recorded and milk samples were collected once a month from March to July. Data were analysed by ANOVA (post hoc: Tukey); the model included the fixed effects of birth weight and average daily gains by life day 10, 20, and 30. Significance was set at $P \leq 0.05$. The significant differences in milk yield during the first lactation were observed between BW1 and BW2, BW3 the birth weight groups ($P=0.0066$). Concerning daily weight gains, the highest milk yield (207.78, 223.77, 232.24 L) was observed in animals with NDG by life day 10, 20, and 30, resp., while the lowest yield (193.58, 166.36, 179.87 L) was recorded in animals with MDG by life day 10, 20, and 30. However, a significant difference was observed only between animals with NDG and MDG by life day 20 ($P=0.0007$). The only significant differences in SCS were observed by life day 10 between daily gain groups NDG (1.89) and MDG (2.32) ($P=0.0467$). In conclusion, only the birth weight and the weight gain by life day 20 influenced milk yield during the first lactations, while weight gain by life day 10 affected SCS. This publication was written as part of the APVV-21-0134 project.

Session 22

Poster 11

The Cathelicidin Gene Cluster in Goats (*Capra hircus*): Organization and DiversityS. González-Acosta^{1,2}, M. R. López¹, P. Asensio-Calavia^{1,2}, A. Otazo-Pérez^{1,2}, J. M. Pérez De La Lastra¹, A. Morales-Delanuez^{1,3}¹ Biotechnology of Macromolecules Research Group, Instituto de Productos Naturales y Agrobiología, Avda. Astrofísico Francisco Sánchez 3, 38206 San Cristóbal de la Laguna, Spain, ² Escuela de Doctorado y Estudios de Posgrado, Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez, SN, 38200 San Cristóbal de La Laguna, Spain, ³ IUSA-ONEHEALTH 4 Animal Production and Biotechnology Group, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Transmontaña s/n, 35413 Arucas, Spain

The innate immune system is the first line of defence against pathogens. Among these, host defence peptides play a crucial role as they have direct antimicrobial activity and modulate the immune response. Cathelicidins are a well-known HDP found in most vertebrates, including goats. These peptides contribute to the immune system by targeting a broad spectrum of microorganisms being involved in immune regulation. Cathelicidins are structurally diverse and are classified according to the secondary structure of their mature peptides, including α -helices, β -hairpin structures, and elongated forms rich in proline-arginine or tryptophan. Their genes are typically organised in genomic clusters, as seen in ruminants such as sheep and cows. The identification of several cathelicidin genes and their organisation is necessary to understand the organisation and diversity of these antimicrobial peptides in the goat genome. This information provides valuable insights into their role in immune defence and disease resistance. The aim of this study was to identify and characterise the goat cathelicidin cluster using bioinformatics tools and analysis of the reference goat genome. Our results show that all cathelicidin genes in the goat genome are grouped in a cluster on chromosome 22 spanning approximately 75 Kb. It was identified a total of seven cathelicidin genes and one potential pseudogene. The average gene size was 1719 bp and ranged from 1309 bp (CATH1) to 1956 bp (CATH3). The first gene of the cluster, MAP34B, encodes a mature peptide containing a CAP-18 domain, a characteristic LPS-binding domain in mammals. Three cathelicidins exhibited a proline-arginine-rich structure, while the other four adopted an α -helix conformation.

High dietary cation and anion ration: Reconsidering the formulation for ruminants fed under high ambient temperature

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It is well known that dairy cows and goats fed under high ambient temperature (HTa) have limited capacity for milk synthesis. There are evidences that the HTa condition influences the animal well-being and that ruminants living under the natural HTa of tropical conditions are at the stage of heat stress. We have investigated the nutritional strategy to mitigate the negative effect of HTa and hypothesize that this strategy should be implemented as the biological coolant for dairy ruminants fed under HTa conditions. This biological coolant is the solution that contains a high degree of cation and anion difference (CAD). We have demonstrated that high dietary CAD (hDCAD) could increase evaporative heat dissipation in dairy goats via increasing respiratory rate (control: 124 ± 10 vs. hDCAD: 148 ± 10 bpm; $p < 0.05$) and that the percentage increment of rectal temperature was lowered (control: $0.76 \pm 0.06\%$ vs. hDCAD: $0.43 \pm 0.05\%$; $p < 0.05$). High DCAD increases nocturnal drinking behavior (control: 6 ± 2 vs. hDCAD: 32 ± 4 mL/kg BW; $p < 0.05$) and tends to increase daily eating behavior (control: 33 ± 2 vs. hDCAD: 37 ± 2 g/kg BW; $p < 0.05$) as well. Under 8 weeks of hDCAD, the ruminal volatile fatty acid and total tract digestibility (control: $74 \pm 0.9\%$ vs. hDCAD: $79 \pm 0.8\%$; $p < 0.05$) were improved. Finally, hDCAD shifted the acid-base balance to the alkaline side within 4th week of treatment and the balance was restored when the treatment was prolonged (within the 8th week of treatment). The later acid-base homeostasis was mainly controlled via kidney functions by increasing the excretion of bicarbonate together with sodium and potassium. We conclude that hDCAD as the biological coolant might be implemented for dairy ruminants living in tropical countries during summer to improve animal well-being and production.

Session 22

Poster 13

Effect of melatonin implants during the dry-off period on blood variables in pregnant dairy goats

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This study aims to evaluate different dry-off strategies based on melatonin implants and milking frequency on blood variables in dairy goats. On day (d) -75 relative to expected parturition, 24 Majorera dairy goats were subcutaneously treated with a 1 mL of saline (SAL group) or with a melatonin (MEL) implant (MEL group). From d -75 to d -60 relative to expected parturition, animals were milked daily. From d -60 to d 0 relative to parturition, animals were not milked. From d -75 to d -52 relative to parturition, blood samples were collected to determine white cells concentrations as well as albumin, B-hydroxybutyrate (BHB), calcium, glucose, free fatty acids (FFA), total protein and urea concentrations. The data was analysed using the PROC MIXED procedure of SAS including implant (IMP; saline vs. melatonin), time (T) and the interaction between both (IMP×T) as fixed effects. The significance was set as $P < 0.05$. Leukocyte concentrations increased (IMP×T = 0.010) in the SAL group compared to the MEL group on d-60 relative to parturition (14.59 ± 1.15 and $10.63 \pm 0.99 \times 10^3$ cells/ μ L, respectively). These changes were caused by monocytes and granulocytes (IMP×T < 0.006) and no changes in lymphocyte concentration were observed (IMP×T = 0.222). Neither albumin, BHB, LDH, FFA nor urea concentrations were affected by either implant ($P > 0.101$) or the IMP×T interaction ($P > 0.34$). Calcium, glucose and protein concentrations were higher (PIMP < 0.048) in the MEL group (8.4 ± 0.20 mg/dL, 58.9 ± 3.34 mg/dL and 7.5 ± 0.17 g/dL, respectively) than in the SAL group (7.6 ± 0.20 mg/dL, 53.8 ± 3.34 mg/dL and 6.8 ± 0.17 g/dL, respectively). In conclusion, using MEL implants during a 15-day dry-off period promotes a smoother transition from late lactation to the dry period in dairy goats. This study was funded by the project PID 2020-113056RA-I00/AEI/10.13039/501100011033.

Effect of milking frequency and melatonin implants during the dry-off period on milk yield and composition in pregnant dairy goats

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This study aims to evaluate the effects of different dry-off strategies based on melatonin implants or milking frequency on milk yield and chemical composition in pregnant dairy goats. On day -75 relative to expected parturition, 24 Majorera dairy goats were subcutaneously treated with 1 mL of saline or with a melatonin implant. From day -75 to day -60 relative to expected parturition, animals were either milked once daily or once every other day. From day -60 to day 0 relative to parturition, animals were not milked. From day -75 to day -60 relative to parturition, milk yield was recorded, and milk samples were collected to determine gross chemical composition (i.e., lactose, fat, protein and total solids) and somatic cell counts (SCC). Data were analysed using the PROC MIXED procedure of SAS, and the significance was set as $P < 0.05$. The results showed a faster milk yield reduction in those animals treated with melatonin implants compared to those receiving saline ($P < 0.001$). In addition, milk yield decreased faster in those animals milked once every other day than those milked once daily. Neither gross chemical composition nor SCC were affected by either the melatonin administration or the milking frequency ($P > 0.05$). In conclusion, it seems that reducing milking frequency as well as the use of melatonin implants during a 15-day dry-off period are effective strategies to promote a faster transition from late lactation to the dry period in dairy goats. This study was funded by the project PID 2020-113056RA-I00/AEI/10.13039/501100011033.

Session 22

Poster 15

Changes in milk yield and composition in dairy goats during kid suckling period

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The aim of the study was to assess the milk yield and kid growth dynamics during the suckling period in Alba de Banat dairy goats. Kids were weighed at birth (M1), at 28 days of age (M2) and weaning at 60 days of age (M3). Milk samples were collected by manual milking at 2, 28, 60, and 120 days after kidding. Milk was analysed for density, added water, freezing point, fat, protein, lactose, solids non-fat and minerals using Lactoscan analyzer. Daily ration of goats consisted of 0.9 kg alfalfa hay, 0.5 kg corn, 0.5 kg wheat bran, 0.05 kg calcium phosphate and salt for licking. Statistical analysis was carried out using Mann Whitney ANOVA in STATISTICA 7 software. Milking capacity of the goats at 28 days after kidding was between 24 and 25.8 kg. Average body weight of kids was 3.12 kg at birth, 7.27 kg at 28 days of age, and 16.00 kg at weaning (60 days of age). Per total growth period, the average daily gain in kids was 0.210 kg. Using Fleishman method to estimate the milk production consumed by kids over the growth period showed that this was between 49.9 and 50.7 kg. When were put on pasture after kids were weaned, the control milking of goats showed a milk yield between 1.485 and 1.800 kg/day. The main chemical components of the goat milk showed an increase from the first to the last control milking, except for the fat content that was significantly lower in M2 than in M1 (3.26% vs. 9.68%, $p \leq 0.05$). After weaning the kids and switching to mechanical milking (M4) a small and non-significant increase ($p \geq 0.05$) for all milk components compared to M3 was observed. There was a negative correlation between milk yield and composition. The highest change in goat milk composition was observed between M1 and M2. Kids growth was associated mostly with milk production of their mothers, therefore it was recommended to make a selection for milk production in Alba de Banat breed, and use the mechanic milking for individuals that have a production higher than 0.5 liters/day.

Subclinical intramammary infection prevalence in Lacaune ewes at early lactation

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There is no clear consensus on somatic cell count (SCC) threshold to detect subclinical intramammary infection (IMI) in sheep, with values from 250×10^3 to 500×10^3 cells/mL. The study evaluated subclinical IMI prevalence and impact on milk quality and identified the main pathogens. Milk samples were collected from half-udders of 83 Lacaune ewes in the 6th week of lactation. Bacteriological samples were aseptically collected in sterile 50-mL bottles and kept refrigerated until cultured on blood agar, and incubated at 37°C for 36 h. Positive (>5 colonies) and uncontaminated cultures (≤ 2 isolates) were identified by MALDI-TOF. Milk samples for quality analysis (fat, protein, lactose, and SCC) were also collected. Milk quality differences between infected (POS) and healthy (NEG) half-udders were assessed with a T-test for independent samples. Most positive samples had 1 pathogen (74%; N = 28) and almost a quarter had 2 pathogens (24%; N = 9). The 36% and 39% of the 61 pathogen isolates were coagulase-negative staphylococci (CNS) and *Corynebacterium* spp. (*C. stationis*, n = 16; *C. efficiens*, n = 4), respectively. The following CNS were identified: *Staphylococcus* sp. (n = 7), *S. simulans* (n = 5), *S. chromogenes* (n = 5), *S. xylosus* (n = 2), *S. ureilyticus* (n = 1), *S. auricularis* (n = 1), *S. microti* (n = 1), and *S. arlettae* (n = 1). Other identified pathogens were: *Escherichia coli* (n = 2), *Bacillus pumilus* (n = 2), and *Brevibacterium senegalense* (n = 1), and *Candida parapsilosis* (n = 1). The 41% ewes had subclinical IMI, 5% both udder halves and 36% one udder half. The SCC was higher in POS ($479 \pm 1.3 \times 10^3$ cells/mL) than in NEG ($174 \pm 1.1 \times 10^3$ cells/mL; $P < 0.05$) samples. However, fat (POS, $5.33 \pm 0.26\%$; NEG, $4.83 \pm 0.14\%$), protein (POS, $5.22 \pm 0.06\%$; NEG, $5.23 \pm 0.04\%$), and lactose (POS, $4.82 \pm 0.07\%$; NEG, $4.95 \pm 0.03\%$) was similar between groups. In conclusion, subclinical IMI prevalence in Lacaune ewes at the beginning of lactation was 41%, mostly one half and due to CNS and *Corynebacterium* sp., and did not modify milk composition despite increasing SCC above 400×10^3 cells/mL.

Session 22

Poster 17

Changes in Fat Nutritional Indices in Cheese from Mountainous Goat Milk During Summer Grazing

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Goat breeding plays a vital economic role in rural mountainous Greece, where milk from upland regions is key to producing superior-quality dairy products. Summer pastures, known for their rich biodiversity, serve as a convergence point for flora from both lowland and alpine meadows. Due to growing consumer concerns about dairy fat quality and its health impacts, this study explored changes in fat nutritional indices in cheese made from milk produced by goats grazing in mountainous summer pastures. To assess changes, two dairy goat (*Capra prisca*) farms grazing in lowlands (March–May) and mountain pastures (June–October) were studied. Brined white cheese (n=12) was produced from milk collected between June and October. Examined indices were: polyunsaturated to saturated fatty acids (PUFA/SFA) ratio, atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic to hypercholesterolemic (h/H) ratio. Differences between sampling periods (months) were analyzed using an ANOVA test. Mean PUFA/SFA ratio, AI, TI, and h/H ratio values were 0.06, 2.64, 2.73, and 0.61, respectively. Significant differences were observed in all indices (PUFA/SFA ratio $P < 0.001$, AI $P < 0.01$, TI $P < 0.001$, h/H ratio $P < 0.01$). The highest AI and TI values, exceeding the recommended threshold of 3, were found in October samples, while the PUFA/SFA and h/H ratio values were lowest. Cheese produced from milk collected in August showed the best values across all indices as a whole: PUFA/SFA ratio (0.07), AI (2.37), TI (2.34), and h/H ratio (0.68). Changes in fatty acid composition are evident about two months after dietary changes. The information on cheese nutritional indices related to the production period is valuable for dairy producers, allowing them to promote their products' health benefits and adjust pricing accordingly. Acknowledgement: AGROTOUR Project (MIS 5047196), NSRF 2014-2020.

Simulating individual milk phenotypic curves from real data and their feedback on dairy goat herd milk production, reproduction and demography

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This study investigates the impact of fertility differences related to milk production profiles on the dynamics of a dairy goat herd using an individual-based simulation model developed by the MoSAR unit. The originality of the model is to integrate individual phenotypic trajectories obtained from real data and then simulate the long-term effects of reproductive, culling, and replacement strategies on kidding distribution, and milk sales. In a previous study on historical data from two French experimental farms, 2 types of primiparous goats were found: type one with low persistence in milk production and high AI success (P-F+) and type two with high persistence in milk production and low AI success (P+F-). AI was used for 30% of the herd, while the rest were bred naturally. Goats that failed AI were then given a second chance through natural mating. The simulator was developed to incorporate this information at the individual level and then simulate two contrasting herd scenarios: one composed exclusively of P-F+ primiparous, and one composed exclusively of P+F- primiparous. Despite a 10% difference in AI fertility between scenarios, the overall kidding distribution remained largely unaffected due to high natural mating success. Sensitivity analysis revealed that significantly reduced fertility could lead to wider kidding spreads and impact herd demography. This study highlights the interest of integrating individual phenotypic trajectories to predict the effects of management decisions on herd performance. The model developed here is intermediate between data-driven and concept-driven approaches because it allows for the simulation of biological mechanisms and management practices at the individual scale, and it also allows for the integration of the real farm data. Future improvements should refine lactation curves and incorporate the effects of AI and natural mating return cycles on fertility. The model could be extended to other dairy goat systems in France to optimize management practices and enhance herd sustainability.

Session 22

Poster 19

Study on the comfort factor of wool in some local sheep breeds in Bulgaria

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The wool comfort factor (CF) is an important standard in the textile industry. This is a relatively new characteristic of wool, which depends on the fiber diameter (FD). The aim of this study was to evaluate the FD and the CF in the wool on some local and locally adapted sheep breeds in Bulgaria. For this purpose, four native Bulgarian sheep breeds and two locally adapted ones were selected: White Maritza, Patch-faced Maritza, Dubene and Central Rhodopa and two locally adapted breeds – Rhodopa Tsigai and Stara Planina Tsigai. These sheep breeds have a uniform wool that could be used in the modern textile industry for clothing production. The samples were taken in 2022 and 2024. The measurement of the FD and the calculations for the CF were performed with Optical Fiber Diameter Analyzer (OFDA) in the laboratory of Südrolle Group in Plovdiv. For each of the breeds, over 100,000 fibers were measured. They were established an average FD and standard deviations (SD) as follows: White Maritza – 30.28 ± 2.51 μm ; Patch-faced Maritza – 34.20 ± 3.06 μm ; Dubene – 32.40 ± 2.55 μm ; Central Rhodopa – 32.78 ± 3.53 μm . The locally adapted breeds Rhodopa Tsigai and Stara Planina Tsigai have a small difference in the FD respectively 29.04 ± 3.95 μm and 31.89 ± 3.95 μm . Four local breeds have a CF as follows; White Maritza – 54.07%; Patch-faced Maritza – 35.27% μm ; Dubene – 44.90%; Central Rhodopa – 45.31% μm . The two locally adapted breeds as Rhodopa Tsigai and Stara Planina Tsigai have a significant difference in the CF of 60.14% and 45.67% respectively. In the wool samples from the White Maritza breed, it was found that there were no significant differences in the FDs and CFs of wool samples from different flocks. There was a weakly pronounced trend in wool from lambs at 7 months of age, the average FD was lower (28.44 ± 2.03 μm), and the CF is higher – 63.70 %. The low CF of wool in the six sheep breeds is an obstacle to the production of finer textiles that are in demand in the market. This requires finding other ways to “soften wool” in order to increase its demand and meet the modern consumer’s preferences for clothing made from natural fabrics made from raw materials with a low carbon footprint.

Untargeted metabolomics describes genetic factors that influence pig metabolism for adaptation traits in a Duroc line

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To enhance selection programs and develop novel breeding strategies, the pig production sector continuously explores new phenotypic traits, expanding the traditional phenotypic landscape, which has typically focused on final phenotypes such as production and performance traits. Final phenotypes are complex, influenced by many external and molecular interactions at the organismal level. These influences can be broken down by studying molecular layers or intermediate phenotypes, such as the metabolome. This study aims to genetically dissect pig metabolism to identify markers associated with the adaptation to heat-stress conditions. About 700 blood plasma metabolites were quantified through an untargeted metabolomic platform on 300 growing male Duroc pigs. Additionally, genotypes for ~40,000 single nucleotide polymorphisms were obtained for the same animals. The pigs were from two groups with extreme and divergent values for feed intake under heat-stress conditions. Genomic heritability estimates and genome wide associations for the level of metabolites were calculated using GEMMA. Heritability estimates ranged from 0.0 to 0.83 (average = 0.18; s.d. = 0.15). We found suggestive ($P < 5.5 \times 10^{-05}$) and significant ($P < 1.3 \times 10^{-06}$) marker associations for 400 and 102 metabolites, respectively. Most of these associations were between markers in enzyme-encoding genes and metabolites that either serve as substrates or final products of the corresponding genes. Overall, these newly identified genetic determinants of pig metabolism serve as (i) a novel source of information for indirectly explaining complex traits, including resilience to heat stress, proxied using feed intake measures; and (ii) new genetic markers for fine-tuning selection programs to enhance the sustainability and resilience of pig production. Acknowledgments: This study received funding from the European Union's Horizon Europe research and innovation program under grant agreement No. 01059609 (Re-Livestock project).

Session 23

Theatre 2

Genetic and heat-stress related environmental influences on pig whole-blood gene expression levels

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Heat stress causes animal welfare and production issues in pig production systems. Tropical pig breeds, such as the Creole breed (a local breed from the Caribbean) are more adapted to heat stress than cosmopolitan pig breeds selected for production and reproduction traits. Large-white x Creole backcross animals were produced both in a tropical (in Guadeloupe) or a temperate facility (in continental France). Pigs from the temperate facility were also subjected to an experimental heat stress. Here, we focus on the environmental and genetic influences on whole blood transcriptome of these backcross pigs ($n = 359$). We identified 2,014 differentially expressed genes (DEGs) between pigs from the tropical and temperate facilities, and 481 DEGs during the experimental heat stress. Through Transcriptome-wide association study, expressions of 156 genes were significantly associated with thermoregulation and production traits. Throughout 18,237 autosome-annotated transcriptomic probes, we detected 9,543 expression quantitative trait loci (eQTLs). Genetic variance was estimated to explain 22.7% of gene expression variance on average, and was higher than 50% for 4.8% of transcripts. Most eQTLs are located in proximal regions (cis-eQTL) while a minority are in distal region (trans-eQTL) to their assigned genes. We identified 131 trans-eQTL hotspots. We further identified six genes associated with genotype by environment interactions through cis-eQTLs. With the addition of molecular QTLs such as metabolite QTLs (metQTLs) and phenotypic QTLs, colocalizations may reveal genomic regions of high interest for genomic selection of pigs for climate accommodation and heat stress resilience. Altogether, these results provide a better understanding of the impact of climate, heat stress and genetic diversity on the pig whole blood transcriptome.

Genomic Prediction of Feed Efficiency in Boars by Deep Learning

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Pork is the most widely consumed meat globally, and significant advancements have been made in the industry through genomic selection. However, traditional linear genomic prediction models fall short for complex traits like feed efficiency, as they mainly capture additive genetic effects and ignore non-additive effects. Deep learning (DL) has the potential to address these limitations due to its capacity to model non-linear patterns in genomic data. This study evaluates the performance of DL methods, specifically Multilayer Perceptron (MLP) and Convolutional Neural Networks (CNN), against linear genomic models for predicting feed efficiency using data from two pig populations. We also estimated the extent of non-additive genetic variance captured by DL methods and its impact on predictive abilities. Linear models were used to estimate the proportion of additive and non-additive genetic variances. To evaluate the DL method's predictive ability, a novel averaging predictions approach was used alongside conventional splits average methods. Additionally, we introduced a novel method to decouple the additive and non-additive predictions captured by DL models. The narrow-sense heritability (h^2) estimates were 0.241 for Duroc and 0.255 for Landrace, using the additive-dominance-epistasis (ADE) model, with small dominance and epistasis ratios. DL models outperformed linear models in Duroc (0.381 for MLP and 0.377 for CNN vs. 0.366 for linear) and showed higher accuracies in Landrace (0.364 for MLP). In terms of non-additive genetic variances captured, MLP captured 0.018 and 0.017 for Duroc and Landrace, respectively, although this hardly impacted overall predictive accuracy. MLP demonstrated the highest predictive ability for feed efficiency, showing improvements of approximately 4.1% for Duroc and 2.8% for Landrace compared to the linear models. DL models were more effective at capturing non-additive genetic variance than linear models, resulting in slight improvements in most cases. Thus, DL methods are recommended for predicting complex phenotypes and total genetic effects, including non-additive components.

Session 23

Theatre 4

Genetic Parameters of Heat Tolerance for Milk Yield in Sarda ewes raised in the Mediterranean region

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In this study the genetic variability of tolerance to heat stress (HS) of Sarda ewes was investigated. The data consisted of 179,943 test-day (TD) milk yield records from 16,249 lactations of 4,940 ewes, collected from 2000 to 2022 in an experimental farm located in the South of Sardinia. The ewes were managed according to the traditional seasonal Sardinian system with a December-July milking period. The temperature-humidity index (THI), derived from local meteorological data, was the climatic parameter considered. To assess the impact of HS on TD yields, a random-regression test-day animal model fitting a reaction norm to the average THI over 4 days preceding each TD (THI4d). We defined HS as a linear function (fHS) of THI4d exceeding the threshold of 20. The model estimated the intercept (general effect) and the linear coefficient (heat tolerance) for individual, genetic and non-genetic, and individual by parity random regressions. Genetic effects and (co)variances were estimated using a genomic relationship matrix from OvineSNP50 genotypes. Using the estimated (co)variances of general and heat tolerance effects, the heritability and the genetic correlation between general effects (fHS 0) and effects under HS (general+tolerance*fHS) was calculated for fHS ranging from 0 to 15 (THI4d >20). A systematic effect of -0.075 l/fHS units was estimated. Correlations between general and heat tolerance were -0.72, -0.80 and -0.81 for individual genetic, non-genetic and individual by parity effects respectively. Heritability was 0.25 at fHS 0, decreased to 0.22 at fHS 5, then increased to 0.25 at fHS 15. The correlation between general and HS-affected genetic effects decreased with increasing fHS, remaining >0.89 until fHS 5 and dropping to -0.04 at fHS 15. This research reveals that HS adversely affects Sarda milk yield, despite seasonal farming systems. The observed fluctuations in heritability and genetic correlations indicate a complex genotype-environment interaction. Therefore, breeding strategies should account for these dynamics to maintain productivity under climate change. Research supported by SCALA-MEDI project (Grant: 2021/SCALA-MEDI/Call 2020 Section 1 Farming RIA), funded by EU (PRIMA).

The genetic basis of thermotolerance varies among lactations in Spanish dairy sheep breeds

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Heat tolerance is inversely related to milk production level, and the level of response to thermal disturbances might show distinct patterns across lactations. The aim of this study was to estimate population thermotolerance thresholds (Thr) for the first three lactations and genetic correlations between indicators of thermotolerance between lactations. For that, data from 261,389 milk records (MR) from 30,306 Latxa ewes (from 2013 to 2024 years, across 48 herds) and from 493,734 MR from 101,966 Manchega ewes (from 2015 to 2020, across 83 herds) were used. Average temperature (Tavg) on the day of MR was used as the thermal load variable, which was obtained for each flock, from the NASA online database, based on their geographical coordinates. A multi-trait linear reaction norm model was applied, considering milk yield in the first three lactations as distinct traits. The model included the environmental effects used in the routine genetic evaluations for each breed, alongside genetic and permanent random effects. Tavg was modeling using Legendre polynomials and Thr were estimated using segmented regression analysis. Results showed slight variation in Thr among lactations in Latxa ewes, estimated at 15 °C, 22 °C and 17°C for the first, second, and third lactations, respectively. The corresponding decline rates were around 1% per degree of increment in Tavg over the Thr. In Machega ewes, Thr ranged from 22°C to 30°C across lactations, with decline rates between 1% and 2% per degree. Genetic correlation for baseline production level between lactations was high and positive (>0.92). Nevertheless, moderate or negative correlations were observed between the lineal and quadratic coefficients of each lactation, as well as among genetic breeding values across Tavg gradient. These correlations confirmed the presence of GxE interaction, which resulted of less magnitude in the Manchega breed, and moderate in Latxa breed.

Session 23

Theatre 6

Breeding for resilience to heat stress

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Heat tolerance in dairy cattle likely deteriorated over time due to unfavorable correlations between production and heat tolerance, with stronger deterioration in later parities. Coupled with climate change, heat stress is felt in increasingly colder areas. The industry responded to the challenge of heat stress by applying managerial modifications, such as better cooling devices, special reproductive techniques and sexed semen. Cooling devices are justified in areas with a long hot season and high milk prices, and less so otherwise. High producing cows seem to be neither resilient nor robust and suffer from poor fertility and survival. Methodology exists to select “robust” cows that better maintain production during the heat stress and also exhibit better fertility and survival, at a cost of lower production. A desirable “resilient” cow would maintain high production during colder conditions while rapidly dropping production at high stress level to maintain fertility and surviving. The drop would depend on the level of heat stress and presence or quality of cooling heat mitigation devices. Resilient cow can be selected by an index including production but excluding test days during heat stress, with a large weight on fertility and survival. Such a selection may already be happening in countries where maintaining healthy cows is as important as production and the period of heat stress is short.

Phenotypic analysis of heat stress effect on first service to conception in Canadian Holstein cattle

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Heat stress is a significant global challenge impacting the dairy industry as high temperatures reduce both dairy cattle production and fertility. While several studies have investigated the relationship between heat stress and milk production, the specific temperature threshold at which fertility is affected remains poorly understood. The objective of this study was to identify the temperature-humidity index (THI) threshold at which heat load affects first service to conception (FSTC) in Canadian Holsteins. Data comprised 2,454,901 FSTC records on 1,393,377 cows across Canada between 2005 and 2018. Climatic data included hourly ambient temperature and relative humidity collected through the NASA POWER database and hourly THI was averaged across each day. Daily THI was averaged across three windows based on follicular and embryonic development: W1 (3 days before to 2 days after first insemination), W2 (10 days before to 2 days after first insemination), and W3 (7 days before to 2 days after first insemination). A single-trait repeatability animal model was fit to adjust the FSTC phenotype for known sources of environmental variation, and the adjusted phenotype was plotted against THI in the four windows. A segmented polynomial was used to identify the THI threshold at which FSTC increases. Across all windows, FSTC increased at approximately 65 THI with a steeper increase observed after approximately 75 THI. While thresholds varied between parities there was no consistent pattern observed across the windows. Estimated thresholds by parity were 66.0, 65.7 and 67.1 (W1); 65.2, 64.9, and 49.7 (W2); and 65.1, 65.0, and 65.2 (W3). The results of this phenotypic analysis suggest an effect of heat stress on FSTC, with thresholds aligning with existing estimates (60 to 70 THI). These results indicate the need for further investigation into the genetic factors underlying heat stress and its impact on fertility outcomes. Next steps of this study will involve a genetic analysis of heat stress effects on FSTC.

Session 23

Theatre 8

Between Heat Tolerance and Resilience: Breeding Against Rising Heat Stress in Dutch Holstein Cattle

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Heat stress is a major environmental challenge in dairy cattle, leading to behavioral changes, production losses, and welfare concerns. While short-term management strategies can mitigate heat stress, genetic selection can aid these efforts long term. However, breeding against heat stress is complex, as it requires balancing heat tolerance—the ability to maintain production in hot conditions—and heat resilience—the ability to reduce production to avoid severe consequences such as morbidity and mortality. Within the projects Re-Livestock (Horizon Europe, GA No. 01059609) and Breed4Food (TKI Agri & Food, LWV20054), data from 1,478 farms including 677,318 cows from 2013 to 2021 across the Netherlands were used to quantify heat stress effects based on the temperature-humidity index (THI). At THI = 75, milk yield, fat concentration, and protein concentration each decreased by 5% compared to thermoneutral conditions, with heritabilities for heat tolerance traits between 0.05 and 0.12. Improving the population through breeding by one genetic standard deviation was estimated to reduce the effect of heat stress by 11% for milk yield, resulting in a remaining relative loss of 4.5% at THI = 75. Even though the same genetic improvement offset 65% of the losses in fat and protein concentration, greater heat tolerance for these traits was negatively correlated with health, resilience, and production. In contrast, heat tolerance based on milk yield showed positive correlations to health, resilience, and longevity with no significant correlations to production. Although heat tolerance traits are highly polygenic, QTLs on BTA5, BTA14, BTA20 were identified through a GWAS using SNPs and haplotype blocks. As milk yield losses increased quadratically with THI and breeding could only offset a small share of production losses, introgression or crossbreeding with more adapted breeds may be required when breeding for more challenging climate conditions.

Developing multi-breed beef cattle international single-step evaluations for feed efficiency

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In beef cattle, novel sustainability traits are often difficult and expensive to measure, leading to small numbers of phenotypes collected on different breeds and crossbreeds in different countries. Thus, new strategies to combine data from different breeds and countries are necessary to improve genomic evaluations for these traits. Previous studies have shown that combining closely related breeds or pure breeds with their crossbreeds in one reference population can improve genomic prediction accuracy. However, current beef cattle international evaluations are pedigree-based, and focus on purebreds and conventional traits. The aim of this study is to develop a multi-breed beef cattle international single-step evaluation for feed efficiency. The dataset includes ~13K phenotyped animals for feed efficiency (of which ~9K genotyped) from more than 15 different breeds and crossbreeds, and ~142K pedigree records from Great Britain, Ireland, and Italy. After imputing all genotypes to a common panel, we will perform a PCA, investigate the persistence of LD phase across breeds, and estimate genetic correlations between breeds and countries to assess their degree of relationship. We will then implement a multi-breed single-step genomic evaluation, where closely related breeds are considered as one population (single-trait approach), and distantly related breeds are considered as different populations (multi-trait approach). Results of this study will contribute to the development of genomic evaluations for novel sustainability traits in beef cattle.

Session 23

Theatre 10

Unveiling mRNA isoform signatures of feed efficiency in Jersey cattle across diverse dietary regimes

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Genetic and dietary strategies to enhance feed efficiency (FE) in dairy cattle hold potential for improving both economic performance and environmental sustainability. Cows that efficiently convert gross energy into production or require less maintenance relative to their body weight typically consume less feed, leading to a lower residual feed intake (RFI). RNA-Sequencing (RNA-Seq) can aid in uncovering the molecular mechanisms behind FE by detecting differentially expressed (DE) mRNA isoforms. The objective of this study was to identify novel and annotated DE mRNA isoforms between low and high feed efficient (RFI; kg DMI/d) Jersey cattle under two dietary treatments. Liver RNA-Seq data (GSE92398) from high-RFI (n=5) and low-RFI (n=5) cows fed both control (C) and high-concentrate (HC) diets were analyzed using CLC Genomics Workbench. Large Gap Read Mapping revealed significantly DE mRNA isoforms (FDR<0.05; |FC|>2) between RFI groups within each diet. Under the C diet, 188 DE isoforms were identified, 120 of which were upregulated in low-RFI animals, while the HC diet revealed 278 DE isoforms, with 158 upregulated in low-RFI animals. Additionally, 104 novel mRNA isoforms were identified across both diets. Noteworthy isoforms, including PRDM2_8 (FDR = 2.7×10⁻¹⁴, |FC| = 5.7) and GIMAP8_4 (FDR = 4.3×10⁻²¹, |FC| = -7.6), were linked to methylation, RNA polymerase II transcription, and the negative regulation of biosynthetic processes. Metabolic pathway analysis using Reactome revealed 238 enriched pathways (FDR<0.05), with 105 under the C diet and 133 under the HC diet. Immune and metabolic processes were enriched in both dietary treatments, with isoform expression varying by RFI group, suggesting RFI-dependent regulation, while cell signalling and reproductive processes were consistently expressed, independent of RFI. Future research will focus on long non-coding RNAs as complementary regulators alongside mRNA isoforms to further explore the genetic regulation of feed efficiency, providing insights for optimizing genetic selection in the dairy industry.

An independence test for deep phenotypes using longitudinal and fixed feed quality data

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Deep phenotypes are inner, invisible traits that could be used to breed dairy cows with high feed efficiency. Nine deep phenotypes were previously assessed using the mechanistic LiGAPS-Dairy model and a genetic algorithm (GA). However, the independence of deep phenotypes to their initial values (base population in GA) while using different feed quality (FQ) data had not been tested. This study aimed to determine whether these nine deep phenotypes were independent of their initial values. Data from 173 Holstein Friesian cows were selected from Dutch experimental farms in Lelystad (n=143) and Leeuwarden (n=30), which included records of fat and protein-corrected milk (≥ 5 parities), liveweight (≥ 3 parities), dry matter intake, and FQ (≥ 2 parities). Deep phenotypes were estimated using fixed feed quality (FQ_fix), representing the average herd FQ from a pilot project and individual, longitudinal feed quality (FQ_ind) data. The model was run twice for each FQ type with two base populations. Assessed deep phenotypes included net energy efficiency for milk (NEEFF), net energy required for maintenance (NEm), maximum body weight (TBW), protein efficiency for milk (PROTEFF), protein required per unit net energy (PROTNE), maximum intake capacity (Int_cap), and the parameters of Wood's lactation curve. The concordance correlation coefficient (CCC) was used to evaluate independence from initial values. For the Lelystad dataset with FQ_fix, CCC values ranged from strong (PROTEFF: 0.94, TBW: 0.93) to weak (PROTNE: 0.12). With FQ_ind, CCC decreased for PROTEFF (0.62) and TBW (0.88) but improved for NEEFF (0.75) and NEm (0.54), while PROTNE remained the lowest (-0.01). Similar trends were observed in the dataset from Leeuwarden, where FQ_fix generally yielded better results than FQ_ind, with PROTNE consistently showing the lowest CCC (~0.0). Overall, most deep phenotypes were independent of initial values except for PROTNE, and individual, longitudinal feed quality data did not improve the independence. Key words: Deep phenotypes, Feed quality, Independence test

Session 23

Theatre 12

Inclusion of susceptibility and resistance to bovine paratuberculosis in the genetic selection of Spanish Holstein cattle

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Bovine paratuberculosis (PTB), caused by *Mycobacterium avium* subsp. paratuberculosis (MAP), leads to significant economic losses worldwide. Genetics plays a crucial role in the response to MAP infection, making genomic selection a viable strategy to control PTB. This study aimed to estimate genomic Estimated Breeding Values (gEBVs) using the genomic Best Linear Unbiased Predictor (gBLUP) model to rank cattle genotyped with the EuroG MD chip based on four traits: presence of diffuse PTB-associated lesions in gut tissues, interferon gamma (IFN- γ) production, MAP survival index in macrophages after ex vivo MAP infection, and positivity to ELISA, qPCR and bacteriological culture. To validate the individual gEBVs, a 10-fold cross-validation was performed in a reference population of 1,046 Holstein cattle. The area under the curve for the diffuse lesions and diagnostic tests was 0.74 and 0.66, respectively. The Spearman correlation coefficient between the gEBVs for MAP's survival index and IFN-The individual gEBVs were used to classify animals as "Resistant", "Susceptible" or "Undefined" based on thresholds established in a larger population of 7,936 Holstein cattle considered "influential" in the Holstein breed, as they include stallions, their parents and embryo donors. An animal was classified as resistant if its gEBVs for at least three traits ranked within the top 20% of the population, with the gEBV for the remaining trait above the lowest 25%. Susceptible animals followed the opposite criteria, while all other animals were categorized as undefined. Resistant animals exhibited lower frequency of lesions (3.1% resistant, 7.5% undefined, 10% susceptible, $p = 0.04$) and were less likely to test positive for MAP diagnostic tests (5.4% resistant, 7.3% undefined, 14.29% susceptible, $p = 0.03$). Additionally, they produced higher levels of IFN- γ , although differences were not significant. Selecting animals with greater resistance to MAP infection could improve overall herd resistance, reduce antibiotic use, and increase longevity.

Current state and challenges in defining and measuring resilience indicator traits for livestock breeding

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The resilience of an animal is the ability to cope with short-term disturbances, including those caused by pathogens, through response and rapid recovery to its original state. To identify what resilience indicators are already applied and analyzed, as well as what different methods of data processing to form a resilience indicator trait, a systematic literature review has been developed for the main livestock species, starting with predefined search strings. This literature review serves as a starting point for further development and refinement of resilience indicator traits. The transferability of resilience indicator traits to other livestock species as well as combining methodology with new underlying data types have been investigated. The applicability of using the methodology of LnVar (natural logarithm of the variance of deviations from an expected curve), which has been used on longitudinal data of production traits, e.g. milk yield in dairy cattle or body weight in fish, was tested for longitudinal behavior data (distance moved) using previous collected data of broilers from two rounds of which one was during a heat wave. Comparing both rounds, the heat wave in the last week before slaughter had less influence on the residuals from the curve fitted on an individual basis than the general decreasing trend in movement during the lifespan. Accordingly, LnVar seems not applicable without corrections for the general trend in activity in broilers. Since most longitudinal activity or production data in livestock have a typical trend over time, considering this in data processing for resilience indicator traits is important. To explore if activity data for other species and stressors are suitable as resilience indicator traits, further data sets will be analyzed and cross-validated with health indicators pre- and post-challenge. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Session 23

Poster 14

Unraveling correlations between milk urea nitrogen and efficiency traits in Holstein cows

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Reducing nitrogen surpluses decreases economic losses and the overall environmental load of the dairy industry. Milk urea nitrogen (MUN) could be an indicator for nitrogen surplus over the amount of nitrogen needed for maintaining a cow's nitrogen balance. Urea is converted from ammonia in the liver and either recycled into the rumen for microbial protein synthesis or excreted in urine, feces or milk. Therefore, MUN might be correlated to dry matter intake (DMI), enteric methane production (MP) and betahydroxybutyrate (BHB). The objective of this study was to estimate genetic parameters among MUN, DMI, MP and milk BHB (as indicator of blood BHB). A total of 28,220 records of 917 first lactation Holstein cows housed at the Ontario Dairy Research Centre were used in the analyses. Multivariate repeated records models were used to estimate variance components using ASReml 4.2. Low to moderate heritabilities were estimated for MUN (0.16 ± 0.08), DMI (0.12 ± 0.08), MP (0.22 ± 0.10), and BHB (0.20 ± 0.10). Genetic correlations among traits ranged from -0.94 ± 0.32 (DMI-BHB) to 0.28 ± 0.07 (DMI-MP). MUN seems to be genetically independent of the other traits, with weak or non-significant, genetic correlations between MUN and DMI (0.15 ± 0.09), MUN and MP (-0.05 ± 0.09), MUN and BHB (0.21 ± 0.39). Phenotypic correlations ranged from -0.11 ± 0.03 (DMI-BHB) to 0.81 ± 0.09 (MUN-DMI). While no phenotypic correlation was found between MUN and MP (-0.05 ± 0.08) nor between MUN and BHB (0.01 ± 0.03), the phenotypic expression of MUN was highly correlated with DMI (0.81 ± 0.09). The high standard error of the estimates in this study indicate that a larger dataset is needed to confirm these preliminary findings. This study represents an initial step toward a more comprehensive understanding of the potential association between MUN and traits related to feed efficiency, methane emissions, and energy balance.

Reproductive efficiency in females of Avileña-negra Iberica local beef cattle breed

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Reproductive efficiency is an indicator of resilience, in addition to be one of the main traits of the economic profitability in beef cattle. In the Spanish local beef cattle Avileña-Negra Ibérica (ANI) under a full extensive production system, the females have to wean at least four calves to overcome the cost of her productive life. Up to now, the objective has been pursued making efforts in decreasing the age at first calving (AFC) to 28 month and reducing the first calving interval (FCI) to 365 days, through the breeding program. However, to get reproductive efficiency the selection must incorporate information associated with the ability of the females to maintain cyclicity all along the reproductive life. The aim of this study was to investigate the genetic components of two reproductive efficiency traits: age at fourth calving (A4C) and number of calvings at 64 months of age (NC64). Overall, AFC, FCI, A4C and NC64 were obtained from 40,387 records in the ANI herd book with age at first calving between 610 and 1,281 days and calving interval between 289 and 600 days. The final number of records were 35,326, 17,238, 8,442 and 21,441 for AFC, FCI, A4C and NC64 respectively, and the whole pedigree contained 52,009 records. The NC64 was analysed as a censored phenotype, with 4 levels. Genomic data were 4,270 genotypes. Two-traits animal models have been run with Blupf90 family of programs. NC64 was analysed under a threshold model. The mean of heritability estimates for A4C and NC64 fluctuated between 0.11 and 0.15 with a high genetic correlation between them. Genetic correlations between A4C and NC64 were low with AFC and high with FCI. These results indicate that incorporating A4C or NC64 as new traits into the breeding objective together with AFC will contribute to maintain the sustainability of the ANI production system as well as improve the individual resilience. Moreover, with the implementation of analysis with censored data and genomics should help to avoid retarding the selection response. This research has been supported by SMARTFARMRANI operational group project, supported by EU strategic plan PAC-FEADER (REGAGE23e00064732005).

Session 23

Poster 16

Effect of selection for resilience on sperm head morphometry in rabbits

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An experiment of divergent selection for residual variance of litter size was carried out on rabbits. The Ve was estimated as the phenotypic variance of litter size within female after correcting litter size for year-season and lactation status. The HO line is selected for decreasing Ve and HE line is selected for increasing Ve. We know that males of the HE lines showed higher temperature than HO line after a stressful stimulus, such as semen extraction, indicating higher stress level and less resilience. Chronic stress has been shown to negatively impact spermatogenesis by affecting hormone levels, particularly testosterone and cortisol, which play crucial roles in sperm development. The aim of this study was to examine the correlated response on sperm head morphometry. A total of 30 semen samples (15 samples by line) were diluted 1:15 and SpermBlue® staining was performed. Automatic analysis of the sperm morphometry with the Sperm Class Analyzer CASA System was used. Each sperm head was measured for four primary parameters (area, perimeter, length, width), and four derived parameters of head shape [Ellipticity (L/W), rugosity ($4\pi A/P^2$), elongation ($(L-W)/(L+W)$), regularity ($\pi LW/4A$)]. Data were analyzed using Bayesian methods. After 17 generations of selection, the HO males showed a lower area of the sperm head than HE males (29.48 μm^2 and 33.54 μm^2 , respectively), being the difference between lines $D = -0.46 \mu m^2$, with a probability of being negative $P = 91\%$. Also, width (4.00 μm and 4.46 μm , $P = 91\%$), length (7.45 μm and 8.33 μm , $P = 91\%$) and perimeter (17.10 μm and 18.96 μm , $P = 90\%$) was lower in HO males. Both lines showed similar results for ellipticity, elongation, regularity and rugosity. In conclusion, selection for increased resilience in rabbits is associated with smaller sperm head dimensions but does not affect overall sperm shape. These differences may be linked to its lower stress levels. We thank the institutions (European Union and Generalitat Valenciana) involved in AGROALNEXT/2022/037 that have provided the necessary funds (PRTR-C17.11) for the execution of the project.

Impact of heat periods on dairy farming in the most affected area of Germany

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Climate change has significantly affected agriculture in recent years, particularly dairy farming. The Interreg project ResKuh investigates how dairy farms can adapt to rising temperatures, more frequent heatwaves and dry seasons. The study aim was to investigate, how weather stations (WS) data can be used to reflect heat stress (HS) in farms nearby. Data from 176 dairy farms located in the Upper Rhine region in Germany at different altitudes and with different breeds (Holstein (HOL) and Vorderwälder (VOW)) were analysed. Climate data from 18 WS between 2021 and 2023 was used. The temperature humidity index (THI) was calculated as HS indicator. QGIS was used for distribution and visualization, while statistical analyses were conducted in R. The results were presented in thematic maps depicting rainfall, THI, and energy corrected milk (ECM)/herd/cow. Rainfall during the study was scarce in low altitudes and tended to decrease, with the most significant drought occurring in summer 2022 (max. 313 mm). The THI map indicates, that lower altitudes had higher THI values than higher elevations. During summer 2022 regions below 500 meters above sea level were affected the most, with highest THI of 72. However, no direct correlation between altitude and ECM was found. For the further analysis of the average ECM/herd/cow, only the two most prevalent breeds in the study region, which are used in different production systems, were compared. Factors such as year, season, altitude and THI were taken into account. The local breed VOW shows with 19.6 kg ECM significant lower average than HOL (27.3 kg ECM). However, considering the variation of ECM during periods of higher THI, ECM of VOW showed less reduction than of HOL (18.6 – 22.1 kg and 8.1 – 44.0 kg respectively). Therefore, the impacts of climate change on ECM are strongly dependent on both: location of the farm (altitude) and the dairy farming system (i.e. breed).

Session 23

Poster 18

Impact of including novel heat stress indicators on the genetic progress of Iberian pigs

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Climate change has increased heat stress (HS) events affecting pig production. Knowledge on impact of including HS resistance traits in current breeding programs is limited. We aimed to examine the impact of including novel HS resistance trait, based on biomarkers, into the breeding scheme of Iberian pigs. Various scenarios were simulated using the MoBPS package in R. Scenarios considered distinct traits for males and females. For males, average daily gain and loin depth were included. Females' traits included litter size, stillborn, wean-to-estrus interval, number of teats, pre-wean mortality, and individual piglet birth weight. Back fat was included for both sexes. Breeding program was based on a maternal selection scheme, focusing on reproductive traits, while other traits were monitored as correlated changes but excluded from selection. Heritabilities, genetic variances, and phenotypic means were obtained from studies or existing Iberian pig breeding programs mimicking the current breeding scheme. Scenarios varied in terms of reference population size (500, 1000, 2000, 3000, 4000, 5000), selection criteria (phenotypic vs. EBV), selection intensity (1:10, 1:20, 5:10), and economic weights assigned to reproductive traits, with and without HS resistance traits. The impact of including these traits on genetic progress was analyzed to determine the best breeding strategy and feasibility of including HS traits in breeding programs for local pig breeds. This work has received funding from the European Union's Horizon Europe research and innovation programme under the grant agreement No 01059609 (Re-Livestock project).

Regulatory role of 8-oxoguanine (8-oxoG) in heat-stressed reproductive cells of Korean beef cattle (Hanwoo) and its implications for fertility

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Heat stress (HS) reduces reproductive efficiency in Hanwoo, potentially by altering the activity of key proteins affecting oocyte quality, early embryo development, and implantation. To investigate the mechanisms and identify potential targets, we selected cumulus, oviduct, and endometrial epithelial cells for analysis. We established primary cultures of them and validated their identity. Cumulus cells exhibited over 99% CD44 positivity while oviduct and endometrial epithelial cells showed over 90% KRT18 and CDH1 positivity by IF and FACS. To determine the optimal HS condition, we examined the levels of oxidative stress, ER stress, and 8-oxoG formation. SOD1 and GPX1 mRNA increased at 12 hrs and returned to baseline after recovery while CAT peaked at 24 hrs. HSP70 was elevated at 12 and 24 hrs. BiP increased at 12 hrs but normalized post-recovery, while CHOP increased at 12 and 24 hrs, suggesting prolonged ER stress. The 8-oxoG levels rose at 4 hrs, normalized after recovery, and remained elevated at 24 hrs. These findings establish 12 hrs as the optimal HS condition for bovine reproductive cells. To explore the regulatory role of 8-oxoG, we performed OG-seq in heat-stressed cells. A 2.5-fold increase in 8-oxoG-enriched genes was observed, with 165 genes uniquely identified. Most 8-oxoG sites were localized near TSS and intronic/intergenic regions, suggesting its involvement in gene regulation under HS. To further investigate the functional impact of 8-oxoG, we analyzed gene expression changes using RNA-seq and proteomics. Many of these genes exhibited strong correlations between 8-oxoG presence, mRNA and protein levels. GSEA identified pathways linked to HS adaptation and reproduction. Further, we aim to experimentally validate the function of 8-oxoG-enriched genes and identify molecules to modulate their activity. By targeting these pathways, we seek to develop novel strategies to enhance reproductive efficiency in Hanwoo for climate-resilient livestock production.

Session 23

Poster 20

Metabolomic-genomic prediction slightly increases ability to predict breeding values for feed conversion ratio in pig breeding herds

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We hypothesised that (i) integrating metabolomic data into metabolomic-genomic prediction models (MGBLUP) generates more accurate EBVs for feed conversion ratio (FCR) than genomic models (GBLUP), and (ii) bivariate MGBLUP including FCR and average daily gain produces more accurate EBVs for FCR than univariate MGBLUP. A MGBLUP model assessed EBV accuracy in an offspring validation scheme using univariate and bivariate models. The study included 8,174 Duroc pigs (4,027 males, 4,147 females) from the DanBred system. Males with FCR records served as the training population, while females without FCR records were the validation population. EBVs of females were validated by correlating them with their offspring's corrected phenotypes. Prediction accuracy improved from 0.385 (GBLUP univariate) to 0.394 (MGBLUP univariate) and from 0.427 (GBLUP bivariate) to 0.432 (MGBLUP bivariate). Comparing bivariate to univariate models, accuracy increased by 10.9% (GBLUP) and 9.6% (MGBLUP), though not statistically significant. Although these accuracy improvements were modest and not statistically significant, we remain optimistic about the potential of metabolomics for two key reasons. First, the observed gains represent incremental improvements in models that already incorporate pedigree and genomic data. Second, these improvements were achieved using an emerging methodology, highlighting opportunities for further refinement. Future enhancements could involve identifying relevant tissue types and sampling times influencing FCR, improving genetic models that integrate metabolomic data, and optimising methods for extracting key metabolites. While current approaches are not yet ready for practical application in breeding schemes, our findings represent an important step toward developing advanced selection criteria. So, we believe that metabolomics holds significant promise for improving prediction accuracy in breeding programs for both plants and animals, with its role expected to expand as we understand metabolite-trait relationships.

Genetic and Environmental Adaptation of European Indigenous Sheep Breeds: Correlation with Climatic and Geographic Data

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European indigenous sheep breeds have diversified over the centuries under the pressure of local environmental conditions and selective breeding for specific traits. These extant, locally adapted breeds, exhibit a variety of phenotypes often within distinct geographic boundaries. Acknowledging the merits of these indigenous breeds led to breeding programs that resulted in the modern genetically improved breeds. Improved breeds such as AfecAssaf, Awassi, Lacaune, Merino, and Texel, were then introduced to new areas and climatic zones across Europe under semi-intensive or intensive conditions. The abiotic factors in these new environments, coupled with imminent climate change, challenge the animals' ability to thrive and maintain high productivity. This highlights the importance of further exploring their adaptive capacity. In this study, we analyzed high-resolution single nucleotide polymorphism (50k SNP) data from approximately 1500 animals representing 30 European and Mediterranean sheep breeds, along with climatic (temperature, precipitation, humidity, rain, radiation), and geographic data (altitude, longitude, latitude) associated with their breeding environments. Our goal was to investigate the correlation between the distribution and evolutionary trajectory of indigenous sheep breeds and the European climate and geographical zones. Ultimately, we aim to identify significant markers and associated genes, revealing the molecular pathways enriched in this process. These findings could elucidate the genetic mechanisms driving the adaptation of the animals in relation to environmental factors and human intervention.

Examining the role of mRNA isoform expression profiles in shaping feed efficiency in Holstein cattle

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Residual feed intake (RFI; kg DMI/day) is a heritable trait affected by genetics and the environment, representing the difference between the actual and predicted dry matter intake, adjusted for maintenance and production. Feed efficiency (FE), vital to dairy cattle profitability and sustainability, is often assessed using RFI and other related measures. RNA-Sequencing (RNA-Seq) offers valuable insights into the molecular mechanisms underlying FE, acting as a tool to support genetic selection targeted at improving production efficiency. This study aimed to identify novel and annotated differently expressed (DE) mRNA isoforms between low- and high-RFI Holstein cattle under two dietary treatments. Liver RNA-Seq data (GSE92398) from nine Holstein cows (n=4 high-RFI, n=5 low-RFI) each fed control (C) and high-concentrate (HC) diets were analyzed. Quality control, alignment, and differential expression analysis were performed using CLC Genomics Workbench. Under the C diet, 121 differentially expressed (DE) mRNA isoforms (FDR<0.05; |FC|>2) were identified, with 38 showing upregulation in low-RFI animals. In contrast, 279 isoforms were identified under the HC diet, 131 of which were upregulated in low-RFI animals, suggesting regulatory influences on FE. Additionally, two novel mRNA isoforms Gene_4240_2 (FDR = 0.04, |FC| = 8.70) and Gene_5818_1 (FDR = 3.08×10⁻⁵, |FC| = -6.16) were detected across both diets, with the highest FC and lowest FDR reported. Functional enrichment analysis revealed that DE mRNA isoforms upregulated in low-RFI animals were primarily involved in RNA stabilization and mRNA metabolism, while those upregulated in high-RFI animals were associated with cell cycle regulation and nuclear division. Pathway analysis with Reactome indicated shared enrichment in organelle division and cellular replication processes across both RFI groups (FDR<0.05). Further investigation will explore long non-coding RNAs as regulators of feed efficiency to uncover genetic factors that can enhance production efficiency through genetic selection.

Exploring the genetic factors affecting feed efficiency traits for Duroc in Japan

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Feed efficiency is an important productive factor that greatly affects profits in the pig production. Recently, automatic feeders have become popular for collecting daily feed intake (DFI) data, making it possible to evaluate genetic effects on feed efficiency. However, in Japan, because of several limitation that the introduction of the automatic feeder systems to the farms require long time, large spaces and higher expenses, those systems have not yet got to be become useful for genetic improvement for feed efficiency. In this study, to improve feed efficiency of Japanese pig populations, we identified the effective SNP markers for the improvement of those traits, based on actual measured DFI values in Duroc pigs in Japan. The data pertaining to individual average DFI, feed conversion ratio and daily gain in test period were collected from 240 male Duroc pigs in this study. The SNP genotypes was obtained using the bead chip by illumina, which contains 75,753 SNPs. We collected QTL information that published on the Animal Genome PigQTL database as SNPs associated with feed efficiency traits, and then the SNPs with matching these regions and having minor allele frequencies > 0.05 were selected. We finally used a total of 185 SNPs for following analysis. We used a simple linear regression model to test significance of each SNP. Statistical significance was considered at a p-value < 0.05. As results, 27 SNP makers were associated with feed efficiency traits. The several genes near the SNPs we found were reported in previous studies as the candidate genes associated with feed efficiency traits for several pig breeds, like TNFAIP3 interacting protein 1, G protein-coupled receptor kinase 5 and Melanocortin 3 receptor. Our findings indicated that some of the previously reported genes associating with feed efficiency would influence on the feed efficiency traits of the Duroc pigs, which could help optimize the design of breeding schemes.

Session 23

Poster 24

Climate effects on fertility evaluated in Maremmana cattle under the open-pasture systems of the Mediterranean harsh bush habitat

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Fertility is a key functional trait in both beef and dairy systems that can be influenced by many environmental and management factors, including exposure to climatic conditions. Heat stress can disrupts reproductive tract performance by altering hormonal balance and reducing oocyte quality. Also, cows in open pasture rearing systems (OPRS) may experience the effects of wind, solar radiation and weather conditions in addition to temperature and humidity. To date, few studies have investigated genotype by environment (GxE) interactions on cattle fertility traits and there is scarce literature on these effects under OPRS. One of the few breeds reared under such conditions in European countries is the Maremmana breed (MA), a local cattle breed belonging to the Podolian strain and reared entirely under OPRS in the Mediterranean harsh bush habitat. The aim of this study was to estimate genetic parameters for fertility in MA, and to investigate the GxE using climatic data. A set of nearly 90000 herd-book records were available for study, referring to about 7000 living cows and dating back to 1950s; calving interval was the studied trait. Genetic parameters were first estimated using a traditional animal model (M1). Then, climate information of the herd communities was obtained from NASA's Climate Data Services, considering the period in which the cows conceived (about 1 month around the conception date). This information, in form of the temperature-humidity index (THI) classical or corrected for wind and solar radiation, as well as the individual variables, were included as environmental covariates in a one-level response norm model (M2) updating M1. Most of the Maremmana parities occurred between January and May and climatic variables explained part of the trait variance, influencing individual breeding values. Results suggest the importance of considering climatic information and GxE in the genetic evaluation of cattle fertility, especially in breeds reared under natural conditions such as Maremmana.

Replacing synthetic Vitamin E with carotenoid-rich citrus by-products in broiler diets

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Bioactive compounds in citrus fruits show potential in animal nutrition due to their antimicrobial and antioxidant properties. Carotenoids, known for their immunomodulatory, anti-inflammatory, antibacterial, and antioxidant effects, were extracted from cold-pressed essential oils of *Citrus reticulata* and included into soy protein to create a feed additive with 3.13 mg of total carotenoids/g. Two hundred 1-day-old Ross 308 broilers were assigned to five dietary treatments with four replicate pens each. The control group (CON) received a basal diet based on corn and soybean meal including synthetic Vit E based on Aviagen's recommendations, while the growth promoter group (GP) received 55 mg/kg feed, zinc-bacitracin. Three other groups were fed diets with 25%, 50%, and 100% synthetic Vit E substitution (C25, C50, C100), by 6.75, 12.5, and 25 mg canthaxanthin eq/kg feed, respectively. At 36 days, eight broilers per dietary treatment (2 per replicate pen) were randomly selected for slaughter. The GP group had higher body weight ($p = 0.013$) compared to the CON, C25, and C100 groups on day 24, and a better feed conversion ratio ($p = 0.029$) compared to the CON and C25 groups over the entire period. Both breast and thigh muscles' malondialdehyde (MDA) content was higher in the C100 compared to the CON group at 2, 4, and 7 days postmortem (meat stored at 4°C). Carcass traits, blood oxidative status, and antioxidant enzyme activities were unaffected. mRNA levels of CAT and GPX1 were lower in the duodenal mucosa of C25 and C100 groups compared to the CON group ($p = 0.028$ and $p = 0.093$, respectively). mRNA levels of TLR4, MAPK, and NFκB were also lower in C25 and C100 groups compared to the CON group ($p = 0.065$, $p = 0.034$, $p = 0.046$, respectively). Partial substitution (50%) of synthetic Vit E by citrus carotenoids was feasible without any adverse effect. The research project is implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union NextGenerationEU (H.F.R.I. Project Number: 16088).

Session 24

Theatre 2

The effect of hominy chop inclusion on diet digestibility in modern broilers

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Feed constitutes the largest cost of broiler production, while competition for food sources further increases the cost of production. The focus of animal nutritionists has therefore shifted to investigating alternative feed sources like milling by-products. The aim of the study was to evaluate the effect of hominy chop on the nutrient digestibility of modern broiler diets. Three dietary treatments were formulated, including (i) a standard maize diet (T1; control), (ii) 10% hominy chop inclusion (T2; isoenergetic and isonitrogenous), and (iii) complete substitution of maize with hominy chop without adjusting the formulation (T3). Birds were floor reared on a control starter crumble and transferred to the metabolic cages on D14 of age. A completely randomized design was used with five birds per cage (15 birds/m²) and six cages per treatment. Birds were adapted to the experimental grower pellets for four days before conducting the digestibility study between D18-22. Feed and water were provided ad lib. Data were subjected to a one-way ANOVA ($P < 0.05$). Dry matter feed intake, start and end weight did not differ, while dried excreta weight was significantly higher for T3 compared to T1 and T2. Dry matter and organic matter digestibility were lower for T3 ($P < 0.05$) compared to T1 and T2. Neutral detergent fibre digestibility decreased ($P = 0.0008$) with hominy chop inclusion. Ash and fat digestibility as well as apparent metabolisable (AMEn) did not differ significantly, but nitrogen retention was higher ($P = 0.0379$) for T3 compared to T1. Lower fibre digestibility of the hominy chop diets was mainly ascribed to the smaller particles of hominy chop ($D_{50} = 0.88$ mm) compared to maize ($D_{50} = 1.32$ mm). Despite the lower dry matter digestibility of T3, improved nitrogen retention indicates an increased efficiency of utilization of nutrients, which could partly be ascribed to the higher dietary fat content of T3. Potential therefore exists to use hominy chop in broiler diets either to partially or completely replace maize. Further research is required to evaluate the effect of hominy chop on broiler performance and variation in hominy chop quality.

Reducing calcium and phytate interactions using marine mineral complex as a source of calcium improves phytate phosphorus digestibility, FCR and daily gain in weaner pigs

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Interactions between dietary nutrients in the digestive tract are known to limit the digestibility of key nutrients that influence health and performance. The interaction between calcium (Ca) and the phosphorous (P) rich phytate molecule has been extensively studied. The addition of feed phytase is a widely implemented solution in the monogastric feed industry. The influence of different sources of Ca (e.g. Marine mineral complex (MMC) and limestone) on this interaction has been evaluated previously in vitro. This demonstrated the ability of MMC to reduce interactions with phytate, leading to more efficient hydrolysis by phytase and increasing digestible P. The aim of this study was to determine if MMC as Ca source can reduce interactions in weaner pigs. A total of 32 piglets (body weight: 10kg) were divided into 4 groups using a complete randomized block design and fed diets A to D. Diet A (High Ca + Monocalcium Phosphate(MCP)), 1.15% Limestone, 0% MMC, and 0.17% MCP; Diet B (Low Ca + MCP), 0.69% Limestone, 0% MMC, and 0.17% MCP; Diet C (Low Ca + MMC + MCP), 0.46% Limestone, 0.25% MMC, and 0.18% MCP; Diet D (Low Ca + MMC), 0.54% Limestone, 0.25% MMC and 0% MCP. Ileal digestibility of P (ID) for diet B (54%) and D (52%) were comparable, but both B and D ID values were significantly ($p<0.05$) higher than those fed diet A (33%) and C (40%). Similarly, total tract digestibility (TTD) of P was significantly ($p<0.05$) higher for diet B (64%) and D (58%). Ileal digestibility of Ca was highest for diet B (59%) compared to A (50%), C (45%) and D (45%). However, for Ca, TTD of diet A (57%) was significantly ($p<0.05$) lower than B, C and D. The feed conversion ratio was 1.31, 1.29, 1.27, 1.27 for A-D, respectively. Average daily gain was 0.45, 0.48, 0.49, 0.49 Kg/d, for A-D, respectively. MMC reduced Ca-phytate interactions, to a level where MCP could be reduced without compromising performance.

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Theatre 4

Estimating the nutritional value of calcium phosphates recovered from bones for phosphorus supplementation in pigs

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There is a need for identifying more sustainable and price-competitive feed phosphates than the traditional non-renewable rock-derived calcium phosphates typically applied in pig's diets. The aim of this study was to determine the apparent total tract digestibility (ATTD) of phosphorus (P) in dicalcium phosphate ("Delfos"-product) and in hydroxyapatite ("Calfos"-product) extracted from bones, in growing pigs (45-50 kg bodyweight) of two sexes. Applying castrates in this type of digestibility study over females with urine catheters inserted for urine collection would lower the risk of urinary tract infections, reduce discomfort for the animal and potentially improve animal welfare. Pigs were fed either a semisynthetic low P diet (control) or the control diet plus either traditional monocalcium phosphate (MCP) of rock origin, Delfos or Calfos ($n=8/\text{diet}$, 4 females and 4 castrates/diet). Pigs were housed in metabolic crates and allowed 4 days of adaptation to diets and crates before total collection of urine and faeces was applied over a six-day period. Female pigs were fitted with a urine catheter whereas urine from castrates was collected directly via a tray into a bucket. There was no significant difference in the ATTD of P between entire diets where either MCP, Delfos or Calfos had been added (80.8, 72.0 and 66.4%, respectively, $P=0.19$) or between castrates and females ($P=0.99$). The ATTD of P in the specific sources of supplemental P was, however, higher for MCP (88.2%) than in the Delfos and Calfos products, which was at a similar level (74.1 and 66.4 %, respectively) ($P=0.03$) but there was no effect of sex ($P=0.77$). Daily P retention was similar for the MCP and Calfos diets (4.4 g P/d), whereas it was higher following the Delfos diet (5.1 g P/d; $P<0.001$). Taken together, the results indicate that bone-derived calcium phosphates, of which Delfos is currently approved for all monogastric animals in the EU, can be used to replace MCP of rock origin for P supplementation in pigs and that male pigs seem as suitable for this type of digestibility study as female pigs.

Effects of feeding nutritionally enhanced protein (Matan XL) on growth performance in weaner/grower pigs
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The ban on pharmacological levels of zinc oxide, is driving piglet nutrition towards reduced crude protein (CP) diets. Diets containing high levels of synthetic amino acids (SAA) can reduce performance due to imbalances created through differing absorption kinetics. Matan XL is manufactured by processing a mixture of proteins and amino acids with sugars under temperature and pressure conditions to create an enhanced protein concentrate that ensures amino acids and sugars are released at a rate commensurate with efficient absorption and utilisation. Findings from 27 unpublished commercial feed trials comparing Matan XL diets with diets formulated using SAA were combined for analysis. In each trial, diets were isoenergetic and balanced for amino acids. Diets were fed over 9-68 Days (Feeding days, FD) and contained inclusion rates (IR) of 2.5-10%. The farms included both low- and high-feed-intake farms; the total intake of Matan XL (TM) was calculated using the formula: (ADFI x FD) x IR. Cohen's d determined the effect size for ADG, ADFI, and FCR, while correlational and regression analyses were performed to assess the influence of FD, IR, and TM. Relative to SAA diets, Matan XL diets significantly increased end weight by 5% (21.51kg vs 20.36kg, $P<0.001$), which was driven by a 10% increase in growth rate ($P<0.001$). Observed effects on feed intake were smaller (5% increase, $P=0.002$), resulting in improved feed efficiency (4%, $P=0.001$) in the Matan XL group. The effect size for the improvements was large (>0.8), indicating biological significance. FD and IR correlated highly with growth rate (0.49 & 0.48, $P<0.1$), while TM had a significantly high correlation (0.73, $P=0.002$). TM accounted for 30% of the variance in growth rate ($r^2=0.298$, <0.05), while FD accounted for 22% ($r^2=0.217$, $P<0.1$). Commercially, this indicates that the inclusion rate of Matan XL should be based on feed intake, with low-intake farms requiring a higher inclusion rate to ensure a high total intake of Matan XL for optimal growth rate improvements. In conclusion, Matan XL in reduced CP diets significantly improves animal performance compared to standard SAA supplementation.

Session 24

Theatre 6

Incorporating agar by-products in European seabass diets: A circular economy approach to sustainable aquaculture

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Under the principles of the circular economy, replacing traditional and imported ingredients with available agro-industrial by-products is essential to promote aquaculture sustainability. This study evaluates the feasibility of incorporating alkaline pre-treated Gelidium macroalgae by-product (GBP) in European seabass (*Dicentrarchus labrax*) diets, alone or in combination with an exogenous carbohydrase complex (Natugrain®). From a control diet (Diet CTRL: CP:44%; EE 16%), three isonitrogenous and isolipidic experimental diets were formulated including 15%GBP (Diet G15), 15%GBP with 1%Natugrain® (Diet G15Nat1) and 15%GBP with 2%Natugrain® (Diet G15Nat2), replacing a mixture of soybean and wheat meals. A total of 360 seabass juveniles (12.7±0.2 g) were randomly distributed into 12 RAS tanks (3 tanks per dietary treatment; 30 fish per tank) and fed twice a day until satiation for 62 days. Final body weight (33.4 g, on average), specific growth rate (1.58% day⁻¹), and viscerosomatic index (2.7%) were similar among experimental diets and diet CTRL ($p>0.05$), whereas feed efficiency and protein efficiency ratio were lower (-19% and -18%, respectively) in fish fed experimental diets compared to those fed diet CTRL ($p<0.01$). Whole-fish carcass composition was not affected by GBP inclusion, alone or enzyme-supplemented ($p>0.05$). Overall, 15% GBP inclusion did not impair growth performance but reduced feed utilisation, whereas the supplementation of Natugrain® did not enhance growth performance or feed efficiency compared to the addition of GBP alone. Future research should evaluate ingredient digestibility, assess the functional properties of GBP, and optimise processing and feeding strategies, to improve nutrient bioavailability and valorise GBP as a novel and sustainable ingredient for aquafeed, increasing the circularity of aquaculture.

The end of win-win solutions in controlling methane emissions from ruminants?

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Ruminant production, which has a recognized high environmental impact, is under pressure to be sustainable. Enteric methane emission is the major contributing factor of the environmental footprint of ruminants and its mitigation has been a priority for at least the last 20 years. Enteric methane is produced by the gastrointestinal microbiome and it is modulated both by the diet and the host. At the first lever of action, mitigation is achieved through good management practices, such as improved nutrition, reproduction and health, which together improve animal performance and farm output, resulting in lower methane yields (kg/kg feed intake) and intensities (kg/kg milk or meat). These well-known practices have a high return on investment and are already being implemented on well-managed, highly efficient farms. More specific dietary and breeding strategies are also available, but alone they will not be sufficient to achieve significant carbon reduction targets for the sector. A second lever that can greatly decrease enteric methane emissions is the direct targeting of the rumen microbiota and the methanogenesis function. For this, there are products already on the market and others in development. In addition, ongoing research programmes to better understand H₂ flows and rumen microbiome interactions will provide new targets to explore. However, there are some caveats and possible trade-offs that need to be considered. For instance, the 'next generation' of antimethanogenic compounds do not yet deliver production co-benefits that can compensate the extra cost and management constraints associated with methane mitigation options. Potential trade-offs with other global goals, such as the UN's SDG 2 Zero Hunger, also need to be weighed. These considerations imply societal choices and go beyond the ruminant production sector alone.

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Theatre 8

Evaluation of grape stalks residues in in vitro rumen fermentations

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In the Trentino Alto Adige region, (North-East Italy), wine cellars face significant costs related to the disposal of grape stalk (GS) residues. GS by-products still contain valuable nutrients and functional compounds that can directly influence the rumen microbiota and could be used as supplement in dairy cow feeding. In this work 3 red GS varieties were collected from a local wine cellar: Lagrein (L), Cabernet Sauvignon (CS), Merlot (M). Using Rumen Simulation Technique (RUSITEC) 5 diets were compared including a control diet (CTRL, no by-product), a grape-seed meal (GSM) diet at 8% and 3 diets with L-GS, CS-GS, M-GS used at 8 % on a dry matter (DM) basis. All diets consisted of 35% hay, 35% grass silage, and 30% concentrate and were made isonitrogenous (16.5%). Two RUSITEC systems were used, each consisting of 5 bioreactors, over 3 experimental runs (n=6 per diet). Each run included 5 days of adaptation, followed by 5 days of sampling. At sampling, one aliquot of the fermentation liquid from each vessel was used immediately for pH, redox, protozoal counts and another aliquot stored at -20°C for other analyses. Gas produced was collected daily in air-tight bags and the gas composition was measured with an infrared detector. All diets were similar in pH (6.46 – 6.53), redox potential (-176 – -192mV), and protozoa counts. L-GS and CS-GS diets showed a lower production of NH₃-N (mmol/L) by 22 and 13%, respectively than CTRL diet (p<0.05), despite similar DM and crude protein degradation. Fermentation gas production was also similar among diets with the range of 1.1 – 1.2 L/d and the gas composition of 14.02-15.80 % CH₄, 76-77 % CO₂ and 0.07-0.09% H₂. Overall, the tested by-products at 8% in diet DM didn't show any detrimental effect on rumen fermentation characteristics investigated. L-GS and CS-GS diets additionally reduced NH₃ production without compromising nutrient degradation. Funding: EU's Horizon 2020 program under the Marie Skłodowska-Curie grant, agreement n° 956265 FoodTraNet and by i-NEST project.

In vitro effects of bakery by-products on rumen degradability, fermentation, and methane yield

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Feeding bakery by-products (BBPs) to ruminants supports circularity and waste management. This study examined in vitro effects of BBPs on rumen degradation, fermentation parameters, and gas and methane output. A control (CON) diet included dried grass silage:wheat grain:soybean meal:corn distillers' grains at 600:300:70:30 g/kg dry matter (DM). Wholemeal crumbed bread (WMB), white crumbed bread (WB), cracker feed (CF), flavoured cracker feed (FCF), and biscuit meal (BM) were added at 205-215 g/kg DM, partially replacing wheat and in WMB, WB, FCF, and BM, also soybean meal. BBP diets had less starch and more fat. CF, FCF, and BM had more water-soluble carbohydrates. Three runs were conducted, each with 1g of diet incubated in Wheaton flasks with rumen fluid (four donor animals; one animal/flask):fermentation medium (10:90) for 72h, as required to model fermentation kinetics. Gas pressure (psi) was recorded and gas samples taken for methane analysis at 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72h. After 72h, flask content was filtered, pH recorded, in vitro DM degradability (IVDMD) measured, and filtered content analysed for volatile fatty acids and ammonia nitrogen. The linear mixed model had treatment as fixed, and animal ID and run as random factors. Fisher's Least Significant Difference test was used for pairwise comparisons. Gas and methane curves were modelled to assess fermentation kinetics. IVDMD was higher ($P=0.007$) in WB than in WMB, FCF, and BM; and in CON than in WMB. Propionate concentration was higher ($P<0.001$) in WMB, WB, CF, and BM than in CON. Valerate concentration was higher ($P=0.004$) in WMB and WB. Acetate:propionate was higher ($P<0.001$) in CON than in WMB, WB, CF, and BM. Methane yield (mL/g IVDMD) was lower ($P=0.045$) in WB and FCF than in CON by 10%. BBP-containing diets maintained effective rumen fermentation compared to the wheat and soybean they replaced, while WB and FCF also showed methane mitigation potential.

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Theatre 10

Effect of the inclusion of orange peel-based ingredients in dairy sheep diets on rumen methanogens

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A potential pathway towards the direct inhibition of methanogenesis in ruminants could be through the inhibition of rumen archaea, which are prokaryotic microorganisms, in their majority, methanogens. The aim of the present study was to evaluate the use of orange peels from a Greek juice industry as a high value secondary feedstuff for dairy sheep, within a circular economy approach. Enzymatically hydrolyzed (processed) and unprocessed orange peels were introduced to 2 groups ($n=12$ /group) of dairy ewes of Chios breed (DIM 42 onwards) at 11% DM inclusion levels (groups P & U), substituting wheat bran and sunflower meal in a typical ration (additionally containing corn and barley grains, wheat bran and soyabean meal), while group C (12 ewes) served as controls. All rations were isoenergetic and isonitrogenous and ewes were individually fed for 84 days. Rumen digesta samples were collected from all ewes on days 0, 42 and 84 of the trial in the morning before feeding, using an esophageal tube (flexible PVG tube of 1.5mm thickness and 10mm I.D.) and a vacuum pump (Selekt Pump, Nimrod). Metagenomic sequencing of DNAs isolated from the rumen fluid samples resulted in the detection of 10, 7 and 11 families and 12, 12 and 16 genera for groups C, Processed and Unprocessed, respectively. The relative abundance of methanogens was overall higher in the experimental groups, compared to the controls, in all samplings. In the processed group, it increased from 28.33% to 48.03% (an increase of 58.9%), in accordance to the peak of production and most dramatically, in the unprocessed group, it doubled down, from 45.57% to 20.70% (a decrease of 54.58%). The steep reduction of the methanogens in the unprocessed group, could be attributed to the tannin content of the feed, and to an undocumented property of orange as a potent inhibitor of the enzyme HMG-CoA reductase. Acknowledgement: EU PRIMA Program (grant agreement n°2013).

Successful use of insect oil as medium chain fatty acid source in calf milk replacer at two inclusion rates

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Producers of milk replacers (MR) rely on coconut oil (CO) to provide medium chain fatty acids (MCFA) C10:0, C12:0 and C14:0 to promote health and performance of calves. Given the global demand for food, exploring alternative sources of oil that can replace CO in MR is important. The fatty acid composition of the Black Soldier fly (*Hermetia Illucens*, HI) oil features high levels of C12:0 and C14:0 making it a viable alternative to CO. The aim of this study was to examine the effects of fully replacing CO by HI at two levels on growth and health of Holstein calves. 400 calves (start weight 50.4±3.5 kg) were assigned to pens within rows and treatments were randomly assigned to rows. The trial lasted 12 weeks and during 9 weeks MR (19.9% CP, 18.8% CF, mixed at 150g/l) was fed in 2 meals of 3l per day with a 3-week step down period. Starter feed and chopped straw was available ad lib. Treatments were a 2×2 factorial with the factors MCFA source (CO vs HI) and level (20% vs 40% of CF). Palm oil was the main oil in all treatments. Calves were weighed at arrival and week 2, 6, 10 and 12. Milk and solid feed intake was recorded per row. Individual fecal scores were recorded from day 0 till 10 and all veterinary treatments were noted. Growth and intake data were analyzed using linear regression including the factors source, level and their interaction. ADG was similar between treatments, with a total ADG of 1094 g/day. A trend ($P=0.09$) for higher ADG post-weaning in groups receiving a MR with 40% MCFA was observed. Calves receiving MR with CO tended ($P=0.08$) to consume more starter feed while 40% MCFA resulted in reduced starter feed intake ($P=0.05$). The source had no effect on diarrhea scores, health treatments and costs. Calves receiving a MR higher in MCFA presented less abnormal feces and a reduction of 35% and 23% for antibiotic usage was observed for CO and HI respectively. Based on these results we conclude that an increased level of MCFA in MR supports performance, despite a reduction in starter intake during the weaning phase and promotes animal health, leading to decreased use of antibiotics. Additionally, insect oil can serve as a viable source of MCFA in milk replacers for calves as an alternative to coconut oil.

Session 24

Theatre 12

Effects of replacing corn grain with whey permeate on the dairy performance of mid-lactation cows

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Feeding whey permeate (WP) to dairy cows will reduce surpluses of solids-not-fat in the Canadian dairy industry. We determined the effects of replacing corn grain with increasing levels of WP in the diets of Holstein cows on dry matter (DM) intake and dairy performance. Twelve mid-lactation cows were used in a triplicate 4×4 Latin square design of 21-d periods. Basal diet consisted of grass and corn silages, corn grain, soybean and corn gluten meals, and mineral and vitamin mix. Four levels (0, 4, 8 and 12% of DM) of dietary WP were tested as a substitute for corn grain. Diets were designed to be iso-energetic (1.72 Mcal/kg of DM) and iso-nitrogenous (16.6% of DM). Models included treatment and period as fixed effects, as well as cow and square as random effects. There were no linear or quadratic effects of WP level on DM intake. Milk yield decreased with increasing WP level ($P_{\text{linear}} < 0.01$) while 4% fat-corrected milk ($P_{\text{diet}} = 0.38$) and energy-corrected milk ($P_{\text{diet}} = 0.31$) were unaffected by treatment. Milk fat ($P_{\text{linear}} = 0.04$; $P_{\text{quadratic}} = 0.07$) and protein ($P_{\text{linear}} = P_{\text{quadratic}} = 0.01$) contents increased, while lactose content decreased ($P_{\text{linear}} < 0.01$; $P_{\text{quadratic}} < 0.01$) with dietary incorporation of WP. Milk fat ($P_{\text{diet}} = 0.50$) and protein yields ($P_{\text{diet}} = 0.32$) were not modified by WP feeding, while lactose yield decreased ($P_{\text{linear}} < 0.01$; $P_{\text{quadratic}} = 0.02$). Milk β -hydroxybutyrate concentration increased ($P_{\text{quadratic}} < 0.01$) with dietary WP level. Milk yield/DM intake ratio decreased with the level of WP ($P_{\text{linear}} < 0.01$; $P_{\text{quadratic}} < 0.01$) due to the decrease in milk yield and the lack of impact on DM intake. There were no linear or quadratic effects of WP level on the 4% fat-corrected milk/DM intake ratio. A linear tendency towards a lower energy-corrected milk/DM intake ratio was observed with increasing WP content. We conclude that replacing corn grain with WP up to 12% of DM does not adversely affect the performance of dairy cows, as 4% fat- and energy-corrected milk remained unchanged.

Use of cocoa byproduct in beef cattle diet: effect on animal performance and health status

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The aim of this work was to evaluate the effect of the inclusion of cocoa bean shells (CBS) in the diet of a local Sardinia cattle breed on animal performance and blood parameters. The experiment was carried out on 30 Sardo Bruna cattle (15 females and 15 males), divided into 3 groups according to sex. A first group was fed the basal diet without any supplementation, a second group was fed the basal diet supplemented with 150 g/head/day of CBS (CBS150), and a third group was fed the basal diet supplemented with 300 g/head/day of CBS (CBS300). The trial lasted six months, during which the weight of the animals were measured monthly, and the average daily gain (ADG) was calculated. Blood samples were collected at the 1st and 6st month of the trial and analyzed for hematological and biochemical analysis. At the end of the experiment, the animals were slaughtered, the carcasses weighed, and the carcass yield calculated. Data were analyzed with a mixed model in which the diet, the sex, the time, and their interactions were used as fixed effects and the animals as random. The inclusion of CBS in the diet did not affect the weight and the ADG of the animals. Males had higher ADG than females (1.4 kg/d and 1.1 kg/d, respectively). No differences in carcass yield were found between treatments. Overall, hematological parameters were not affected by the diets, except for the basophiles, which tended to be higher in CBS300 than in the other two groups. Females had lower neutrophils and higher lymphocytes than males. As regard the biochemical parameters, bilirubin and phosphorous were higher in CBS150 than the other two groups ($P < 0.05$). No effect of the CBS was found in the parameters of liver functions, such as bilirubin, aspartate transaminase, and alanine transaminase. Regarding the effect of the sex, the males had higher bilirubin and creatinine than females ($P < 0.05$). In conclusion, the long-term inclusion of CBS in the diet of beef cattle did not affect the performance of the animals or have a negative effect on their health, as shown by the hematological and biochemical results. Research supported by: NextGenerationEU; PNRR M4C2, CN00000022 AGRITECH.

Session 24

Poster 14

Environmental Impact of feed concentrate: the role of by-products

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Using agricultural by-products in livestock feed can be an effective strategy to optimise land use and mitigate the environmental impact of animal farming. Global deforestation accounts for about 50% of the environmental impact of agriculture, forestry, and other land use (AFOLU). This study aimed to assess the environmental impact using the Life Cycle Assessment approach for three different concentrated livestock feeds, with and without agricultural by-products and with and without the soybean from deforestation. The three concentrated feeds are homogeneous in terms of energy and crude protein and have been classified as By-Product Feed (BPF), Conventional Concentrate Feed (CCF), and Conventional Concentrate Feed with Soybean meal from Deforestation (CCFSD). Using the commercial software openLCA 2.0.1 and the Agribalyse v. 3.0.1 database, the ReCiPe mid-point (H) method was used. The Functional Unit was 1 ton. of concentrate feed. The main impact categories considered were Climate Change (CC, kg CO₂-eq), Terrestrial Acidification (TA, kg SO₂-eq), Freshwater Eutrophication (FE, kg P-eq), Fossil Resource Scarcity (FRS, kg oil-eq), and Land Use (m²a crop-eq). The BPF shows a 37.4% reduction on environmental impact in terms of CC compared to the CCF, while the reduction reaches 70.3% when considering soybean sourced from deforested areas. Regarding TA, the impact reduction is 21.0% compared to CCF and 32.2% for CCFSD. For FE, the reduction is approximately 65% for both concentrated feeds. Regarding non-renewable resources (FRS), the impact decreases by 43.7% and 48.8% compared to CCF and CCFSD, respectively. Land use is also reduced by about 60% when concentrated feeds containing agricultural by-products are used. Incorporating agricultural by-products into concentrated livestock feed offers a potentially sustainable alternative to using raw plant-based materials that could otherwise compete with human food resources and also to reduce deforestation.

Can leeks be included in a dairy sheep diet to replace conventional ingredients?

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Spain ranks among the top ten leek producers worldwide. Sulfur compounds in leeks may influence ruminal fermentation, and some have been associated with a reduction in methane production. The aim of this study was to evaluate the in vitro ruminal fermentation of dairy sheep diets with increasing amounts of leeks. Four diets (40:60 forage:concentrate), isoenergetic and isoproteic, were formulated: a control diet (CON) and with 8, 16 and 24 % DM of leek (L8, L16 and L24) replacing part of the concentrate of the CON diet. 0.3 g of substrate were incubated in batch cultures of ruminal microorganisms at 39 °C for 24 hours (n=4). Gas, volatile fatty acids (VFA) and CH₄ productions, ammonia concentration and dry matter disappearance (DMD) were measured. Increasing levels of leek in the diet were associated with higher total VFA production (P<0.05, linear effect) with values of 2200, 2594, 2729 and 2568 µmol for CON, L8, L16 and L24, respectively. Acetate and propionate production also shown a linear increment (1203, 1475, 1587, 1514 µmol and 662, 776, 814, 759 µmol for CON, L8, L16 and L24). The proportion of acetate increased linearly (P<0.05) with the inclusion of leek in the diet (53.9, 56.3, 57.6 and 58.7 % for CON, L8, L16 and L24). However, there were no differences (P>0.05) between diets on the proportion of propionate or acetate/propionate ratio. DMD was similar (P>0.05) in all diets (mean value 53.8 %). Replacing conventional ingredients with leek involved a linear reduction (P<0.05) of methane production and CH₄/VFA ratio (277, 283, 268, 253 µmol and 2.84, 2.46, 2.24, 2.21 mL/mmol for CON, L8, L16, L24, respectively). The results indicate that leeks could replace up to 24% of the cereals and high-protein ingredients in a high-cereal concentrate and decrease methane production without affecting the in vitro rumen fermentation of the diet. Further research is required to evaluate the increased risk of sulfur toxicity or the possible modification of the flavor of milk in practical conditions. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged.

Session 24

Poster 16

Inclusion of different levels of carrot in dairy sheep diets: in vitro studies

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Carrot is a worldwide important crop. At least 25-30% of all carrots are out-graded, mainly rejected due to physical/aesthetic defects, being a paradigmatic example of food waste. The aim of this study was to evaluate the in vitro ruminal fermentation of dairy sheep diets with increasing amounts of carrots. Four diets (40:60 forage:concentrate ratio) were formulated: a control diet (CON) and with 8, 16 and 24 % DM of carrots (CAR8, CAR16 and CAR24) replacing part of the concentrate of the CON diet. The diets were isoenergetic and isoproteic. 0.3 g of substrate were incubated at 39 °C for 24 hours (n=4) in serum bottles containing 30 mL of buffered ruminal fluid. Gas, volatile fatty acids (VFA) and CH₄ productions, ammonia concentration and the dry matter disappearance (DMD) were measured. The gas and total VFA productions increased linearly (P<0.05) with the inclusion of carrot in the diet (46.3, 51.8, 50.7, 53.6 mL and 2200, 2568, 2665, 2905 µmol for CON, CAR8, CAR16 and CAR24, respectively). The same linear increment (P<0.05) was observed for acetate (1203, 1524, 1640 and 1857 µmol) and propionate (662, 717, 735 and 786 µmol) productions and for the acetate proportion (53.9, 59.1, 61.2 and 63.5 %). On the contrary, the propionate proportion decreased linearly (P<0.05) when the proportion of carrot in the diet increased. Consequently, a linear increase (P<0.05) was also observed in the acetate/propionate ratio, with the highest ratio for CAR16 and CAR24 (2.24 and 2.34). DMD also increased linearly (P<0.05) with the inclusion of carrot in the diet (52.7, 54.7, 55.6 and 57.4 % for CON, CAR8, 16 and 24). CH₄ production was higher in CAR8 and CAR24 (294 and 320 µmol, respectively; P<0.05) than in the other diets, although no differences (P>0.05) in CH₄/VFA ratio were detected. In conclusion, dried carrots could replace up to 24% of the cereals and high-protein ingredients in a high-cereal concentrate without affecting the in vitro rumen fermentation of the diet. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged.

Nutritional evaluation of carrot, leek and bell pepper as alternative ingredients for ruminant diets

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Spain is the leading producer of fruits and vegetables in the EU. This high production is associated with large quantities of waste and by-products that must be removed from the environment. The objective of this work was to evaluate the chemical composition and the *in vitro* ruminal fermentation of carrot (CAR), leek (LEE) and bell pepper (PEP) as a first step for their nutritional assessment. Chemical composition of CAR, LEE and PEP, and four reference foods included in the study for comparison (corn, barley, soybean and beet pulp) was analysed. Ingredients were incubated in batch cultures of ruminal microorganisms at 39 °C for 24 hours (n=4). At the end of incubation, gas production, volatile fatty acids (VFA), CH₄, ammonia concentration, and substrate dry matter disappearance (DMD) were measured. The DM content of CAR, LEE, and PEP did not exceed 10%. The sugar content was higher in the three vegetables than in the conventional feeds (P<0.05). The CP content was different in the 3 by-products (P<0.05), being higher in LEE than in CAR (19.0 and 8.58%, respectively), with intermediate values for PEP (13.5%). LEE had the highest NDF content (22.6%), although its lignification was lower than in PEP (19.8% vs 30.1%). Total VFA production was higher (P<0.05) in the vegetables than in the reference materials. The proportion of acetate was similar (P>0.05) in the 3 vegetables and the beet pulp and higher (P<0.05) than in the other conventional feeds, whereas the proportion of propionate was similar in all substrates. The highest acetate/propionate ratio of the vegetables was observed with LEE (P<0.05). DMD was higher (P<0.05) in the vegetables than in the conventional ingredients. CH₄ production varied depending on the substrate and was related to its chemical composition. The lowest CH₄/VFA ratio was for the CAR (2.33) and the highest for corn (3.56). The results obtained indicate the potential of carrot, leek and bell pepper as alternative ingredients for ruminant diets. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged.

Session 24

Poster 18

Inclusion of different levels of bell pepper in dairy sheep diets: *in vitro* studiesI. Mateos^{1,2}, N. Merino^{1,2}, A. Díaz^{1,2}, C. Saro^{1,2}, R. Garcimartín¹, S. López^{1,2}, M. J. Ranilla^{1,2}¹ Universidad de León, Campus de Vegazana, s/n, 24071 León, Spain, ² Instituto de Ganadería de Montaña, Finca Marzanas, 24346 Grulleros, Spain

Bell pepper (PEP) production is important worldwide and in the province of León (Spain). This production involves a high volume of waste and by-products that could be used as an alternative ingredient in dairy sheep diets. The aim of this study was to evaluate the *in vitro* ruminal fermentation of diets for dairy sheep with three different levels of PEP to replace conventional ingredients. Four diets were formulated with a forage:concentrate ratio of 40:60: control diet (CON) and three with 8, 16 and 24 % of PEP (DM basis; PEP8, PEP16 and PEP24) replacing part of the corn, barley and soybean in the CON diet. The diets (isoenergetic and isoproteic) were incubated in batch cultures of ruminal microorganisms at 39 °C for 24 hours (n=4). Gas production, volatile fatty acids (VFA), methane, ammonia concentration, and dry matter disappearance of the diet (DMD) were measured. The replacement of dietary ingredients with bell pepper was associated with a linear increase (P<0.05) in total VFA production (2200, 2662, 2874 and 2986 µmol for CON, PEP8, PEP16 and PEP24, respectively). Acetate and propionate production also increased linearly (P<0.05). The proportion of the different VFA was also affected (P<0.05) by the inclusion level of PEP, increasing that of acetate and decreasing the rest. As a consequence, the acetate/propionate ratio also increased linearly (P<0.05) with pepper content in the diet (1.83, 2.06, 2.22 and 2.26 for CON, PEP8, PEP16 and PEP24, respectively). DMD was higher in PEP diets than in the control, with a linear increase (P<0.05) (52.7, 55.3, 57.9 and 58.8 % for CON, PEP8, PEP16 and PEP24). Methane production also increased linearly (P<0.05) although it was similar in CON and PEP8 (277 and 289 µmol; P>0.05). The results indicate that replacing up to 24% of the conventional ingredients of a diet for dairy sheep by bell pepper improved *in vitro* ruminal fermentation compared to the control diet, encouraging further research as an alternative feed for ruminants. Funding from the Spanish State Research Agency (AEI) and the European Regional Development Fund (Project PID2021-124648OB-C22) is gratefully acknowledged.

By-products from the Spanish gazpacho industry as alternative ingredients for sheep diets: In vivo digestibility and N balance

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The industrial production of gazpacho, a traditional Spanish cold soup, has grown significantly in recent years, reflecting its increasing international popularity. Recent data show that the consumption of gazpacho at national level is more than 100 million tons per year, contributing approximately 2,000 million euros per year. However, this large-scale of consumption and production generates substantial amounts of by-products (BGP), primarily from tomatoes, peppers, and cucumbers, which can globally amount to millions of tonnes per year, present both environmental challenges and opportunities for resource recovery. The 5th range food industry is increasingly focusing on revalorizing these by-products, which retain significant nutritional value and bioactive compounds, for use in foods and as ingredients in human and animal consumption, aligning with principles of circular bioeconomy food production. This study evaluated the effects of including 15% of BGP in Segureña sheep ration on in vivo ruminal fermentation, digestibility, and N balance. Eighteen sheep (57.2 ± 2.73 kg) were divided into two groups (n=9): a control group (CTL) fed a 60:40 forage (barley straw) to commercial concentrate diet, and a second group (C4F) fed the same diet with 15% BGP inclusion in the concentrate. A digestibility trial was conducted using metabolic cages, following the methodology described by Belanche et al., 2023. Samples of faeces, urine, feed refusals, ruminal fluid and blood, were collected and analyzed. Data analysis using ANOVA (SPSS 29.0.0.0) revealed no significant differences ($p>0.05$) in nutrient intake, apparent digestibility, N balance or ruminal fermentation parameters (ammonia nitrogen, lactic acid, and volatile fatty acids) between the two groups. Thus, the inclusion of 15% SGP in sheep diet did not affect voluntary intake, reduce rumen fermentative capacity, or impair the utilization of major nutrients. These findings suggest that this approach represents a promising alternative that warrants further investigation and could to support circularity and enhance the sustainability of the sector without compromising animal production and health.

Session 24

Poster 20

Effect of the conservation method of Brewer's Spent Grain on nutritional and microbial composition

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Evaluate nutritional and microbial composition of Brewer's Spent Grain (BSG) in silage and pellet. Study was carried out from Feb-2024 to Jan-2025. BSG was collected at D'OLBEK brewery and transported to a sun-dryer at INIA Tamei Aike, where 60 trays with up to 15 kg of BSG were arranged and dehydrated. BSG samples were taken daily to evaluate moisture loss and drying time. Once BSG obtain 50 and 15% dry matter (DM), it was processed into silage and pellet using 25 kg bags. Silage bags were hermetically sealed using a vacuum cleaner to extract oxygen. For pellet, a power classic machine with a capacity of 50 to 80 kg/h was used. Samples were taken at 30-, 60-, 90- and 120-days post-conservation to evaluate nutritional and microbial composition. Nutritional and microbial composition was statistical analyzed RStudio software, using ANOVA test for repeated measurements. Results did not indicate significant differences between post-conservation days (30, 60, 90 and 120). However, significant differences were observed on the conservation method for nutritional composition. For silage, higher contents of ash, CP, NDF and EE were obtained, contrary to the pellet, with higher contents of DM, WSC, digestibility and WSC/CP ratio. Regarding to microbial composition, high concentrations of fungi and bacteria were observed in both conservation methods (silage: 292 bacteria and 308 fungi; pellet: 178 bacteria and 348 fungi), without significant differences between post-conservation days. However, there are differences in fungi identified, since 2 genera of dominant fungi were observed (Aspergillus and Penicillium) in the samples isolated from pellet, compared to the silage samples, where 80% of the samples are yeasts. These results demonstrate the importance of knowing even more about mycotoxigenic fungi present. BSG presents a high nutritional quality in both conservation methods. while pellets have a better WSC/CP ratio. Thus, drying process may lead to elevated fungal concentrations with potential mycotoxin contamination.

Effects of protein-reduced supplementation on dairy cow production in an organic herbage-based feeding system

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This study investigated the effects of protein supply on production in a herbage-based feeding system over a three-year period. The study included 142 lactations from 53 Holstein and 41 Swiss Fleckvieh cows from the organic farm 'Ferme Ecole de Sorens' in Switzerland. The winter ration consisted of moderate-quality hay (5.2 ± 0.15 MJ net energy for lactation [NEL], 118 ± 4.8 g crude protein [CP] per kg dry matter [DM]). From the end of March, the cows were continuously turned out to graze during the day and at night (herbage: 6.1 ± 0.25 MJ NEL, 178 g CP per kg DM). During the first 90 days in milk (DIM), cows were fed 3 kg of concentrate daily, and after a 10-d adaptation period, 1 kg up to 150 DIM. In total, 340 kg of concentrate were available to the cows during the first 150 DIM ($\square$ 5% of the annual ration). One half of the cows received the CERE concentrate (7.8 ± 0.14 MJ NEL, 135 ± 7.5 g CP per kg DM) while the other half received the PROT concentrates (8.0 ± 0.15 MJ NEL, 225 ± 7.3 g CP). The calving period lasted from December to May, with a mean calving date around February 15th. Before the calving period, the cows were paired based on breed, lactation number, and anticipated calving date. The biweekly milk control recordings were allocated to the respective lactation (L) stages: L-start (< 90 DIM), mid-L, and L-end (> 200 DIM), and averaged for evaluation. The statistical evaluation of the recordings was carried out using a mixed linear model (package lme4) in R, with fixed factors (treatment, lactation stage and their interaction), while the experimental animal was considered a random factor. In L-start, the cows with PROT exhibited higher energy-corrected milk production (28.9 vs. 27.0 kg, $P = 0.002$) and had higher milk urea concentrations (20.7 vs. 16.6 mg/dl, $P < 0.001$). However, no significant differences were observed in mid-L and L-end. Milk components, including fat, protein, and lactose, as well as somatic cell count showed no significant differences between the PROT and CERE treatments. As expected, the lactation stage proved to be highly significant for each investigated characteristic. The interaction between treatment and lactation stage reached statistical significance for all characteristics except for milk protein content.

Session 25

Theatre 1

One big success: management of endangered livestock breeds in Austria

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In situ conservation of local endangered breeds of livestock is worthwhile for several reasons. Overall genetic diversity of a species is maintained, as are genetic variants of potential future importance. Local breeds play an important cultural role, farmers and people of a region being attached to their features. Soon after joining the European Union in 1995, Austria established an EU co-funded programme for the promotion of an agricultural system that is environmentally sound, extensive and protective of natural habitats (ÖPUL). One funding stream supports farmers keeping endangered breeds of livestock, providing money per active male and female breeding animal. Rules and regulations were developed and frequently updated by ÖNGENE, a body comprising members from regional chambers of agriculture and two universities and supervised by the Ministry of Agriculture. A gene bank with routine collection of semen of male animals of endangered breeds has been established. Virtually all breeds supported have substantially increased in population size, allowing a move from exclusively minimizing increase in inbreeding levels to a combination of inbreeding avoidance and improvement of quantitative traits. The current program supports breeders of nine cattle breeds, eight sheep, seven goat, two pig and one horse breed. Its newly established routine parentage test for all breeding animals, mostly using high throughput SNP markers, provides the opportunity to actively avoid genetic disorders and to check for the breed integrity of animals. To date, avoidance of inbreeding mating is being done using pedigree information. For this purpose, farmers receive lists of potential male mating partners for their female animals based on the inbreeding coefficients of potential offspring. A study of using SNP genotype data instead of pedigree for such mating suggestions indicated that the additional benefit in terms of genetic diversity was very limited and ÖNGENE decided not to move from pedigree to genomic information. Yet, using the SNP genotypes for moving from routine pedigree based genetic evaluation for many traits to single step genomic prediction for the bigger endangered breeds of cattle is suggested.

Icelandic dairy cattle: successful conservation but how can the breed keep profitable?

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Milk production in Iceland is based on around 25,000 cows of the Icelandic Dairy Cattle (IDC) breed. The IDC has a unique genetic makeup, acceptable rate of inbreeding and considerable genetic gain. Import of dairy cattle genetics is not allowed but beef semen and embryos are imported. Despite the restrictions, dairy farmers are interested in importing more productive dairy genetics to improve their farms' profitability, by crossbreeding with IDC or replacing them. The production cost of kg milk could be reduced 0.12-0.13€ by having Holstein or Nordic Red cows instead of IDC. For the whole country, new breed could reduce costs by over 20 million €, which can be seen as the cost of protecting IDC by limiting import of genetics. This cost can be limited by genetic improvement within IDC. Genomic selection is already in place. The aim of this study was to evaluate genomic breeding plans for a small, isolated population with some use of natural service sires (NS), like IDC, in terms of genetic gain (ΔG) and rate of inbreeding (ΔF). We simulated a dairy cattle population mimicking the Icelandic population and compared 80 breeding programs. The use of NS in the population reduced ΔG but that can partly be alleviated by genotyping the potential NS. A multiple ovulation and embryo transfer (MOET) program increased ΔG regardless of the use of NS. Application of X-semen needs to be combined with MOET or controlled such that enough bull calves are produced for genomic selection of AI-sires. The ΔF can be managed with the selection of AI-sires, ideally based on optimum contributions, otherwise using at least 20 new AI-sires annually. The rapid ΔG in the larger breeds increases the cost of keeping IDC as the only milk production breed in Iceland. Maximizing ΔG while maintaining genetic diversity within IDC increases the possibility of IDC to keep its position. Implementing a MOET donor scheme, combined with sexed semen, and optimum contribution selection are effective ways for that.

Session 25

Theatre 3

Genetic diversity of Turopolje and Magalica pig breeds in Austria

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The Turopolje and Mangalica are endangered breeds in Austria, kept as local populations. Due to the small population size the management of genetic diversity is of high relevance in both breeds. The diversity management is done using through analysis of conventional pedigrees, but also routine genotyping with single nucleotide polymorphism markers (SNP) of all animals used for breeding. The most notable use of SNP markers is for assessment of inbreeding levels and for avoidance of admixed animals for breeding. SNP genotypes are also used for parentage testing (based on the number of mendelian inconsistencies between parent-offspring pairs), because the former method based on microsatellites was not sustainable due to the high inbreeding levels in both breeds. The aim of this study was to assess the overall inbreeding levels and admixture proportions in both breeds. A total of 265 Turopolje and 214 Mangalica pigs were genotyped using a custom chip with 77,122 SNP. In the quality control, SNPs and individuals with more than 10% missingness were removed. Additionally, SNP with less than 5% minor allele frequency were removed for admixture analysis. After quality control a total of 262 Turopolje and 211 Mangalica pigs and 70,099 autosomal SNP remained for genomics inbreeding (52,906 for admixture analysis). The genomic inbreeding was assessed based on runs of homozygosity (ROH) with the cgaTOH software (common ancestors up to 6 generations in the past, minimum length of ROH more than 8 Mb). The average inbreeding coefficient was 0.144 (sd 0.053) in the Turopolje and 0.165 (sd 0.084) in the Mangalica breed. Several crossbred animals up to 27% admixture from foreign breeds were identified in Turopolje and up to 50% in Mangalica. It is recommended that crossbred animals not be used for breeding. The continued use of SNP chips for the routine management of genetic diversity is recommended.

Population genomics in Reggiana cattle to manage diversity within and across farms

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Reggiana is an autochthonous cattle breed mainly raised in the Emilia-Romagna region, in the North of Italy. This breed is important for economic and traditional reasons due to production of mono-breed branded Parmigiano-Reggiano cheese, which provides essential economic incomes for the sustainable conservation of this valuable genetic resource. The breed population includes around 4,500 animals registered in the Herd Book. In this study, more than 4,000 animals have been genotyped with GGP Bovine 150k and GGP Bovine 777k SNP chips. The animals included in the dataset cover almost the entire breed, including 280 sires, and come from 50 different farms. Genomic data were used to refine pedigree information and trace back the individual history of specific animals. Genomic inbreeding was estimated using runs of homozygosity (ROH). Admixture and multi-dimensional scaling analyses were carried out to check population genomic structure and differences in farm clustering. Among the most relevant results indicating variability between farms, F_{st} had a wide range of values, with an average of 0.018 ± 0.0043 and a maximum of 0.65 for some specific regions. Inbreeding levels were compared between different farms and showed various inbreeding patterns indicating differences in the management of mating. Farm-specific data were collected to evaluate among farms genetic diversity, monitor breeding practices and guide choices in farm management. These findings will support mating plans and conservation strategies for the Reggiana cattle breed, allowing tailored decisions to be made and contributing to the sustainability of this animal genetic resource. Research funded by the European Union – NextGenerationEU under the National Recovery and Resilience Plan (PNRR) – Mission 4 Education and research – Component 2 From research to business – Investment 1.1 Notice PRIN 2022 PNRR (DD N. 1409 del 14/09/2022), proposal code P2022ALHF4 – CUP J53D23018270001

Optimizing mating strategies in a critically endangered antelope conservation program: the case of the mhorr gazelle (*Nanger dama mhorr*)

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Threatened species suffer a greater loss of genetic diversity due to their small size and increased relatedness between individuals. Therefore, conservation programs seek effective mating strategies that retain maximum genetic variability of the population and guarantee its long-term survival. The objective of this study was to compare the performance of different breeding schemes in a real captive population of mhorr gazelle (*Nanger dama mhorr*) via computer simulations. From a total pedigree of 3059 records, 87 living animals (25 males and 62 females) formed the selected reference population of Almería (breeding stock). A total of 22 mating strategies were tested according to different assumptions: the use of parents coancestry or offspring coancestry, the use of the coefficients themselves in calculations or their increases, and the number of males and females involved in the mating. Fifty replicates of each strategy were conducted during 15 discrete generations. The evolution of genetic diversity was assessed by the effective population size value. All the analyses were computed using the ENDOG program v4.9. The results showed that the best mating strategies to maximize genetic variability in the short and long-term of this reference population were those that minimize both parents and offspring coancestry (5% and 95% respectively and 50% each one gave similar results), and when all females were expected to contribute to the next generation, but not all males. The strategies that only minimize the coancestry of the parents improved when all females and all males were forced to participate. In the case of the strategies that only minimize the coancestry of the offspring, the best effective population size values were obtained when not all females and not all males participated with offspring for the next generation. The results of this study will help to improve the effectiveness of genetic management in this captive population.

Preliminary analysis of survivorship of captive-reared Northern bald ibis (*Geronticus eremita*) reintroduced in the wild

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The northern bald ibis *Geronticus eremita* (NBI) was extinct in Europe since the 17th century. Nowadays, thanks to the Proyecto Eremita and the Waldrappteam programs, the NBI has been reintroduced in southern Spain and Central Europe, respectively. The Proyecto Eremita has been developed since 2004 to date by Zoobotánico de Jerez. The aim of this study was to analyze the risk factors in survival after release of the reintroduced NBI individuals. The database contained information about 694 birds born from 1991 to 2020. After data editing a total of 406 individuals were used for the analysis. The time of survival after release was analyzed by fitting a Cox proportional hazard regression model adjusting for the sex (male and female), type of rearing (parent-rearing and hand-rearing), origin of birth (Germany, Austria, France, Netherlands, Hungary, England, Italy, Portugal, Czech Republic, Switzerland, Spain, Zoobotánico de Jerez and Retín), total time in the aviary before the release (in days), age at release (in days) and the month of releasing. The software used was the package “survival” of the R environment. The median of the survival in the wild was 0.44 ± 1.49 years. Only the type of rearing and the month of release reached significant effect ($p < 0.05$) on the survival in the wild. Thus, the risk of mortality was higher when the birds were released in February, June, July, August and December compared to November. Similarly, the risk of mortality of parent-reared birds was significantly higher (59.70%) than hand-reared birds ($p < 0.05$). Therefore, it was concluded that human management increased the survival in the wild of individuals. Our results provide some important cues to improve the releasing technique methodology.

Session 25

Theatre 7

Did we store sufficient genetic material in the gene bank to effectively reconstruct breeds in case of extinction?

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How much genetic material should be stored in a gene bank to effectively reconstruct a breed? Gene banks can serve, next to other purposes, as a backup to restore breeds in case of extinction. Consequently, it is important to assess whether sufficient genetic material is stored for this purpose. The effective population size of the reconstruction program, which depends on the number of individuals involved, will determine how much of the genetic diversity is lost at the end of the program. The type of genetic material also determines how many doses, embryos or cells should be stored. Cryoconservation of primordial germ cells (PGCs) is the most recent development and indications of required quantities to be stored are needed. Other things to consider are the required purity of the recreated breed, the viability of cryoconserved material, pregnancy rates, litter sizes and whether or not sexed semen or embryos are available. All of these aspects of breed restoration are combined in a mathematical model, and have been implemented in an easy-to-use spreadsheet, called the conservation planner. This enables the user to evaluate for every breed the number of samples, to be stored in a gene bank to allow for breed reconstruction. In previous research, for example, the conservation planner indicated that the number of doses of semen needed to be stored was at least 890 doses for cattle. Nine of the 11 Dutch cattle breeds or breed varieties had sufficient semen stored at that time. For the 31 Dutch chicken breeds or varieties only one had sufficient number of doses stored. These figures are now supplemented with the number of embryos and PGCs needed to be stored to enable reconstruction of breeds.

Added value of sustainable breeding advice and use of genebank material to support conservation of Dutch native breeds

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To identify breeds at risk in time, their status is monitored annually in the Netherlands. Using indicators such as, population size, population trend, number of breeding males and females and progeny born. These are supplemented with extensive genetic population analyses at regular intervals. Based on this information, CGN gives advice to breed societies to optimize their breeding programmes. CGN also manages the national animal genebank with the aim to support the long term conservation of local breeds. A recent study on Dutch native goat breeds provides an example of the positive impact of breeding programme advice. In 2016, the rate of inbreeding per generation was above 0.5% for all breeds. Urgent advice was given to use less related breeding males and a higher number of bucks per generation. In response, the breed societies adapted their protocols for selecting bucks. A new analysis in 2024 showed decreased rates of inbreeding (<0,25%) and a wider range of bucks used. Groningen White Headed cattle (GWH) provides an example of the benefit of the use of genetic material from the national genebank to increase the genetic diversity of the in-situ breeding population. The inbreeding rate was 1.34% per generation between 1999 and 2004. The GWH breed society then started collaboration with the genebank on the promotion and use of genetic material from bulls stored in the genebank. Semen of in total 26 bulls was used between 2005 and 2016, fathering 553 calves. The use of genebank material contributed to a decreasing the rate of inbreeding below 0.25% between 2019 and 2024. These examples illustrate the relevance of both science based monitoring and optimization of breeding programmes in combination with the use of genebank collections to support conservation of local breeds.

Session 25

Theatre 9

Optimizing Relatedness Penalties in Multi-Stage Optimal Contribution in Dairy Cattle Breeding Programs

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Inbreeding levels in dairy cattle populations have increased due to intensified selection pressures, raising concerns about inbreeding depression and its negative impact on key traits. Optimal contribution selection (OCS) has been proposed to balance genetic gain and inbreeding, but its use in multi-stage breeding schemes remains underexplored. This study investigates how penalties on relatedness in OCS can be optimized across selection stages. We hypothesize that stage-specific penalty adjustments—higher penalties for early-stage selection and lower for final selection—enhance genetic diversity while preserving genetic progress. We employ stochastic simulations with AlphaSimR, using a dairy cattle population model. The breeding scheme includes a 60-year burn-in phase followed by 10 years of scenario evaluation. Each gender and age group are simulated separately, modelling 10,000 annual matings to reflect realistic breeding structures. Estimated breeding values (EBVs) are used for selection and matings, incorporating both pedigree-based BLUP and single-step genomic BLUP (ssGBLUP). OCS is implemented with inbreeding constraints of 0.5% and 1% to optimize genetic contributions. Three scenarios are evaluated: (i) genomic selection without OCS, (ii) single-stage OCS at bull selection, and (iii) two-stage OCS at calf selection for genotyping and bull selection. The impact of each scenario on genetic gain, inbreeding accumulation, and the number of bulls required for sustainable breeding is analyzed. This study provides practical guidelines for implementing OCS in commercial dairy breeding by optimizing penalties on relatedness across selection stages. By refining OCS strategies, breeders can enhance sustainable genetic management, ensuring the long-term viability of dairy populations. The results contribute to sustainable breeding programs by mitigating inbreeding risks while maximizing genetic progress.

Effects of restructuring breeding programs to bring in external diversity - a simulation study

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Closed populations are gradually losing genetic diversity due to drift over time which may limit the long-term genetic gain that can be achieved. This barrier may be lifted by establishing a continuum to genetic material outside of the elite nucleus which allows diversity to flow into the active breeding population. We hypothesize to do this by restructuring breeding programs into an elite component and several layers that upcycle external genetic material introduced by so called “diversity donors”. The role of the upcycling-component is to explore external diversity by phenotyping new material, and to exploit it, i.e., bringing the useful variation into the genetic background of the elite. We test the performance of restructured breeding programs in a simulated idealized genomic selection swine breeding program for 20 generations. For simplicity, candidates for diversity donors were former selected elite sires of which frozen semen is stored. The performance of restructured breeding programs is compared to ordinary programs run with optimum contribution selection (OCS). Restructured breeding programs always showed lower genetic gain compared to the control scenario which can be attributed to the lower number of animals used to advance the elite. For example, the control breeding program achieved genetic progress of 19.5 genetic standard deviations (gSD) over 20 generations whereas breeding programs investing 25% of resources on upcycling achieved gain of about 17.4 gSD, meaning 10% less gain. Despite lower gain, restructured breeding programs retained more genetic diversity in terms of genetic variance, number of beneficial alleles, haplotypes and inbreeding level. The observed lower inbreeding levels were surprising as restructured breeding programs and the control scenario were run with the same inbreeding restriction in OCS which should have resulted in the same inbreeding rate. Restructured breeding programs were more efficient, i.e., for a given reduction in diversity they achieved more genetic gain. Our findings might help to guide diversity management of real breeding programs especially if more variance is needed to support breeding goal changes or if genetic material of other lines is introduced.

Session 25

Poster 11

Low selection ability for IGF2 and MC4R markers in Polish Landrace population

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IGF2 and MC4R aspire to be the most significant genes following RYR1 in pig breeding. IGF2 plays an essential role in muscle growth by regulating myoblast proliferation, apoptosis, and differentiation (Saltiel & Kahn, 2001), and MC4R indirectly regulates feed intake (Harrold et al., 1999). These genes are known to affect pigs' growth traits and meat content (Oczkiewicz et al. 2009). IGF2 marker is associated with a mutation in intron 3 (Van Laere et al. 2003), which changes the binding site for ZBED6 transcription factor (Huang et al., 2014) and IGF2 expression level. In turn, in MC4R, missense variant G1426A (Asp298Asn) (Kim et al. 2000), causes dysfunction in the MC4R protein. For several years, the Polish Ministry of Agriculture commissions the National Research Institute of Animal Production Polish Landrace pig population monitoring for IGF2 and MC4R markers. Therefore, 1196 Polish Landrace pigs were scored from 2020-2024. All individuals were free of RYR1 mutation. Genotyping of MC4R and IGF2 mutations was performed using the allelic discrimination method on Quant Studio 7 flex. Results of MC4R and IGF2 present significant overdominance of alleles related to higher meat content G and A, respectively. Over the years, the distribution of minor alleles (G) in IGF2 decreased from 22 to 10% and in MC4R (A) slightly increased from 18 to 25%. Similarly, the presence of a minor genotype (GG) in IGF2 lowered from 6 to 1%, and in MC4R (AA) increased from 4 to 7%. In both particular, minor genotypes were associated with higher fat content. In conclusion, although high meat content in pigs is associated with low IMF% and subcutaneous fat related to worse pork flavor and technological parameters, this state is maintained in Polish Landrace breeding due to higher economic benefits. Thus, with the low frequency of minor alleles, a poor selection possibility exists, which would include IGF2 and MC4R markers. Founding Polish Ministry of Agricultural 503-180-709

How can cryopreserved genetic resources be used in domestic animal populations?

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Genetic diversity is essential for the adaptability of animal populations and is thus a central pillar of the agro-ecological transition. However, part of this genetic variability is inevitably lost within populations and efforts are needed to limit this phenomenon. In this context, the use of ex situ genetic resources, i.e. cryopreserved semen in gene banks, could be a valuable management tool to maintain or restore genetic diversity. Thus our study aims at evaluating the value of ex situ genetic resources to manage genetic diversity in populations undergoing contrasted breeding programs, in order to make recommendations for their efficient use. We used simulations (MoBPS program) of livestock populations being selected or not, and experiencing a conservation program or not. Selection targeted two negatively correlated traits and was simulated to maximize either breeding values or genetic diversity, using OCS for optimizing the use of ex situ genetic resources. We show that the appropriate use of ex situ genetic resources differed radically depending on the characteristics of the target population and its management objectives. For conserved populations, a regular use of many cryopreserved sires helped maintaining genetic diversity across generations, slowing the increase of inbreeding. For populations under selection, the strategy depended on the breeding objectives: when genetic progress is prioritized, occasional use of a few cryopreserved sires increased genetic variance, allowing for sustainable selection. However, for populations with a strong change of breeding goal, the use of old sires proved relevant provided it is carefully managed to avoid decreasing breeding value for key traits. This study highlights the strategic potential of cryopreserved genetic resources for the management of genetic diversity and genetic progress. However, the use of cryopreserved semen in animal populations requires a large amount of material: for selected breeds because the number of offspring is high, and for conserved breeds because the frozen semen of many sires is used repeatedly over a long period. Consequently, gene banks should keep a large choice of material to support livestock breeding effectively.

Session 25

Poster 13

Maximizing genetic diversity in gene banks: the examples of Dutch goats and sheep

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Maximizing genetic diversity in gene banks: the examples of Dutch goats and sheep. Maximizing the genetic diversity of cryoconserved material stored in gene banks is essential to sustain the role of gene banks in conservation. The examples of Dutch goat and sheep breeds illustrate how this can be achieved. The basic idea is to minimize the average kinship of the animals stored in the gene bank. This was done for several goat breeds by choosing animals for storage in the gene bank based on their mean kinship with animals already stored in the gene bank. Optimal contributions also minimize kinships, but with this method the kinship of all candidates for storage and those already stored are taken into account in a single step. For the Dutch landrace goat semen was originally stored in 2005 when pedigree information at that time was incomplete. An effort was made to avoid storing material of related animals, by sampling animals of different breeders but how much genetic diversity was stored in the gene bank was not clear. In 2024 the material stored in the gene bank could be supplemented, and now almost the complete pedigree information was available. Optimal contributions could be calculated to identify the group of individuals that would minimize the average kinship in the gene bank. Storing material from the indicated animals, could decrease the average relatedness in the gene bank from 0.199 to 0.100. However, not all animals indicated were available for sampling and when this was taken into account the average relatedness decreased to 0.167 only. For the Mergellander sheep optimal contributions were employed as well, but here special care was needed to avoid storing animals with incomplete pedigree information. In conclusion, using mean kinships and optimal contributions are suitable to select candidates for storage in a gene bank, but practical considerations need to be considered, before doing so.

Stability of Effective Population Size Estimates: A Genomic Approach Using Different SNP Densities and Sample Sizes

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Effective population size (N_e) is a key parameter in population genetics, as it directly influences stochastic variation in allele frequencies, inbreeding, and linkage disequilibrium. However, estimating N_e from genomic data can be biased due to factors such as admixture, a small number of non-randomly selected individuals, or low SNP chip density. This study aimed to evaluate the stability of N_e estimates under varying sample sizes and SNP chip densities. N_e was estimated using gametic/linkage disequilibrium information, as implemented in the GONE software. Three horse breeds – Old Kladruher, Lipizzaner, and Arabian – were included in the analysis. Two breeds were genotyped using medium-density SNP chips (60k), while one was analyzed using high-density SNP chips (300k). Stability was assessed by randomly selecting different numbers of individuals (ranging from 10 to 300) and by systematically reducing SNP chip density. For medium-density chips, SNP counts were reduced in 10k increments from 10k to 50k, while for high-density chips, SNP counts were reduced from 50k to 300k. The results showed that SNP chip density had a more significant impact on N_e estimate stability than sample size. A minimum SNP density of 30k was identified as a threshold for reliable N_e estimation, with higher SNP densities providing even greater stability. Similarly, a minimum of 30 individuals was required for robust N_e estimation. Overall, this study highlights the reliability of N_e estimation from genomic data and provides practical recommendations for study design. This research was supported by GACR 24-14325L, QL25020022, APVV-20-0161, and the NextGenerationEU project “NEXT GENERATION ANIMAL PRODUCTION” (NPOO.C3.2.R3-11.04.0141), which funded Mario Shihabi and Ino Curik.

Session 26

Theatre 1

Fat quality in carcasses of organic pigs: Influencing factors in feed, farm and animal health

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Since 2022 the EU organic regulation requires growing-finishing pigs to be fed with 100 % organic feed. Until 2021, it was permitted to use up to 5 % conventionally produced feed components which typically included potato protein with a favourable amino acid profile. A disadvantage of alternative organic components, such as soy cake, is that they often contain high concentrations of polyunsaturated fatty acids (PUFA). In Switzerland, slaughterhouses apply a penalty to carcasses with PUFA concentrations in backfat exceeding 15.6% in order to reduce oxidation and maintain good processing properties. The goal of the study presented here was therefore to evaluate factors influencing PUFA concentrations in carcass fat. We visited 18 organic farms with growing-finishing pigs, which were classified as either “PUFA high” or “PUFA low” based on routine PUFA measurements from 2022 and 2023. During the farm visits, we assessed animal health (tail and ear lesions, diarrhea, lameness, skin lesions), farm management (feeding technique, climate, pen cleanliness, water and roughage provision), collected faecal samples for parasitological analysis, and feed samples for nutrient content analysis. Additionally, we collected individual fat samples after slaughter from five farms (analyzed via NIRS) and recorded abattoir findings such as discarded organs and abscesses. We performed linear mixed-effects models with PUFA level (high vs. low) as a fixed factor and farm as a random effect. Data from the on-farm animal health assessments showed that “PUFA high” farms had higher incidences of diarrhea, ear lesions, and short tails. Results from the individual fat analyses indicated a large variation in PUFA concentration within batches of carcasses from the same farm, suggesting a strong individual and age effect. Furthermore, there was a positive association between PUFA concentration in the grower feed and PUFA concentration in the carcass. These preliminary results suggest that farms with more health issues are more likely to have high PUFA concentrations in their carcasses and thus face penalties from slaughterhouses. Final results from all aspects of the study will be presented at the conference.

Grape stem inclusion in fattening young bull slightly modified the meat fatty acid profile

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The use of agro-industrial by-products in animal feeding is a sustainable strategy to reduce feed costs and environmental impact. Grape (*Vitis vinifera*) stems have high fibre content (35% NDF, 26% ADF) and low protein content (7% CP) but are rich in polyphenols and proanthocyanidins, which can influence lipid metabolism. This study evaluates the effect of different proportions of grape stem and straw in the concentrate on meat fatty acid (FA) profile of young bulls. Iso-energetic (1.04 CFU/kg DM) and iso-proteic (11.2% DM) concentrates were formulated with 0% stem:6% straw (0% stem), 3%stem:3%straw (3%stem) and 6%stem:0%straw (6%stem). Young bulls (162 d, live weight (BW): 173 kg) were fed ad libitum straw and one of the three concentrates during 17 weeks (the 4 heaviest from each concentrate) and 20 weeks (the remaining animals). The longissimus thoracis muscle was used to study the FA composition by gas chromatography. Sums of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n-3, n-6 and branched chain (BCFA) were calculated. The live weight at slaughter and the carcass weight were similar among concentrates (503 and 284 kg, respectively; $P>0.05$). The inclusion of stem decreased C18:1 c9 ($P<0.05$) and increased C18:2 n6 and C18:3 n3 ($P<0.001$) in the concentrates. In the meat, the total FA tended to be affected ($P=0.09$) by the inclusion of stem, being slightly greater in 0% than 3% stem (229 vs. 182 ± 14.7 mg FA/g DM, respectively). The inclusion of 6% stem in the concentrate tended to decrease the percentage of C16:0 ($P=0.08$) and increased the percentages of C18:2 n6, C20:2 n6, and consequently the total PUFA and PUFA n6 compared to 0% ($P<0.05$), while the 3% stem presented intermediate percentages. The percentage of conjugated linoleic acid c9t11 was higher in 0% than in 3% and 6% stem (0.23 vs. 0.20 %). The percentage of MUFA tended to be affected by the inclusion of stem ($P=0.08$), presenting 0% greater values than 3% stem. Total SFA, PUFA n3 and BCFA were not affected by the inclusion of stem in the concentrate. Therefore, the inclusion of up to 6% grape stem in diet of finishing young bulls could be advisable, as it slightly modifies the FA profile of the meat.

Session 26

Theatre 3

Seasonal off-flavours in milk on Swedish dairy farms

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Milk from Swedish dairy farms are sometimes classified to have a sweet odour and flavour reminiscent of fruits, the taint is often identified as blueberry taste. This milk taint "blueberry off-flavour" (BOF) have been an issue mainly during winter in northern Sweden. This study aimed to investigate the causes of BOF with three approaches: on farm investigations, analyses of milk and silage; a laboratory trial to mimic volatile organic compounds (VOC) combinations in milk associated with BOF; and a pilot feeding trial with a experimental round bailed silage (ES). The farm investigations revealed that the problem could have several causes, with uneven silage quality being one of the most significant. The laboratory tests identified that the off-flavours associated with BOF were linked to a combinations of VOC, specifically ethyl 2-methylbutanoate and ethyl caproate. The feeding trial involved 10 late-lactation dairy cows, starting with a covariate period on a control silage, followed by a 20-day period on experimental bailed silage (ES) and ending with a second period with the control silage. Milk samples were analysed for chemical composition, VOC, and sensory evaluation, while both silages were assessed for VOC, chemical composition, and hygiene parameters. Preliminary results showed large differences for sugar, ethanol and VOC concentrations between the control silage and ES. The levels of VOC in the ES varied considerably among individual bales. Feeding ES, altered the milk's concentration of VOC, with some samples exhibiting BOF, though other off-flavours, including feed, oxidation, sugarcane, and sour flavours. Observations suggested that feeding and milking time can affect VOC levels in the milk and the off-flavour perceptions of the sensory panellists. The VOC transfer from silage to milk was evident, however more studies are needed to explore the role of internal metabolism of VOC.

Feeding dairy cow with alternative forage may have different effects on cheese properties according to the cheese type

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Farmers in grassland areas, develop strategies to cope with the more frequent summer forage shortages. In particular, they may cultivate alternative forages more adapted to droughts than native grasslands, which raises questions in the PDO cheese sector. During a 4-week experiment, 3 groups of 14 dairy cows were fed 25% grazing with 75% of alternative forage indoor: (i) local permanent grassland hay (HA), (ii) freshly foraged cereal-legume mix (ME), or (iii) Teff grass hay (TG) while a control group only grazed on a permanent grassland (GR). At the end of the experiment, soft (SC) and uncooked pressed cheeses (UPC) were made from blended raw milk of the 4 groups. Sensorial cheese attributes and fatty acid (FA) composition of milks and cheeses were analysed. A significant decrease in milk production was observed for all groups throughout the experiment, but the ME cows better maintained their production, while HA and TG cows exhibited the greatest losses. The GR milks and cheeses had the lowest proportion of saturated FA and the highest proportion of polyunsaturated FA compared to the other groups. In line with the literature, GR cheeses were yellower than HA ones in both cheese types. Compared to HA ones, GR UPC were also softer, in line with their FA profiles. The ME UPC were very different from GR ones, except for yellow colour, less aromatic than GR and HA ones, but closer to the attributes of the dry forage cheeses (HA, TG). In contrast, GR SC were less sour, less bitter but chalkier and tended to be runnier than HA ones. The ME SC were similar to GR ones while TG ones were less yellow, melting, runny and sticky than all the others. Our study showed that alternative forages had a significant impact on milk production, milk and cheese FA profile and on cheese sensory attributes. Nevertheless, the effect of alternative forages differed according to the cheese type, which suggests that their use in the PDO cheese sector should be addressed locally.

Session 26

Theatre 5

Effects of Sulla Flexuosa Hay as Alternative Feed Resource on Goat's Milk Production and Quality

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Sulla flexuosa (*Hedysarum flexuosum* L.) is an endemic legume growing in some Mediterranean areas in rainfed and cold mountainous conditions. It could be used in goat diets as an alternative protein source instead of alfalfa to supplement forest rangeland. This study aimed to test the effects of incorporating *Sulla flexuosa* (SF) hay in the diet of Beni Arouss goats on their milk production and quality. The hay was introduced at two levels, i.e., 35 or 70% (SF70), on a DM basis; it partially or totally replaced the alfalfa hay of the control diet. *Sulla flexuosa* incorporation did not affect milk production or physicochemical composition. However, milk FA content varied in proportion to the percentage of SF incorporation. The SF70 diet was associated with increased milk levels in C18:1n-9, C18:2n-6, C18:3n-3, and C22:6n-3 and total monounsaturated, polyunsaturated, and n-3 fatty acids. As a consequence, atherogenic and thrombogenic indices were improved. Additionally, better antioxidant capacity was observed in SF70.

Impact of 3-nitrooxypropanol and whey on technological properties of milk and cheese in Holstein cows
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Feeding 3-nitrooxypropanol (3-NOP) is known to reduce methane emissions of cows, yet its impact on milk's technological properties remains largely unexplored. Meanwhile, whey inclusion in dairy diets is recognized for enhancing milk coagulation properties. This study aimed to investigate the effects of 3-NOP, with or without whey powder, on milk and cheese-making quality in Holstein cows. A 2×2 factorial design experiment was conducted with 24 cows, which received either a basal TMR (BAS) or whey-mixed (WHM; 15% DM), with either 3-NOP (80 mg/kg DM) or a placebo (PCB). The study lasted 15 weeks, with milk evaluations and cheesemaking conducted during weeks 9 and 13. Milk samples (AM and PM) were collected over 2 consecutive days, pooled daily for each treatment, thermized and transformed into Appenzeller-type cheese, yielding 16 cheeses. Milk pH was measured before rennet addition, while cheese pH was assessed at 2, 4, and 24 h. Cheese at 24 h was analyzed for yield, galactose, and lactic acid production. After 120 d of ripening, a trained sensory panel will conduct blind descriptive sensory analysis, alongside physicochemical analyses (e.g., elasticity, and fatty acids profile). Data were analyzed using mixed model in R, with 3-NOP, whey, their interactions, period, k-casein variant (AA, AB, AE, BE), and cow as a random effect. Results showed that 3-NOP had no impact on milk clotting properties (r , A30, k20) and fat, protein or urea content, but reduced lactose content (-3.3%, $P<0.01$). In contrast, whey increased milk lactose content (+3.8%, $P<0.01$). Notably, cheese from the BAS 3-NOP group had the highest lactic acid content at 24 h, while the BAS PCB group had the lowest, though cheese pH remained unaffected at all time points. Whey enhanced curd firmness (A30, +13.8%, $P=0.02$) but had no effect on coagulation time (r) or rate (k20). As expected, milk from cows carrying the AB k-casein genotype had the best clotting properties (r , A30, K20). This study provides new insights into the effects of 3-NOP and whey inclusion on milk and cheese properties.

Session 26

Theatre 7

Effect of replacing corn silage with alternative crops on cheese composition of Italian Simmental dairy cows
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The substitution of corn silage in dairy cow diets is crucial for the economic and sustainability of the livestock sector. However, research on the effects of using alternative crops as replacement for corn silage on cheese composition is scarce. The trial involved 15 Italian Simmental cows (days in milk: 145±80; parity: 3.2±1.7; milk yield: 24.7±4.7 kg/d) raised in Northern Italy and was conducted over 3 consecutive 21-day periods, with 12 days transition phases to gradually modify the diet. Three isonitrogenous and isoenergetic diets (14.5% CP, 38.7% NDF, 22.5% starch, DM basis) were used: a diet based on corn silage (P1), a diet where corn silage was replaced by sorghum-soy silage (P2), and by sorghum silage (P3). For each period, milk from 2 days during the last week of diet administration was used to produce 6 cheeses following the "Latteria" cheese procedure. Cheese yield after 60 days of ripening was unaffected by diet, averaging 7.03%. Dry matter (60.97% on average) and ash (3.72% on average) showed no differences between diets. Cheeses from P2 had the highest fat content (30.08%), but the lowest protein content (25.29%). Color and physical traits were significantly affected by diet. Cheeses from P2 and P3 showed higher luminosity (L^* : 80.65 and 81.66; $P<0.001$) and yellowness (b^* : 17.42 and 17.63; $P<0.001$). Compared to P1, P2 and P3 cheeses had higher values of hardness (37.69, 70.84 and 62.74 N; $P<0.001$) and cohesiveness (0.69, 0.76 and 0.76; $P<0.001$) and lower values of elasticity (0.99, 0.64, and 0.59 mm; $P<0.01$). Cheeses from P2 and P3, compared to P1, contained higher levels of MUFA (25.11, 24.75 vs. 21.97%; $P<0.001$) and lower levels of SFA (70.61, 70.94 vs. 73.77%; $P<0.001$). The n6:n3 ratio, an important health index, was low and similar (3:1 on average) across diets. To conclude, replacing corn silage with alternative crops increased cheese luminosity and yellowness, decreased protein content, but favorably enhanced fat content by increasing MUFA levels. These findings suggest that sorghum and sorghum-soy silages could serve as viable alternative crops in dairy cow diets, contributing to improved cheese quality.

Effect of plant-based additives in finisher diet on dietary quality and oxidative stability of pig meat

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The aim of the study was to determine the effect of a feed mixture containing 2% dried herbs or fruit with or without the addition of 3% linseed on the quality of pork meat in terms of dietary value and oxidation susceptibility. The study included 50 fattening pigs of the primitive Złotnicka Spotted breed. In the last phase of fattening, i.e. from 90 kg to 140 kg body weight, the animals were divided into 5 dietary groups: group A (control) and 4 experimental groups, the diets of which were supplemented with 2% dried herbs (H), 2% dried fruits (F), 2% dried herbs and 3% linseeds (H+), 2% dried fruits and 3% linseeds (F+). After slaughtering the animals, an assessment of the dietary quality of raw Longissimus lumborum and Semimembranosus muscles (LLM, SM) and raw matured Longissimus lumborum muscle (RMLLM) meat was performed based on the fatty acid profile. In addition, the lipid susceptibility to oxidation (TBARS) was determined in LLM and RMLLM meat. The use of H and F additives had a statistically significant effect on increasing the content of PUFAs (including C18:2 n-6, C20:4 n-6 and C22:6 n-3) and decreasing the content of MUFAs, primarily in SM raw meat. An increase in PUFA content (including C18:2 n-6 and C22:6 n-3) was observed in RMLLM from group F. The addition of linseeds to the mixtures significantly improved the dietary value of the pork. The SM, LLM and RMLLM meat from the H+ and F+ groups was characterized by a significantly increased content of mainly PUFA n-3 and lower PUFA n-6/n-3 and thrombogenicity index (TI) values. In addition, the use of the F+ additive had the beneficial effect of increasing the content of hypocholesterolemic acids (DFA) in the LLM meat of fattening pigs. At the same time, it was shown that RMLLM from the groups fed dried fruit (F and F+) had a statistically significantly increased susceptibility to lipid oxidation (TBARS). Research financed as part of statutory work of the National Research Institute of Animal Production, project no. 01-11-04-11

Session 26

Poster 9

The Effect of Camelina Cake in Chicken Feed on Muscle Fatty Acid Profile

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The trial aimed to assess the effect of adding Camelina cake to the chicken diet to improve the muscle fatty acid profile while maintaining growth performance. The trial was conducted with 5% and 10% Camelina cake inclusion levels in the diet. A total of 210 one-day-old, sex-unrelated chickens were assigned to three dietary treatment groups. The control group received a diet consisting of wheat and soy meal, while the treatment groups had part of the soy meal replaced with 5% Camelina cake (CAM5) or 10% Camelina cake (CAM10). The results indicated that Camelina cake positively affected the production of healthier breast and thigh meat. Specifically, the levels of various Omega-3 fatty acids, which are considered beneficial for human health, were significantly higher in the treatment groups compared to the control group. The greatest increase was observed in α -linolenic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids, which increased by as much as 1.7 to 2.67 times ($p \leq 0.05$) in both treated groups. Additionally, the total n-3 PUFA content increased by 2.36 – 3.36 times ($P \leq 0.05$), while the n-6/n-3 PUFA ratio, TI index, and total trans fatty acids decreased. The inclusion of 10% Camelina cake had a greater effect on n-3 PUFA content compared to the 5% inclusion level. Analysis of the chickens' growth showed that neither 5% nor 10% Camelina cake supplementation did not affect their growth performance. In summary, the inclusion of 10% Camelina cake in chicken feed can enhance the nutritional profile of the meat, particularly by increasing n-3 PUFA content, without negatively affecting the chickens' growth.

Age, feeding and breed determines bodyweight in male dual-purpose and layer chicken

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Dual-purpose chicken (DPC) breeds and rearing male layer chickens (MLC) are of interest in poultry production to address consumer and political demands for higher animal welfare and organic products. Unfortunately, information regarding the optimal rearing conditions, particularly in terms of feeding regimes, is scarce. Previous research has indicated that DPC and MLC maintain a uniform performance level, even when diets are not balanced, compared to high-performing breeds, which may be more sensitive to dietary imbalance. We aimed to investigate how DPC and MLC breeds perform under organic rearing conditions and how their organ weights, performance, meat quality, and behavior are affected. A total of 320 male chickens (160 ÖTZ Coffee (DPC); 160 ISA Warren (MLC)) were raised under organic conditions (EU Reg. 2018/848). At 9 weeks of age, the chickens were randomly allocated to either the control group fed standard diets (19.4% CP, 0.9% lysine, 0.33% methionine) or the trial group receiving nutrient reduced diets (16.2% CP, 0.7% lysine, 0.31% methionine; 40 animals/breed/group, 2 replicates/group). Bodyweight (BW) and feed intake were recorded weekly. Chickens were monitored on a weekly basis. Week 12-16, 6 animals/trial were sacrificed and meat quality was investigated. No differences were observed regarding BW and bodyweight gain (BWG) between chicken from the same breed and different feeding groups ($P>0.05$), but DPC showed a significantly higher BW and BWG compared to MLC ($P<0.001$). Breed related differences were observed for the weight of breast fillet in relation to the BW ($P<0.001$) but not for feed ($P>0.05$). MLC control groups had higher ratio of appendix/BW ($P<0.001$). The MLC and trial groups used the pasture more frequently ($P<0.001$). No differences were observed regarding bone deformations. DPC had lower cooking juice losses ($P<0.001$).

Session 27

Theatre 1

From Big Game to Animal Agriculture: How Humans Evolved to Need Animal Food

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Around 2.6 million years ago, *Homo habilis*, began hunting in the opening savannas of Africa. Their successor, *Homo erectus*, increasingly targeted large animals like elephants, likely due to their higher fat content. Because human metabolism can derive no more than ~35% of daily caloric intake from protein, hunters had to obtain some 65% of their energy from either plant carbohydrates or animal fat. Archaeological and physiological evidence suggests that whenever possible, humans selected fat-rich animals to meet these energy demands. Over the next two million years, large prey populations declined, which forced humans to adapt to hunting progressively smaller animals. As prey became smaller and more fat-depleted, humans faced a dietary challenge that ultimately led to the need to raise animals for meat and fat and cultivate plants for carbohydrates—laying the foundation for agriculture. However, human physiology retains adaptations favoring meat and fat consumption, highlighting the enduring importance of animal-sourced foods in human diets.

The Story of the Story of Domestication

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The majority of the 8 billion people on Earth are dependent in some way, for sustenance, or labour, or companionship, upon the ~30 billion chickens, the nearly 1 billion cattle, the 1 billion dogs, the 700 million pigs, and hundreds of millions of other domestic animals, and the 2.5 billion metric tons of domestic grain. The domestication process that has led to this reliance has also allowed the 4 million or so individuals who lived on earth 10,000 years ago to become more than 8 billion today. This story of the rapid increase in human population size and the commensurate technological development has typically been told from a positivist perspective. Our ancestors were smart people who foresaw how great the world would be so they domesticated plants and animals. This position has led to the development of narratives to support this assumption, often without any empirical evidence to underpin the stories. Here, I unpick the classic origin stories of domestic animals including cats, rabbits, chickens, and others to reveal our tendency to invent stories that flatter our ideas of what the origins of the modern world should be.

Session 27

Theatre 3

Agricultural revolutions: How animals paved the way for modern civilization

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Most scholars and public still think of the domestication of livestock as an event that happened rapidly at the beginning of the Neolithic (c. 8000 BCE) despite the wealth of zooarchaeological data to the contrary. Yes, there were revolutions, but they were not sudden nor were the new breeds immediately adopted by many societies. They were revolutions only in the sense that lifestyles were completely transformed. But these changes took time and are the cumulation of many small incremental changes in livestock exploitation strategies. In this paper, I will outline some of the changes that transformed domestic livestock (sheep, goat, cattle, donkeys, pigs, chickens and others) exploitation over time based on zooarchaeological studies. These changes helped create the basis for productive intensity in livestock exploitation that underline and support the evolution of complex societies around the world, which rely upon the dramatic increase in surplus food production provided by a small part of society.

A genomic history of our pets and livestock: from ancient origins to modern breeding

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The long-term co-evolution of humans and domestic animals, spanning over 10,000 years, has profoundly altered their genomes. While domestication unfolded gradually, the 19th and 20th-century industrial and green revolutions accelerated selective breeding, potentially causing significant genomic shifts. However, reconstructing pre-revolution evolutionary dynamics and quantifying the impact of recent intentional breeding on the genome has been challenging. Modern palaeogenomics now allows for ancient genome sequencing, offering the possibility to analyse powerful genomic time-series. This presentation will showcase recent large-scale studies that leverage palaeogenomics to directly trace livestock and pet genomic evolution, and address fundamental questions about their biology and evolutionary history.

Session 28

Theatre 1

Farm level incentive-driven genetic decisions are key to addressing value chain demand for beef and dairy product sustainability attributes

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Beef and dairy production systems have made dramatic productivity gains over the last century and, as a result, produce more meat and milk per animal than at any point in human history. Genetic improvement has played a key role in this progress and, due to the cumulative and permanent nature of its effect, remains a critical intervention to support more productive and sustainable food systems. An increasing focus on product sustainability attributes, particularly those related to environmental impact, is signaling the need for credible farm-level solutions that can be scaled up to address entire supply chains. Selective beef and dairy breeding programs can be designed to meet the needs of value chain stakeholders by targeting improvements in efficiency, product quality and overall system profitability, while, at the same time, addressing animal health, wellbeing and environmental footprint. Though genetic gain is the most visible metric used to determine the success of breeding program execution, and is an important driver of ongoing performance improvement, optimization of mating decisions, herd structure and dissemination strategies are all critical factors that must be combined with genetic improvement to ensure maximum impact at the commercial level. Minimal integration in many beef and dairy supply chains has led to limited investment in genetic approaches that do not provide obvious near-term farmer benefits, even if the net benefit for all stakeholders is positive. Recent value chain commitments to reducing the environmental impact of beef and dairy production provide opportunities to incentivize farm-level decision makers to access and implement bespoke genetics and genetic strategies that improve long term on-farm profitability and credibly reduce environmental impact per unit of product produced. Once the impact of genetics is quantified and verified, genetic interventions can be quickly scaled up to meet the sustainable sourcing requirements of a beef or dairy supply shed.

Long-term use of virtual fencing - how animals' interactions with the technology evolves over time

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Virtual fencing is an emerging technology the interest of which is spreading across Europe. Because of its recent uptake there is still limited research on the long-term impact of virtual fencing on animal behaviour. This paper – which will use Nofence (founded in Norway in 2011 and first commercially available virtual fencing company) as a case study – has the objective of filling this gap identified in the existing literature. There are conclusive findings from research institutions in Europe about the reduced impact of the technology on animal stress levels and on positive data with regards to comparing the effects of virtual fencing with those of physical electric fences. Sweden is one of the countries in Europe where virtual fencing is not yet authorised and Swedish state-owned research institute RISE has been conducting research on virtual fencing including Nofence collars since 2022 to provide data to inform local decision-making. There are also studies looking into the training period, the adaptability of animals to learn how to use the system. These investigate the stress level of animals over this phase. Few studies investigate how animals remember and settle back into the virtual fence during the next grazing sessions after spending the winter in the barn. Through its system, Nofence has collected over 700 million hours of grazing data. RISE decided to partner with Nofence for this research to investigate a larger dataset – based on anonymised data from customers in EU countries where Nofence is commercially available namely Spain and Ireland. This paper will analyse data from customers with over multiple years of use to analyse trends in animal behaviour, which will provide insights into virtual fencing's impact on animals use of the system over a long period.

Effect of housing system on selected functional traits in dairy farms

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In Austria, dairy cows are mainly kept in loose housings, where they can move freely throughout the year. However, particularly in mountainous areas where farming conditions are challenging, the so-called combined housing, i.e. tie-stalls in winter and grazing during summer, is still widespread. This work aimed to determine how different systems, combined housing and loose housing with and without outdoor access affect selected functional traits. The database consisted of 6,969 annual reports from the years 2019 to 2021 from 2,345 dairy farms. Statistical analysis was performed using linear or logistic mixed models including the random effect of farm and the fixed effects of herd size and herd lactation yield. For fertility traits, farms with loose housing were superior to those with combined housing. Calving interval, days from first to successful insemination, and fertility related veterinary diagnoses were significantly lower in both loose housing systems, while non-return-rate after 90 days was significantly higher in case of outdoor access only. Combined housing performed better in terms of claw and leg health (veterinary diagnoses) and the proportion of cows with elevated fat to protein ratio. However, the latter results may also be due to different feeding practices. For udder health, the effect of the housing system was limited; loose housing systems performed best for first lactating cows and the traits somatic cell count and the proportion of cows with an elevated somatic cell count, respectively. However, the loose housing system with outdoor access did not differ significantly from that without. The comparison of functional traits based on the annual reports shows that no housing system is clearly superior to the other in terms of the functional traits analysed. However, there is a tendency for loose housing, particularly with outdoor access, to be beneficial. In addition to housing systems, many other factors influence functional traits and it should be noted that not all aspects of animal welfare and well-being could be considered in this study. Funding by the COMET-Project D4Dairy, handled by the FFG is gratefully acknowledged.

Genomic innovation and herd management for sustainable dairy farming: A case study of Littleton Farm
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Industry is advancing sustainable agriculture by integrating innovative practices at the farm level. Here we examine Littleton Farm, a 1,130-ha dairy operation in Dumfries and Galloway, Scotland. The farm sustains a herd of 1,750 dairy cows, producing 16.5 million L of milk annually with a C footprint of 1.047 kg CO₂e/L, below the industry average of 1.18 kg CO₂e. Through privately driven strategies, Littleton Farm has achieved notable sustainability outcomes, offering a replicable model for dairy systems. Since 2020, Littleton Farm has partnered with Promar International to embed genomic testing and genetic indices (EnviroCow and Profitable Lifetime Index) into herd management. With a 25% annual replacement rate, the farm produces approximately 437 replacement heifers each year from the top 25% of genetically ranked cows. The remaining 75% are bred with Wagyu sires, yielding premium, low-emission crossbred calves sold to local beef farms. This approach accelerates genetic progress, enhances feed and N use efficiency, and supports the regional economy by creating jobs. Over 3 y, these efforts have reduced annual emissions by 652 t CO₂e from a 2020 baseline, with potential to inset 1,486 t CO₂e yearly as efficiency advances. Sustainability efforts also encompass nutrient management. Manure from high-genetic-merit cows contains 24.4% less N and 15.3% less phosphate, reducing eutrophication risks and improving soil health when applied to fields, thus protecting local water quality. Approximately 90% of cull cows are processed through Pickstock's abattoir, ensuring traceability within the system. Littleton Farm's achievements demonstrate the impact of private innovation and industry collaboration. By utilising genomic tools, diversifying outputs with premium calves, and optimising nutrient cycles, the farm maintains productivity while reducing its environmental footprint. This case study highlights how targeted, self-directed strategies can decarbonise dairy operations and enhance resource efficiency, offering practical insights for farmers and industry stakeholders. Littleton Farm illustrates the potential of farm-level innovation to deliver measurable environmental benefits.

Session 28

Theatre 5

The importance of accurate phenotypes to achieve sustainability goals

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Genetic selection is a powerful tool for achieving livestock sustainability goals because genetic progress is cumulative and permanent. With the implementation of genomic selection (GS) based on genome-wide DNA testing, the rate of genetic gain for important traits has increased substantially. For example, in U.S. dairy cattle, GS has doubled the rate of progress for traits related to production, longevity, reproduction, and health. Genomic selection has also enabled the inclusion of new and economically important traits in national genetic evaluations providing an opportunity to accelerate sustainable dairy and beef production through the reduction of methane emissions in cattle. However, successful genetic progress requires accurate phenotypes. Beyond accurate phenotyping, it is important to use technology that promotes automatic collection of large amounts of data from individual animals without interfering with natural animal behavior and feeding patterns. The GreenFeed system (C-Lock Inc., USA) has been used globally to measure methane emissions from individual animals to construct reference populations for genetic selection programs and enable emissions prediction in young animals based on genotype data. Studies have reported that methane emission traits—such as methane production, methane intensity, and methane yield—are moderately to highly heritable, indicating that a significant proportion of phenotypic variance is explained by additive genetic effects, making methane emissions suitable for genetic selection programs. Additionally, methane emission traits have been shown to be favorably correlated with feed efficiency (residual feed intake), which can accelerate progress toward sustainability, as selecting for low methane emissions will favor more feed-efficient animals without negatively impacting production. Industry-wide research efforts are providing this affordable and powerful tool to farms for breeding lower methane-emission herds. Ongoing collaborative research between academia, industry, and funding institutes are working to expand the availability of GreenFeed to further accelerate genetic progress.

Sustainable Dairy Production Through Effective Calf Rearing Systems

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A major challenge in dairy farming is ensuring calves develop into resilient, high-yield dairy cows that remain productive for multiple lactations. A case of Bovine Respiratory Disease (BRD) in a calf may cost just €100 to treat, but the long-term impact is far greater. Heifers with BRD face delayed first calving, reduced first-lactation milk yield of around 121.2kg, and a lifetime production loss exceeding 2,000 kg. They are also more likely to be culled before their second lactation. Considering fertility challenges, lower productivity, and early culling, the lifetime economic loss can reach €1,000 per animal. Calf-rearing systems are often housed in repurposed or outdated buildings that fail to meet modern welfare and management standards. Inadequate ventilation, poor drainage, and suboptimal hygiene contribute to higher incidences of respiratory disease, slower growth rates, and increased labour demands. Research has shown that poor environmental conditions in early life can negatively impact immune function, feed efficiency, and long-term productivity. The Cost-Effective Ventilated Environment for Calves (CEVEC) project was developed to design, construct, and evaluate a low-cost, automated calf-rearing facility with real-time environmental monitoring. The system aimed to improve calf health, welfare, and growth performance while significantly reducing labour input. While group housing with auto-feeders offers nutritional and social benefits, it also heightens the risk of disease transmission. Therefore, a holistic, evidence-based approach was used to develop a purpose-built facility that optimises ventilation, hygiene, drainage, lighting, and social interaction, ensuring both high welfare standards and operational efficiency. A full-scale trial in Scotland compared this system to traditional hutches, demonstrating enhanced calf performance and farm profitability. Calves were lung scanned before and after rearing in both systems, to determine improved respiratory health. Labour demands were reduced by 60% per calf, and calves reared with the automated feeder showed improved daily live weight gains. The project has resulted in a modular calf-rearing building, adaptable to herd size and designed for standardised, repeatable, and efficient management on any farm.

Session 28

Theatre 7

Biochar for sustainable livestock systems

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Biochar is an invaluable tool for improving sustainability at the farm-level. As demand for cleaner and more regenerative livestock production grows, biochar is situated well to provide versatile solutions to tough problems, capable of improving soil health and feed efficiency while reducing greenhouse gas emissions, directly sequestering carbon, and contributing to farm-led regenerative strategies. We will explore the use of biochar in livestock systems with a brief review of recent literature, an overview of key case studies, and a brief look at the current and emerging options for green finance of biochar projects. The prospect of immediate carbon removal coupled with enhanced soil sequestration and reduced emissions over time presents high carbon offset potential from well-integrated biochar-livestock systems. Further benefits to the farm system include enhanced animal productivity, reduced disease risk, and significantly increased nutrient efficiency & retention. The central aim for this segment will be to showcase the significant potential for biochar to introduce sustainable solutions to problems faced by livestock farms, from environmental impact, to animal welfare, to economic viability.

The role of calcareous algae on rumen microbiota homeostasis and its effect on greenhouse gas emissions
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Calcareous algae precipitate carbonates up to 80% in their body weight through a biomineralization or biocalcification process. They accumulate biominerals mainly into three forms of crystals, namely, calcite (CaMgCO₃), aragonite (CaCO₃), and dolomite (CaMg(CO₃)). Lithothamnium calcareum is a calcareous alga that belongs to the group of red or rhodophyte algae of the Corallineaceae family. In addition, it is a renewable source of macro and micro minerals. These algae are abundant and widespread in coastal areas throughout the world and their abundance, size, shape, and species composition varies considerably depending on their growth location. Modern ruminant production systems have led to the increased use of highly fermentative carbohydrates in their diets and cows are often found to have a low rumen pH. Therefore, diet formulation needs to be done carefully to achieve optimal rumen fermentation, aiming to aid in the neutralization of volatile fatty acids produced in the rumen. If ruminal acids are not adequately monitored and buffered, it might reduce the productivity and possibly harm the health of the animal. Recently, a series of trials have been co-developed with farmers to test Lithothamnium sp. in its capacity to support rumen health. The addition of this mineral source to the diet of dairy cows has shown its capacity to neutralize the acids created by the ingestion of carbohydrate in the rumen, shifting the volatile fatty acid concentrations in rumen liquor, maintaining an adequately balanced ruminal bacteria population, optimizing fibre digestion, nitrogen utilization, and overall feed conversion as result of optimal buffer supplementation. As consequence of a better rumen homeostasis, the farmers who instigated the trials have been benefited with improved milk production and its components, better pasture utilization, reduced water pollution due to improved nitrogen utilization, and animal welfare throughout a significant decrease of subacute ruminal acidosis. This has also positively impacted the environment by reducing the contributing to greenhouse gas emissions, primarily through changes in rumen microbial populations leading to altered methane production.

NEU.rind – A User-Friendly Tool for Sustainability Assessment on Austrian Dairy Farms

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NEU.rind is a user-friendly yet scientific tool for assessing sustainability on dairy farms. It integrates existing farm data from multiple sources (e.g., Central Cattle Database, IACS data) and applies advanced methodologies to evaluate key indicators on animal health, global warming, air quality, fossil energy use, protein efficiency, biodiversity, and economic viability. The assessment provides results per kg milk, per hectare, or per cow and year. The tool was initially designed to provide farmers with guidance for improving farm management. It asks for a wide range of data, including feeding (energy requirements), housing systems, manure management, fertilizers, infrastructure (buildings, feed/slurry storage), farm machinery, and energy use. Minimal manual data entry is required due to integration of already collected data. Collection typically takes 1 to 2 hours per farm. After pilot testing on 200 Austrian dairy farms, NEU.rind is now being implemented by Austrian dairies to support sustainable production management and comply with the Corporate Sustainability Reporting Directive (CSRD). This can give the high sustainability of Austrian dairy production an additional boost, making it even more of a global pioneer in 'green production'. The tool enables systematic, data-driven, and transparent sustainability assessments for individual farms and farm groups. Farmers receive a comprehensive overview of their strengths and weaknesses and can benchmark their performance against other farms. Where weaknesses appear in both product- and land-based assessments, targeted recommendations are provided. NEU.rind is designed (programmed) to be flexible and expandable. Results indicate distinct farm clusters with specific sustainability profiles, with individual strengths and weaknesses. This confirms the need for individualized farm advisory, as farms differ in site conditions and structural constraints.

Incentives to reach net-zero: Evidence from UK dairy farmers

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The introduction of climate-smart technologies is crucial to achieve net-zero in the UK dairy sector. Along with key stakeholders from the dairy supply chain we identified nine promising climate-smart innovations and management practices to help farmers to reduce their carbon footprint and improve their systems sustainability. We carried out an online survey with UK dairy farmers (n=184) on the adoption and constraints to adopt these practices. We document a low adoption of emission-based practices, such as, technologies to capture CH₄ and N (5% of farmers) or to reduce emissions from slurry and manure (24% of farmers); and the usage of anti-metanogenic feed supplements (8% of farmers). We document a high number of farmers that have adopted nature-based practices (herbal leys, cover crops and silvopasture) to boost soil health and ecosystem services (66%); technology-based practices, including sexed semen and genomic selection (90%) and replacement of soy in the animals' diet (62%). For those farmers who did not adopt a certain practice, we evaluated the their preferred option of three financial reward schemes: 1) a milk Price Incentive (PI), 2) a Grant Scheme (GS), and 3) an Income Reward Scheme (IRS). For more than 90% of respondents, the financial support in the form of PI is deemed essential to increase the uptake of anti-metanogenic supplements, but GS was preferred for increasing adoption of technologies to capture CH₄ and N from slurry or manure. However, cost-based mechanisms, such as GS, or IRS, are preferred for To enhance the adoption of more sustainable practices or technology, targeted support mechanisms are needed. Tailored financial incentives aim to lower adoption barriers, making it feasible for farmers to implement innovative practices and contribute to achieving net-zero emissions.

Session 28

Poster 11

An innovative approach to create the optimum dairy herd housing

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Sustainability in dairy farming depends on optimising animal welfare, enhancing productivity, and reducing manual labour. Galebreaker has leveraged funded research to implement innovative environmental solutions for the dairy cow and their resilience to heat stress and climate change. The Animal Centred Controlled Environment for Dairy (ACCED) project developed a proof of concept using artificial intelligence driven motion sensors in ear tags to detect early heat stress in cows, triggering an adaptive ventilation system. This mitigates heat stress effects, improving feed intake, milk yield, and overall herd health. The first phase of the project developed machine learning models to assess cow behaviour (e.g. lying, standing, feeding, ruminating) via the ear tags. Combined with milk yield data and recorded temperature/humidity, these analyses evaluated heat stress impact. High-yielding cows in peak lactation showed the greatest declines in milk yield (>20%), lying (>79%), and feeding (>90%), requiring 20 days to recover and losing an average of 50 litres of milk each. The second phase implemented a full-scale proof of concept, where cow behaviour directly controls natural ventilation side curtains and positive pressure cooling tubes, allowing cows to regulate their own cooling and airflow. Further research as part of a second project explores the feasibility of a digital modelling tool to assess livestock buildings' thermal and ventilation performance. This tool will evaluate how external environments impact animal welfare and test building resilience to climate change. By simulating building modifications and ventilation strategies, it enables cost-effective, optimized solutions. Using a digital twin approach ensures efficient, timely implementation, further enhancing farm sustainability. By integrating artificial intelligence, digital modelling, sensor technology, and automation of innovative ventilation devices, these projects have shown how optimising the environment for housed livestock, whilst reduce labour dependency can enhance sustainability, animal welfare, and farm efficiency. This holistic approach optimises resource use, minimizes environmental impact, and supports the long-term viability of dairy farming.

Machine learning applied to milk FT-IR spectra reveals regional fingerprints

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Fourier Transform Infrared (FTIR) spectroscopy offers a rapid, easy, non-destructive and cost-effective tool in the dairy industry to determine milk composition and to predict various milk and cheese characteristics. Moreover, FTIR is routinely used in food industry for food fraud detection. Using data provided by the Italian Livestock Environment Open Data (LEO) for 10k samples, we explored the potential of FTIR to provide additional insight into characteristics and origins of milk. We demonstrated that the unique molecular fingerprints of different regional milks can be identified using machine learning. A neural network, trained on 70k samples and tested on 30k, reached 98% precision and recall in the test set in identifying milk from the ten largest Italian milk-producing regions. The effectiveness of the algorithm was mainly driven by the two main milk-producing areas, Lombardy and Piedmont, although other regions such as Emilia-Romagna and Veneto also produced milk with distinctive spectra, suggesting regional markers within the FTIR spectra that correspond to unique environmental or dietary factors influencing milk composition. The region where the algorithm performed worst was Liguria, where milk spectra were highly similar to those of Piedmont, explained by the geographic proximity and overlap in farming practices. This research highlights the utility of artificial intelligence in saving costs associated with milk and cheese production by eliminating the need for multiple tests when an existing easily sampled biofluid can offer much more information than assays currently used.

Session 29

Theatre 2

Prediction of mastitis via mid infrared analysis of milk: Validation through experimental approach

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Mastitis infections in dairy cows result in significant economic losses due to costs associated with reduced milk production, milk disposal, veterinary treatments, increased labor, and premature culling animals. Furthermore, mastitis can alter milk quality by increasing the somatic cell count (SCC) and altering other milk components. Mastitis is detected through SCC, usually analyzed by flow cytometry. But a potential alternative using mid infrared (MIR) spectroscopy have been studied through different projects as HappyMoo or MastiMir. The advantages of using MIR analysis could be cost-efficiency, faster and more versatile analysis. On the other hand, SCC alone is not always a perfect indicator of mastitis, given that there are cases where mastitis may not immediately lead to a notable increase in SCC, especially in the early stages of infection when SCC remain high over long period without infection. It is consequently interesting to investigate complementary measurement methods besides SCC counting. This alternative via spectral standardization could be used on benchtop apparatus, but potentially also on miniaturized MIR spectrometers. The objective of this work will be to evaluate the potential of MIR to detect mastitis by inducing mastitis in an experimental context. The experiment used four different concentrations of *S. aureus* bacteria, each concentration across five distinct cows. Daily analyses during an eight-day period have been conducted with MIR spectroscopy and SCC analysis on each udder alongside the measurement of bacterial concentration on the infected udder. MIR analysis indicate a significant distinction between infected and non-infected udders, through the prediction of lactose, NAGase, sodium and lactate dehydrogenase.

Enhancing and combining MIR models to provide individual cow environmental score

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Holicow is an Interreg NWE project focused on developing a holistic herd management tool for farmers, leveraging more than 60 million milk recordings and over 500 existing predictive MIR models. In the frame of this project, our work aimed to assess the performance of individual dairy cows according to environmental aspects, with the final objective to provide an environmental score for each cow. One of the primary challenges was the identification and the valorisation of existing data and models providing insights on environmental impact of dairy cows. A total of 20 relevant MIR indicators were identified, and corresponding models were run on the MIR database. Several new indicators were also created based on the existing models. Those 20 indicators were merged into five dimensions: methane emissions, nitrogen efficiency, energetic efficiency, longevity, and grazing. These indicators are combinations of carefully selected pre-existing models that have been trained on cleaned data. The days in milk at milk recording time was found to significantly influence the indicators, prompting the adoption of a longitudinal approach to account for the days in milk artefact. To deliver synthesized information to farmers, two methods were developed: a spider graph for visualizing the dimensions and an agglomerative hierarchical clustering approach for refining these inputs. Those tools will enable the comparison of cows within herds, of farms within regions. Together with methods for assessing milk production, milk processing, cow welfare, fertility, and heat stress, this method constitutes a comprehensive, collaborative tool accessible to farmers and advisors.

Session 29

Theatre 4

Using MIR Data to Predict Individual Global Scores for Walloon Holstein cows

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Mid-infrared (MIR) spectroscopy has been widely applied in the dairy industry for milk quality analysis. Extending its application to health and welfare monitoring could provide significant benefits. The aim of this study was to explore the potential of using MIR data to predict the health and welfare conditions of dairy cows. Two individual welfare scores (SGI-I and SGI-II) were defined based on the Welfare Quality© Protocol. SGI-I was based on feeding, housing, and health, while SGI-II included behavioral data in addition to feeding, housing, and health. The dataset consisted of 3,291 records on 2,381 animals for SGI-I, and 1,473 records on 1,229 animals for SGI-II. The mean (SD) SGI-I and SGI-II were 69.92 (10.28) and 65.90 (9.65), respectively. 75% of the records for each SGI were used for training, with the remaining 25% used for testing the models. Data from 212 MIR spectra, along with parity, milk yield (MY), fat/protein percentage (FP, PP), somatic cell score (SCS), and days in milk (DIM) were used to optimize the prediction models. The root mean square error (RMSE) and mean absolute error (MAE) for the developed models were 9.21 and 7.25 for SGI-I, and 8.51 and 6.78 for SGI-II, respectively. The developed models were used to predict SGIs for a large number of animals for DIM when MIR data were available: The number of predicted SGIs records (animals) for the 1st, 2nd, and 3rd lactations were 169,708 (30,202), 157,939 (23,107), and 115,671 (16,126), respectively. The mean heritability for direct SGI-I and SGI-II were 0.22 and 0.27, respectively, while for MIR-based predicted SGI-I and SGI-II were 0.22 and 0.24, respectively. The mean genetic correlation between direct SGI-I and SGI-II was 0.88, and between MIR-based predicted SGI-I and SGI-II was 0.78. Both direct and MIR-based predicted SGIs were negatively correlated with MY and SCS and positively correlated with FP and PP, indicating that higher MY and SCS are associated with lower health/welfare conditions.

Estimating methane emissions in Angler cattle using existing prediction equations based on milk fatty acid composition

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Several models have been developed to predict methane emission from mid-infrared spectra of milk in dairy cattle and seem promising to estimate individual methane emissions at a large scale. However, most research has been done in Holstein cattle, whereas there is a lack of studies for small local breeds like Angler. Thus, we aimed to analyse the transferability of the prediction equations obtained in the Holstein breed. For this purpose, monthly milk samples from approximately 2,700 cows were taken repeatedly over a period of more than one year at commercial farms keeping either Angler cattle or Angler and Holstein cattle. Milk components and milk fatty acid composition were quantified using mid-infrared spectroscopy in order to estimate individual methane emissions (g/day) using two existing prediction models. Linear mixed models were applied to analyse how fixed effects (e.g. farm or lactation number) influence estimated methane emissions per breed and equation. Animal was included as a random effect and we found moderate correlations between predicted animal effects obtained with this procedure for both breeds, Angler and Holstein cattle. In addition, animals were assigned to ten different emission groups (very low to very high) and we tested whether grouping based on the two different prediction equations resulted in similar rankings. The best agreement was found for the extreme groups indicating that the prediction equations can help to identify very low emitting and very high emitting cows. A comparison between measured and estimated individual methane emissions is crucial and additional recording of real data is currently implemented. For this purpose, infrared gas analysers are installed at one of the farms with robotic milking and both, Angler and Holstein cattle.

Session 29

Theatre 6

Prediction of methane using milk mid-infrared spectra

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In the project breed4green the genetic potential for reducing methane emissions in Austrian Fleckvieh and Brown Swiss cattle is being explored. Data collection, which includes measurement of methane using the GreenFeed system on commercial farms, is ongoing and will continue until 2027. In this study, we aimed to develop a prediction model for methane based on milk mid-infrared spectra. Currently, methane measurements of dairy cows from 9 commercial farms are available, with 6 farms having data from two distinct time periods. The analysis included milk mid-infrared spectra from cows that had at least 3 GreenFeed methane measurements taken within the same week. The final dataset included 1,574 milk mid-infrared spectral records from 337 cows with additional information on parity, days in milk, milk yield and body weight. A partial least squares regression model was applied using 5-fold cross-validation ensuring that repeated records from the same cow remained within either the calibration or validation data set. The results showed that including milk yield and body weight alongside milk mid-infrared spectra significantly improved model performance. In the calibration data set, the model that included milk mid-infrared spectra, parity, days in milk, milk yield and body weight achieved the highest R^2 value (0.47). The corresponding R^2 value for the validation data set was 0.34. The first results look promising. Future research will focus on the inclusion of additional information (e.g. feeding information) to improve the accuracy of the model.

Prediction equations for individual methane emissions in Swiss cows using milk mid-infrared spectra
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Prediction equations for methane (CH₄) emissions using individual cow's milk mid-infrared (MIR) spectra have been developed in some countries, offering a cost-effective solution to predict individual cow's CH₄ emissions. Currently, Switzerland does not have its own MIR equation. Therefore, the current study aims to develop prediction equations for CH₄ emissions using milk MIR spectra of Holstein Friesian cows raised in Switzerland. The GreenFeed system was used to measure CH₄ emissions from 1 research and 3 commercial farms; MIR spectra were available from the routine milk recording collection or from Agroscope experiments. CH₄ emissions were averaged over at least 10 GreenFeed measurements collected during 7 days around the date of the milk sampling. The final dataset included 1,175 records from 179 cows. The mean, standard deviation, minimum, and maximum for daily CH₄ emissions (g/day) were 486, 65.8, 250, and 713, respectively. The partial least squares regression prediction algorithm was used to develop the prediction model using the milk MIR spectra and the milk yield as prediction variables. In 4-fold independent-cow cross validation, R₂ ranged from 0.37 to 0.50, the slope from 0.79 to 1.04, and the ratio of performance to interquartile range (RPIQ) from 1.58 to 2.06. During the second phase of the analyses, recordings of 3 farms were included in the reference to develop the prediction model, while the data from 1 randomly selected commercial farm was used to validate the predictions. This evaluation procedure has been undertaken twice, using each time a different farm to validate the predictions. These 2 farms had 28 and 19 records, respectively. For this validation scenario, the R₂ was 0.24 and 0.29, the slope was 0.92 and 0.82, and the RPIQ was 1.27 for both farms. Even if prediction results in external validation were moderate to poor, more data are planned to be recorded with the aim to improve the model's prediction accuracy. Nonetheless, high and low emitting cows were correctly identified both in cross-validation and in external validation.

Session 29

Theatre 8

Development and validation of predictive models for early-lactation energy status of dairy cows based on milk mid-infrared estimated fatty acids and spectra

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The extent of negative energy balance (NEB) in post-partum dairy cows is reflected by the concentration of plasma non-esterified fatty acids (NEFA), where greater levels are associated with increased risks of metabolic and reproductive disorders. The objective of this study was to evaluate the predictive ability of milk mid-infrared estimated fatty acids (MIRFA) and spectra (MIRS) for elevated NEFA. Preprandial blood samples were collected from the coccygeal vessel of 297 Holsteins ($4 < \text{DIM} \leq 50$) across 24 commercial Canadian dairy farms (2-31 (min-max) cows per farm) visited in the morning of the official milk recording day. Plasma NEFA concentrations (mM) were determined by enzymatic method and were used to classify cows with elevated (NEFA ≥ 0.6 mM, $n = 72$) or normal (NEFA < 0.6 mM, $n = 225$) lipomobilization. Animal parameters (DIM and parity), MIRFA, and MIRS were subsequently associated with the corresponding classification based on plasma NEFA concentration. Partial least squares discriminant models were trained using nested cross-validation (CV) composed of an inner 10-fold CV for hyperparameter tuning and an outer 5-fold CV for performance evaluation. In both inner and outer CV, different farms were used to create train and test folds. Balanced accuracy (BAC) was used to assess predictive ability. The fixed effect of predictor type (MIRFA, MIRS, or both MIRFA+MIRS), class imbalance correction (down-sampling, up-sampling, or synthetic minority over-sampling (SMOTE)), inclusion of animal parameters and their potential interactions on BAC was evaluated by a mixed model where the left-out fold was considered as a random effect. There was no interaction between fixed effects. Using MIRS alone as predictors ($0.75_b \pm 0.008$, LSM $\pm$ SEM) resulted in lower ($P = 0.03$) BAC than MIRFA (0.77_a) and MIRFA+MIRS (0.77_a). Down-sampling ($0.78_a \pm 0.009$), up-sampling (0.79_a) and SMOTE (0.79_a) resulted in higher BAC ($P < 0.01$) than using no imbalance correction (0.69_b). Inclusion of animal parameters did not increase BAC ($P = 0.14$). These findings highlight the potential of milk MIR spectroscopy analysis as a non-invasive tool for monitoring energy status of dairy cows.

Mid-infrared predicted traits for energy balance and their genetic parameters in dairy cows

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Negative energy balances (EB) impair health and performance of dairy cows. EB is therefore a valuable trait for herd management and genetic evaluation systems to improve health of dairy cows. As EB cannot be recorded on farms, milk mid-infrared (MIR) spectra are used to predict phenotypes for EB and related traits as an alternative. The present study aims to predict EB, energy intake (EI) and dry matter intake (DMI) from milk MIR spectra and estimate corresponding genetic parameters in dairy cows. Prediction equations were developed by applying partial least squares regression analysis, utilizing 212 selected MIR spectra, milk yield and parity as predictors. The calibration dataset comprised 67,400 records collected on two research farms, with correlation coefficients ranging from 0.71 to 0.75 for EB, DMI and EI in external validation by cow. Developed prediction equations were subsequently applied to spectral test-day records collected within the routine milk recording system of Austria, resulting in 290,000 MIR-predicted EB, DMI and EI records from 22,700 Fleckvieh dairy cows. Heritability and genetic correlations were estimated using a repeatability animal linear mixed model for EB, DMI and EI and milk yield, fat and protein contents, for several lactation stages. Heritability for MIR-predicted EB ranged from 0.14 to 0.20, for DMI from 0.23 to 0.29 and for EI from 0.24 to 0.27, with variation between lactation stages. EB was negatively genetically correlated with DMI and EI (-0.35 – -0.37). Genetic correlations between MIR-predicted traits and milk traits were mostly positive (0.03 – 0.80), apart from EB and milk yield, which exhibited a strong negative correlation (-0.73 – -0.76). The results indicate that MIR spectra may be utilized to generate large-scale phenotypes for EB and related traits, which exhibit moderate heritability and could thus be useful in genetic evaluation systems for breeding healthier and more efficient dairy cows.

Session 29

Theatre 10

Evaluating the Potential of Milk Mid-Infrared Spectroscopy as a Proxy for Genetic Relationships in Dairy Cattle

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Infrared spectroscopy has been proposed as an alternative to genetic markers in marker-assisted selection, mainly in plant breeding. This approach, called phenomic selection, uses high-throughput phenotyping to capture genetic similarities between individuals and perform predictions. This study proposes to use mid-infrared spectra (MIRS) from milk samples in dairy cattle to build a (hyper)spectral (H) relationship matrix. The objectives were to assess if MIRS can serve as a proxy for genetic relationships by comparing H to the genomic relationship matrix (G), and evaluating the stability of H across lactation stages and parities, determining the similarity of milk MIRS for long-term use in breeding program decisions. From 2013 to 2024, 113,822 milk samples were collected from 2,354 Canadian Holsteins. MIRS, obtained from spectrometers with 1,060 wavenumbers (5,008 to 925 cm⁻¹), were narrowed to 512 by removing water absorption-related noise. The H matrix was defined as $S \cdot S^T$, where S is a centered and scaled matrix for each wavenumber, divided by the number of wavenumbers. First, H and G were compared using Pearson correlation of off-diagonal elements and covariance matrix analysis, with eigenvectors of each sample to explain the variation in the other. Then, H matrices were compared across lactation stages (Start of lactation: 4-103 DIM, Middle: 104-205, End: 206-364) and parities (1, 2, 3), using the same metrics. The population structure of G was not captured using MIRS, resulting in low correlation (0.09) and high differences between the two covariance matrices. Across lactation stages, correlations ranged from 0.42 (Start-End) to 0.65 (Middle-End), with higher values between consecutive stages. Spectral similarity was relatively stable between 1st and 2nd parity, with a moderate correlation (0.56) and small differences in H covariance matrices, suggesting a temporally consistent MIRS signal. Using spectroscopy to improve phenomic prediction may require hybrid models that integrate genetic and spectral signals. Future work will evaluate these hybrid models for phenomic prediction.

Genomic prediction integrating indirect breeding values from MIR spectra data in dairy cows

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Mid-infrared (MIR) spectroscopy provides a high-throughput, cost-effective phenotyping method that captures molecular information from milk samples. This proof-of-concept study evaluates the integration of MIR spectral data into genomic prediction models to improve the estimation of breeding values for production, health, fertility, and morphological traits in three French dairy breeds: Holstein, Normande, and Montbéliarde. A total of 6,861 cows were analyzed, with genotypes from a 50K SNP chip and MIR spectral records. Heritabilities of MIR spectra varied across breeds, with estimates reaching 0.45 in Holstein, 0.42 in Normande, and 0.30 in Montbéliarde. We compared four genomic prediction models: (1) GBLUP (genomic relationship matrix), (2) FBLUP (MIR-based relationship matrix), (3) F+GBLUP (genomic and MIR-based relationship matrices), and (4) GOBLUP (genomic model incorporating indirect breeding values derived from the genetic effects of MIR). Prediction accuracies improved when MIR data were integrated. For instance, in Holstein, protein content prediction accuracy increased from 0.56 (GBLUP) to 0.60 (GOBLUP), while in Montbéliarde, fertility traits showed a gain of up to 0.05 in prediction accuracy. Across-breed prediction was more effective with MIR data than with genomic data alone. These results highlight the potential of MIR spectra as an auxiliary tool to enhance genomic predictions in dairy cattle breeding programs.

Feasibility of visible/near-infrared spectroscopy to predict amino acid content and chemical composition of dairy cattle total mixed rations

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The potential of near-infrared spectroscopy (NIRS) to predict amino acid (AA) content and chemical composition of dairy cattle total mixed ration (TMR) was evaluated. A total of 130 TMR samples were collected from 32 Holstein herds fed hay-based diets (n. 86 lactating, n. 33 dry cows and n. 11 heifers). Diets formulas were recorded and samples chemically analyzed for DM, CP, EE, aNDFom, ADF, ADL, ash, Starch, NFC, uNDF and NDFD24. Total AA were determined after acid hydrolysis, except for sulfur AA – oxidated with performic acid before hydrolysis – and Trp – for which alkaline hydrolysis was performed. The procedure was optimized for sample homogenization and substrate to solvent ratio. Samples were analyzed by RP-UPLC/ESI-MS in the SIR scan mode. The AA content was estimated using the internal standard method (Trp), and an external calibration curve (all other AA). The predictive equations were subjected to validation procedures, incorporating both cycling 5-fold cross-validation and an external validation procedure (ExV). For the ExV the dataset was randomly divided as 75% of the data as a calibration dataset and 25% of the data as validation set. Strong estimation accuracies, indicated by the coefficient of determination in ExV (R²ExV), were achieved for CP, EE, Starch and NDF content (R²ExV > 0.80). Moderate predictions were obtained for humidity, ash and NFC parameters (R²ExV > 0.65). The best predictive performances for AA composition were obtained for Ile (R²ExV = 0.57), Leu (R²ExV = 0.65), Phe (R²ExV = 0.56), Arg (R²ExV = 0.63), Tyr (R²ExV = 0.59) and Met (R²ExV = 0.56). All the other nutrients and AA showed poor prediction accuracy. The NIRS technology appears to be a promising technique for the prediction of specific nutrients and AA. Additional sample sets are needed to improve calibration accuracy. This project has received funding from the European Union's Horizon 2020 research and innovation programme, under the G.A. No 777974.

Innovative integration of phenomics, genomics, and machine learning into dairy cattle breeding in Croatia
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In response to emerging challenges in animal breeding, we have launched a project to introduce cutting-edge technologies into dairy cattle breeding in Croatia. Our approach integrates the latest advancements in phenomics and genomics to explore innovative methodologies that could be routinely applied in dairy breeding programs. A key component of this project is low-pass whole genome sequencing (lpWGS) performed on milk samples. Beyond providing genomic SNP data, this approach enables the retrieval of complete mitogenomes, insights into milk microbiota composition (e.g., mastitis pathogen detection), and the estimation of mtDNA copy number, a potential novel phenotype associated with milk production and health. We also apply machine learning techniques to analyze large-scale FTIR spectral data, focusing on milk coagulation traits and key milk components. This approach offers a robust model for managing big data with direct applications in dairy breeding and cheese production. While the project has a strong applied focus on integrating novel breeding tools into Croatian dairy farming, we also investigate underexplored inheritance mechanisms, particularly the influence of sex chromosomes and mito-nuclear interactions on quantitative traits. The PhenoGeno-IP-2022-10-6914 project (<https://angen.agr.hr/en/group/112/PhenoGeno>) is funded by the Croatian Science Foundation.

Session 30

Theatre 1

How do we measure welfare? Indicators, proxies and gold standards

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Animal welfare is what the animal experiences and spans very negative to very positive emotional states. Welfare is influenced by nutrition, the environment, health and the behavioural interactions that an animal may have, integrated through the impacts that these have on the mental state of the animal. Whilst we can often readily measure and describe the conditions under which the animal is kept, and even the physical state of the animal within these conditions, these are not sufficient to tell us what the animal is experiencing. The Gold Standard for animal welfare, therefore, would be a measure that tells us something about the mental state of the animal. Most scientists believe that this is not, currently, a directly measurable entity, and thus welfare assessment relies on a series of measurable outputs, or indicators, that serve as proxy indicators for welfare state. In these cases, we infer the mental state and welfare of the animal from the measure. For example, an animal may be inferred to be experiencing pain, and hence poor welfare, if we see changes in physiology, EEG, posture, facial expression, etc that appear after a potentially pain inducing stimulus and disappear after provision of effective analgesia. Similarly, we might infer a positive mental state in an animal that is relaxed and engaging actively with the environment and/or in an affiliative way with conspecifics. We cannot know for certain that this is what the animal feels, but we consider based on the evidence that this is the most likely outcome. Many of these measures rely on assessment of behaviour, as the most useful and sensitive proxy for the animal's mental state. These may rely on assessing ongoing changes in response, such as facial expression, circadian rhythms or Qualitative Behavioural Assessment, or complex patterns of behaviour, such as preferences, motivational priorities, or perceptions, which allow deeper views into what is the likely mental state of the animal. Many of these measures are time consuming and challenging to assess, but some may be amenable to use of sensors or automated methods. These require an understanding of what is being measured and how this relates to the animal's mental state. This presentation will discuss what is currently feasible and options for future developments.

The opportunities and challenges with welfare monitoring with Precision Livestock Farming

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Precision Livestock Farming (PLF) is a framework for livestock farming that integrates sensor technologies and artificial intelligence for continuous, real-time monitoring of animal health and welfare. There are some examples of this available in the literature, such as the use of sensors, computer vision, and acoustic monitoring in the early detection of disease, stress, and behavioral deviations. However, most are still under research and the real evidence of how these tools can improve herd management, and minimizes production is still somewhat scarce. Barriers that limit adoption include lack of standardisation during technology development, data interpretation... This presentation will discuss the challenges and opportunities for the application of PLF in modern livestock production, with a focus on the role PLF it can play in welfare monitoring.

Data Standards in Precision Livestock: Challenges and Opportunities in Large-Scale AI Integration

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The rapid advancement of artificial intelligence (AI) in agriculture presents unprecedented opportunities for precision livestock farming, enabling data-driven solutions for animal health monitoring, welfare assessment, greenhouse gas emissions tracking, and labor efficiency. However, leveraging AI technologies such as computer vision and natural language processing at scale requires robust data processing pipelines and standardization frameworks. One of the major challenges in this domain is the curation and benchmarking of large datasets before they can be integrated into open-source platforms. Unlike structured data, AI-driven livestock systems rely on vast amounts of image, video, and text data, requiring efficient annotation, metadata management, and interoperability to ensure reliable and scalable datasets. The lack of standardized data formats, annotation protocols, and benchmarking tools hinders reproducibility and slows the adoption of AI solutions across the industry. In this talk, we will explore the technical and logistical challenges associated with processing large-scale computer vision and text-based datasets in livestock systems. We will also discuss emerging efforts to establish data standards and open-source benchmarks, which are essential for developing fair, transparent, and widely applicable AI models. While these challenges are significant, they also present new opportunities for collaboration between researchers, industry, and policymakers to drive innovation in precision livestock farming.

Sensor data cleaning for research, dairy herd management, and breeding

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Data cleaning is essential when using data from dairy sensor technologies. Based on data from different sensor types collected within an international project (D4Dairy) and relevant literature we discuss important considerations and steps for data cleaning to support professionals involved in the collection, management and analysis of sensor data. Before starting with the data cleaning itself, it is crucial to consider context and intended purpose of the data use. For the cleaning process we recommend following these five key steps: 1) Validate the data merging process. Animal identifiers and sensor data have to be accurately aligned and errors such as duplicate assignments of one animal to multiple devices, or the other way around, and time zone mismatches have to be corrected. 2) Understand the data including its type (raw vs. processed data), units, whether values are aggregated or single measurements, and sampling rates. Visualization is recommended to identify patterns and distributions. 3) Check completeness of the data. Duplicated data must be resolved and strategies for dealing with gaps, which are a common issue in time series data due to e.g. sensor malfunction or low battery, determined. 4) Evaluating the plausibility of sensor measures and detecting outliers are the most critical step. Decisions may be based on biological meaningful ranges, cross-checking additional information, statistically based thresholds, or advanced modeling techniques. Removal of outliers that may hold valuable insights must be carefully omitted. 5) Check for technology related noise such as sensor drift, calibration issues, and updates, which may lead to inconsistencies in the data. If available, information should be requested from the sensor company. To ensure quality and reproducibility, it is essential for data users to document their approach throughout the process. Following these guidelines may contribute to ensure data for further analysis is accurate, consistent, and reliable, ultimately supporting high quality research, more informed decision-making for herd management and improved breeding results for dairy herds.

Session 30

Theatre 5

Data annotation for animal behavior identification and analysis

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Accurate assessment of individual-level behavior in farm animals is crucial for health and welfare monitoring systems. Understanding behavioral needs is also vital for optimizing husbandry practices. This talk highlights areas where standardization is required when annotating behavioral data in different species and discusses approaches for large-scale labeling. Traditionally, behavior analysis relies on human observation guided by pre-defined precise ethograms. Reliable assessment requires trained observers with high inter- and intra-observer reliability. Ideally, the same expert standards should be applied when creating the 'gold standard' annotations used to develop and validate automated behavior monitoring systems. However, ethology expertise is often not available during the development process, and universal standards for animal behavior data annotation are lacking. As a result, the reliability of many 'gold standard' datasets used for automated tools is unclear. Building machine learning models for routine monitoring demands large-scale behavioral labeling, which is especially challenging for data streams other than video (e.g., accelerometers, RFID, location tracking, audio). Moreover, there can be discrepancies between the sampling frequency of human assessment and sensor-based behavioral monitoring. Human observers may miss subtle behavioral details detectable by machines and it is unclear to what extent labels based on human observation limit monitoring tool accuracy as studies cross-validating human and algorithm detection are rare. I suggest considering the following questions in the context of behavioral analysis for welfare assessment: Is the behavior a validated indicator of welfare? Is there a detailed ethogram for this behavior described by ethologists? Are there opportunities for refining the ethogram by combining multiple data sources? Is the start and the end of the behavior clearly defined? Can this behavior be detected reliably by human observation? Does the annotated data contain variations of the behavior and conditions under which it may occur? Is it possible to distinguish individuals performing the behavior? In addition, methods for large-scale behavioral labeling with different data modalities will be discussed.

Overcoming Hardware and Data Challenges in Sensor-Based Animal Behavior Research

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For over a decade, Idele has conducted research using sensors to monitor animal behavior, from detecting health issues in young beef cattle with industry sensors to developing custom solutions for small ruminants, where no suitable sensors existed. This presentation shares the challenges and obstacles encountered at the hardware level and in data valorization. At the hardware level, working with technology providers presents obstacles such as poor communication regarding raw data properties, undetected time drift, and unexpected hardware modifications. These setbacks highlight the need for sensor standardization to ensure compatibility with research objectives and mostly reproducibility between different data collections. Machine learning is then widely used to classify time-windowed signals into behaviors, yet several challenges hinder the development and scalability of AI models in this field. Data variance is a major limiting factor in machine learning model performance. While research often prioritizes data volume and quality, variance is critical for generalizability. Current datasets are highly imbalanced, overrepresenting common behaviors while underrepresenting rarer ones, making data collection costly and inefficient. While targeted sampling can help, avoiding the collection of redundant data remains difficult. One potential solution is merging datasets across studies to enhance variance. However, the lack of standardized sensors and protocols introduces more reproducibility noise than meaningful variance, complicating model training. This issue is even more pronounced in computer vision-based behavior monitoring, which is now a hot topic in the field. By sharing our experiences—including technical and methodological challenges—we advocate for greater collaboration and standardization in sensor-based behavioral research. Establishing shared frameworks for data collection and processing is essential for improving machine learning applications, ultimately advancing the field toward more scalable and reproducible solutions.

Session 30

Theatre 7

Discussion and key work collection

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This session focuses on advancing the standardization of tools and methodologies in Precision Livestock Farming (PLF). As part of the starting SENSTARA Working Group (“Standardization of PLF Tools and Methodologies for Welfare Assessment”), we aim to establish best practices and harmonized standards for data collection, sensor development, and analytical approaches across species and systems. Researchers are invited to join this discussion related to sensor technology, data integration, welfare assessment, and methodological frameworks that contribute to consistent, scalable, and impactful PLF applications.

Prediction of lactation performance based on cow characteristics before pregnancy for on-farm decision support on the voluntary waiting period of individual dairy cows

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Extending the voluntary waiting period (VWP) for dairy cows is of interest to extend the calving interval (CInt) and reduce the frequency of critical calving events. Not all cows seem suited for an extended VWP related to a low milk yield (MY) at the end of lactation or low MY in the total lactation. The aim of this study was the development of prediction models for fat-and-protein corrected milk yield (FPCM) in the final six weeks before dry-off (FPCMend) and FPCM per day of CInt (FPCM/CInt) based on cow characteristics before pregnancy using test-day data of 40 Dutch dairy farms. Lactation curves were fitted with a non-linear model using a Wilmink curve with a negative gestation effect starting after 5 months of gestation. Subsequently, data of 50% of the farms was used to create the prediction models using mixed models with backward selection. The data of the other 50% of farms was used to test the prediction models. Both models included several MY variables before pregnancy and the breeding value for persistency. 70-80% of the variation in FPCMend and FPCM/CInt was explained by the mean MY in the week before pregnancy, where a greater MY before pregnancy was associated with greater FPCMend and greater FPCM/CInt. Predictive performance of the model for FPCMend was limited for both the train and test datasets ($R^2=0.42-0.47$, RMSE=4.90-5.04 kg/d), but was much better for FPCM/CInt ($R^2=0.87-0.89$, RMSE=1.77-1.84-5.04 kg/d). Overall, prediction performance of FPCMend was limited but FPCM/CInt could be predicted rather accurately, and mean MY in the week before pregnancy seems to be an important indicator in determining the optimal VWP of individual cows.

Session 31

Theatre 2

Evaluating Dairy Cow Interactions with AMS: Behavioral Analysis and Genetic Implications

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Automatic milking systems (AMS) are of increasing relevance in dairy farming. Hence, improving cows' behavioral response to technical environments (cow-AMS interactions) gains importance. To ensure smooth cow traffic, cows should visit the AMS freely but milked only at pre-set intervals. Behavioral responses to the technical environment (AMS traits: rejected milkings, udder scan time, pre-treatment time, connecting time) can be associated with learning behavior and temperament, and can be automatically gathered by AMS. AMS data from 24 German dairy farms were analyzed. Data comprised 15,684 Holstein cows with 41,581 lactations documented from 2007 – 2024 and was aggregated on a daily basis to form 12,186,928 daily cow-AMS interactions. Stage of lactation was defined as time periods for days in milk. To assess the degree of learning we used two traits (training before 1st lactation at AMS; days milking at AMS). Training before 1st lactation at AMS was defined as groups 0=no training, 1=training 1-7x, and 2=training >7x. Days at milking at AMS were grouped to define 'experience groups'. Analyses were conducted in SAS using a mixed linear model with fixed effects daily milk yield, the combined effects of parity*training before 1st lactation at AMS, parity*milking at AMS, and farm*season. Cow effects were included as random. Independent of parity, cows in training group 2 had the highest rejected milkings per day, i.e. they tried to be milked too often. This points to an overtraining effect before 1st lactation at AMS. In further analyses, these overtrained cows were excluded. Rejected milkings for cows in groups 0 and 1 clearly declined with age and days milking at AMS. Connecting time and pre-treatment time both declined with age and slightly with days milking at AMS, while udder scan time only declined with age. Estimations of heritabilities are still ongoing, and will reveal a better understanding of genetic mechanisms influencing learning behavior and temperament in dairy cows.

Relationship between mastitis prevalence and farm management practices for mountain dairy farms in the western Alps

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Logistic limitations in mountain areas make hard to assure assistance and veterinary services for dairy farms, inducing a high incidence of mastitis. Here, the way to improve herd's health is prevention. Our study aimed at identifying the relationship between mastitis prevalence and farm management practices for mountain dairy farms in the western Alps. We selected 8 dairy farms of Lanzo's Valleys differing for their farming practices. Each farm was visited before transhumance period (T0), 3 to 5 days after transhumance (T1) and approximately 1 month later (T2). At each visit, individual milk samples were collected for somatic cell count (SCC) and microbiological analysis to investigate the presence of contagious pathogens (CoP). Individual cow mammary gland, animal and bedding cleanness were evaluated through a score range between 1 (insufficient) and 3 (optimal). During visits, we surveyed data on animal characteristics, herd and farm management. The prevalence of CoP was then calculated at a herd level. A principal component (PC) analysis was run on microbiological data and SCC and cleanness scores, using farm management practices as non-active descriptive variables. The PC 1 was positively related to cleanness scores. Among descriptive non active variables PC1 was positively correlated to variables related to intensification level of the farms (milk yield, stocking rate, dietary concentrates, dairy cows/workforce unit, low crossbreed proposition). The PC2 was positively related to the prevalence of the CoP and milk SCC. Among non-active variables, PC2 was positively related to extensification level of the farm (altitude, grazing time/day, dietary pasture proportion, grazing season duration, age at first calving, lameness prevalence, lactation rank), suggesting that specialisation on dairy production better focus farmers attention on mammary health. The prevalence of CoP and SCC increased after transhumance, especially in T2, suggesting that the lack of veterinary assistance prevents farmers to monitor and control mastitis on upland.

Session 31

Theatre 4

Machine Learning to Understand Relationships Between Farm Practices and Milk Fatty Acids across Diverse European Dairy Farms

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We studied the relationship between farm practices and milk fatty acids (FA) profile while accounting for complex, nonlinear interactions between practices through a dual-model machine learning workflow. Bulk tank milk was collected from 75 dairy farms in 5 EU countries (CH, DE, FR, IE, IT) with 3 summer and 2 winter samplings, pooled per season, resulting in 144 pooled samples for FA analysis by gas chromatography. Farming systems varied from very low to very high intensity based on inputs on lands and animals. Farm data were collected at each visit via questionnaires (29 features on feeding, herd, management and land use). Random Forest models (RF) were trained and validated on a train-test split. Hyperparameters were tuned on the train set via cross-validation. Feature selection combined permutation importance and the Boruta algorithm. Given the low interpretability of RF, the top 5 features were selected to fit a Conditional Inference Tree (CIT) to rank feature significance, with the same RF workflow. This resulted in top-performing models for alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), n-6 polyunsaturated FAs (PUFA), n-6:n-3 PUFA ratio, and linoleic acid (LA) (RF: $R^2 \geq 0.80$; CIT: $R^2 \geq 0.70$ and $\Delta R^2 < 0.03$). LA and n-6 PUFA increased with higher concentrate intake and higher livestock units (LSU/ha), but decreased with longer grazing time. The n-6:n-3 ratio increased with higher concentrates intake, annual milk yield, and LSU/ha. ALA and n-3 PUFA increased with lower total dry matter (DM) and maize silage intakes and under organic production. EPA decreased with higher concentrate and DM intakes, and with higher annual milk yield. We propose this approach as a flexible, evolving framework for identifying key farm practices influencing other intrinsic milk quality traits.

Modelling feed efficiency in dairy cows for selection and management decisions

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Selective breeding and herd management practices are two important levers to improve feed efficiency in dairy cattle production. However, different approaches are used to model this trait and make decisions in both fields. The objective of this study was to compare two variables used for genetic evaluations (Residual feed intake, RFI) and the simulation of feed efficiency in the SimHerd software (FESim). This comparison was carried out using a dataset collected at the Danish Cattle Research Centre. It comprised lactations 1-3 of Holstein cows for which weekly averages were available from 1-44 weeks in milk for dry matter intake (DMI), energy corrected milk yield (ECM) and body weight (BW). After cleaning, data were available for 1732 lactations recorded on 908 cows. The FESim variable was computed by dividing expected by observed net energy intake. Expected energy intake was estimated using an equation with ECM, BW and BW change (BWc) as energy sinks and energy coefficients from nutritional studies. RFI was derived using a multivariate analysis of DMI, ECM and BW longitudinal records with random regression (RR) linear mixed models. Estimated variance components were used to calculate regression coefficients for the prediction of expected DMI based on ECM, BW and BWc. RFI was derived as the deviation between observed and expected DMI. Thus, efficient cows had higher FESim and lower RFI values. RR mixed models were also run to estimate covariances between FESim and RFI. Repeatability values were low for RFI (0.12-0.25) but consistent with previous estimates. Repeatability of FESim was low until week 37 in milk (0.06-0.25) and moderate in weeks 37-44 (up to 0.43). Correlations between the cow effect of FESim and ECM were moderately positive over most of the lactation, and moderately negative between FESim, BW and DMI. Correlations between the cow effect of FESim and RFI were moderately to strongly negative, i.e. favourable, over the whole lactation (-0.50 to -0.95). Hence, decisions made using RFI and FESim will be rather consistent, but re-ranking of cows will occur especially at the start and end of lactation.

Session 31

Theatre 6

Encapsulation of bacteria and plant extracts for enhancing safety and health in fresh cheese

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This project focuses on developing a novel fresh cheese enriched with microcapsules (MC) containing microbial strains with bioactive/antimicrobial properties and plant extracts rich in functional compounds to enhance health benefits, safety and shelf-life. MC were formulated with co-emulsified microbial strains of *Lactococcus lactis* (LcL), *Lacticaseibacillus paracasei* (LbP) and *Lb. rhamnosus* (LbR) species and functional plant extracts (blackcurrant, black carrot, elderberry, Moringa, Alchemilla, grape stalk) using chitosan or whey protein coatings at different concentrations. 108 MC combinations were analyzed over 30 days of storage at 4°C, assessing encapsulation efficiency (EE%), microbial viability, and polyphenol retention. The highest EE% was observed for LcL and LbP together with blackcurrant and moringa coated with chitosan, and LbP with elderberry and whey proteins coating. After 30 days at 4°C, viability losses were around 1.9 log CFU/g, and LbP with moringa and blackcurrant in whey protein exhibited the best performance in terms of EE%. Grape stalk and blackcurrant extracts showed the highest polyphenol retention. Based on these results, further work will focus on Alchemilla, elderberry, and moringa for LcL and Alchemilla, elderberry, and blackcurrant for LbP/LbR using whey protein as coatings. These MC will be used for model-cheeses production that will be analysed by Nuclear Magnetic Resonance for the delineation of their characteristic profile. Alongside a consumer survey for 1,000 participants will assess acceptance of novel dairy products toward innovation, risk-benefit perception and willingness to adopt sustainable food technologies. This study was carried out within the ONFoods2 and received funding from the European Union Next-GenerationEU (PNRR-MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.3-D.D. 1550 11/10/2022, PE000000003).

1H NMR metabolomic fingerprinting to authenticate feeding systems and altitude origin of bulk milk
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The dietary forage sources might affect the nutritional and organoleptic quality traits of milk and dairy products, especially in the comparison between lowland indoor and outdoor mountainous feeding systems. The aim of this study was to investigate the capacity of high-resolution proton nuclear magnetic resonance (1H NMR) to differentiate the metabolomic profile of milk sample from three Italian dairy chains based on: a mix of grass-legume and maize silages (silage), grass and lucerne hay (hay) and highland pasture (pasture) as main forage components of the diet fed to lactating dairy cows, respectively. Raw bulk samples (n = 72) were analyzed for the polar fraction by Varian Inova-600 MHz NMR spectrometer equipped with a 5-mm triple resonance inverse probe. A total of 92 NMR variables were detected and submitted to a supervised machine learning LDA (linear discriminant analysis) using the train set (n = 51). The reliability of the LDA model was assessed arranging a confusion matrix on the test set (n = 21), and the classification performance evaluated by a pool of descriptive statistics. The 1H NMR spectra were dominated by resonances from lactose, amino acids, sugars, organic acids; however, a restricted pool of NMR variables was able to discriminate the three milk feeding systems. The exploratory targeting model attributed a significant discriminant role to few secondary metabolites such as creatinine, orotate, fumarate, choline, and some specific amino acids. Both the LDA scattergram and the classification performance resulting from the confusion matrix showed an accurate discrimination between the lowland intensive (silage and hay treatments) and the alpine extensive (pasture) dairy systems highlighting the significant role of the forage from perennial polyphytic swards in modulating the milk metabolic fingerprinting. A small pool of polar secondary compounds can act as powerful and reliable biomarkers to authenticate milk from different feeding systems and altitude.

Session 31

Theatre 8

Smoothing Quantile Evolution in Day-in-Milk Data: A Novel Approach to Modelling Lactation Curve Persistence

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Lactation curves depict milk production from calving to drying-off. The most significant phenomena are peak production, the highest point of the curve, and lactation curve persistence, the ability to sustain yield over time. These parameters are critical indicators of dairy performance, offering insights into biological and management factors. Unlike accumulated milk yield, which can be skewed by external, unrecorded factors, peak production and persistence are less prone to such artifacts, providing clearer understanding of physiological and environmental drivers. Analyzing these metrics enables targeted strategies to address productivity variations. The objective of this work is to study the lactation curve parameters to describe the dynamics of milk production in cows from Castilla y León, the largest and most homogeneous subset of Spanish dairy cattle, characterized by consistent management, feeding, climate, and other factors, using data from five million milk yield tests. Our approach involved applying spline smoothing to a set of quantiles within day-in-milk subsets, across the lactation curve. This method strikes a balance between a heuristic, data-driven approach and distribution-agnostic modelling, allowing for flexible yet robust analysis of lactation trends without imposing strict assumptions on the underlying data distribution. The mid-lactation slope of a smoothed curve is a reliable indicator of persistence. Greater persistence allows for flexible drying-off dates, stabilizes daily milk output, and enhances robotic milking efficiency. Parity significantly impacts persistence, with first-lactation cows showing better persistence than multiparous cows. Persistence declines linearly until the third lactation and stabilizes thereafter. In the top 5% of high-producing cows, strong peak production compensates for lower persistence, underscoring its importance. Higher-yielding farms exhibit less persistent curves with higher peaks. First lactations feature later peaks (+25 days), lower peak yields (-8 kg), and greater persistence (losing 50 g less per day). No link was found between peak timing and overall productivity.

Factors associated with increased resilience to heat waves in French dairy herds - a long-term study

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Livestock systems are increasingly challenged by climate change. In this context, improved herd management is increasingly being sought to enhance coping capacity. We investigated short-term stability (resilience) and long term stability (robustness) in 3393 herds that were monitored for 12 to 23 years. Four annual herd performance indicators were analysed: average milk yield per cow, average productive longevity (first calving to end of last lactation), average herd size (dairy cows number) and in-calf rate. Resilience was evaluated as the deviation from an estimated performance trend in response to an annual heat waves index. Robustness was quantified using coefficients of variation in performance over time. We first described impact of heat waves on herd performance, then analysed the factors influencing herds sensitivity to heat waves, including breed, calving season, production level, herd size, herd robustness and climatic region (hot oceanic, cold-wet oceanic, semi-mountainous). We found that annual average milk yield and in-calf were significantly reduced in years with heat waves (average losses: -1% milk; -5% in-calf). The following year, average cow longevity and dairy herd size were also reduced (-5% longevity; -8% herd size). Herds with the top 25% robustness levels were twice as resilient to annual heat wave disturbances compared to the average. The climatic region also showed a major effect: herds located in semi-mountainous and hot oceanic areas were twice as resilient to heat waves compared to those in cold-wet oceanic climate. These results indicate that (1) ability of dairy herds to cope with specific challenges is associated with their overall robustness, and (2) the sensitivity of dairy herds to heat waves varies according to their geographical context. Herds previously exposed to heat waves and temperature fluctuations may have developed coping mechanisms to face climate change through cattle acclimatisation and/or husbandry practices. Understanding why some herds fare better than others could lead to the identification of levers for adaptation of livestock systems to climate change.

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Assessing sustainability and resilience of three dairy value chains in Southern Mediterranean countries

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The challenges faced by dairy value chains in Southern Mediterranean countries are multi-faceted. The identification of possible solutions requires a holistic analysis, including environmental, economic, social and institutional aspects. This study is a preliminary analysis of the challenges faced in three case studies in Morocco (Kenitra), Turkey (Marmara) and Egypt (Giza). The analysis considers the point of view of (i) local experts, (ii) international experts and stakeholders, (iii) local stakeholders, addressing (i) with structured interviews, (ii) with a roundtable, (iii) with focus groups with local stakeholders. Identified challenges spanned all parts of the dairy value chains and were common to the three studies or specific to some of them. Feed provision is subject to droughts affecting provision quantity and timing, it is subject to currency fluctuation where coming from abroad. Farmers' challenges include limited access to resources, education, veterinary cares, markets; low negotiation power; gender inequality; poor knowledge about animal welfare practices; cattle diseases. Transportation faces challenges such as inadequate storage facilities and long transportation distance. The three case studies pointed out the existence of a formal and informal dairy value chain. Informal value chains involve direct selling to transporters with high negotiation power and sometimes involved in fraudulent practices. The inaccessibility by farmers to milk collection centres favours informal markets with prices often unfavourable. Policy challenges were also identified, such as policy schemes changing too often and unfavourable choices about import/exports. On the consumer side, a challenge is the perception that fresh milk from the informal market is safer than from the formal value chain. This analysis constitutes a basis for further discussion about possible strategies at the systemic or at the local level and further activities in the SUPREM-MILK project.

Utilizing machine learning for the genomic selection of feed efficient Holstein dairy cattle

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Genomic selection to enhance feed efficiency (FE) in cattle presents a viable strategy to reduce cost of production while maintaining milk yield by identify cattle with favourable genetic traits for FE. Machine learning (ML) has emerged as a powerful tool to integrate these high dimensional genomic datasets to improve the identification of genetic markers associated with FE and enhance the predictive accuracy of genomic selection models. In this study, ML algorithms were applied to genomic data from 195 Holstein-Friesian dairy cattle to predict residual feed intake (RFI), a key indicator of FE. Predictions of RFI were conducted using conventional Genomic Best Linear Unbiased Prediction (GBLUP) alongside ML algorithms, including Decision Trees (DT), Support Vector Machines (SVM) and Random Forests (RF). The RF model achieved the highest accuracy, outperforming other ML models and the GBLUP approach. Random forest, SVM, and decision tree models achieved accuracies of 0.76, 0.74, 0.58, respectively and GBLUP had the lowest accuracies of 0.51. These results showed the potential of using ML to effectively predict RFI offering a more robust method for genomic selection. A total of 7 markers were identified as significant markers using the RF model feature importance value, which were linked to candidate genes GPRASP2 and MUC2. Novel genes MUC2 is expressed abundantly in the gastrointestinal tract of mammals, expression of this genes has been linked to animal nutrition and feed utilization. While GPRASP2 plays a role in neuron signalling, particularly in glutamatergic synapses which regulated feed efficiency in pigs via appetite. In conclusion, we have found that machine learning has enhanced genomic prediction in dairy cattle for identifying feed-efficient animals and can explain the linked genes associated to FE.

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Determining Iodine Status in Bovines

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Iodine is an essential trace mineral used for the synthesis of thyroid hormones. Deficiency of iodine can lead to decreased fertility, retained placentas and stillborn foetuses in cattle. However, very little evidence is available surrounding testing for iodine status. The direct measurement of iodine in the thyroid gives the greatest overview of an animal's iodine status. However, it is rendered of little use due to there being no reference interval currently published. This study aimed to develop a greater understanding of the iodine status of cattle by establishing a normal reference range for thyroid iodine. Thus, allowing for better utilisation of these tests by clinicians in practice. Samples were collected from cattle from different abattoirs within Leicestershire, n=120. Only samples from 'healthy' animals were included in the study. A healthy animal was defined as 'an animal that had been declared fit for human consumption'. A 0.8-gram sample was dissected from the center of the tissue and freeze dried. The samples were then analysed via ICP mass spectrometry, following oven extraction with tetramethylammonium hydroxide. From these results a normal distribution curve was developed and a reference interval created using distribution statistics and percentiles. A retrospective questionnaire was sent to farmers whose cattle had been included in the study. Data around feed composition, additional iodine supplementation and, for dairy cattle, use of iodine-based teat sprays was collected to allow for the compounding effects of these on the reference range to be accounted for. Preliminary results indicate a thyroid iodine reference interval for a healthy ruminant of 5902 – 9915 mg/kgDM. By using two standard deviations this reference interval will include 95% of clinically healthy animals. These preliminary results have been obtained from a pilot trial of only 55 animals. Results from the main study samples are still currently being analysed. In conclusion, establishing the reference range for thyroid iodine will allow this test to be better utilised. An animals true iodine status is now able to be measured allowing for accurate diagnosis and supplementation. Future research is needed to focus more in depth on specific sub-populations such as bulls vs cows vs youngstock.

The Use of Vicia Faba Meal in the Finishing of Holstein-Friesian Bulls

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*Correspondence: zena@uwm.edu.pl; phone: +48 792717240 In the countries where beef cattle populations are small, beef production is based on the fattening of young Holstein-Friesian (HF) dairy bulls. The fattening phase ends with finishing to improve carcass and meat quality. Rapeseed meal (RM) is commonly used and legume seeds are sporadically used as protein sources in cattle diets. The aim of this study was to evaluate the effect of RM and Vicia faba meal (VF) on the fattening performance, carcass quality, and meat quality of young HF bulls. Twenty HF bulls aged 17 months, with an initial body weight of 500 kg, were divided into two groups fed isonitrogenous and isocaloric diets differing only in the protein source: VF vs. RM. Group VF received maize silage, VF, ground triticale, and premix (0.62/0.17/0.18/0.03). Group RM received maize silage, RM, ground triticale, and premix (0.62/0.14/0.21/0.03). After 5 months of the finishing phase, the animals were slaughtered, carcass quality was assessed, and samples of the longissimus lumborum muscle (LL) were collected to determine the sensory quality and fatty acid profile of meat. Statistical analyses of experimental data, including analysis of variance (ANOVA) were performed using Statistica 13.3 software, at a 1% and 5% level of significance. The experimental diets had no effect on the average weight gain of bulls (RM – 1.266 kg, VF – 1.170 kg). Significant ($P < 0.05$) differences in favor of group RM were observed for carcass conformation (EUROP system). Sensory analysis revealed that meat samples from the loin of VF group bulls scored higher for juiciness ($P < 0.01$) and taste ($P < 0.05$) than meat samples from the loin of RM group bulls. Fat extracted from the LL of RM group bulls had a higher proportion of polyunsaturated fatty acids (PUFAs), including trans-vaccenic acid (C18:1), CLA (C18:2 c9 t11), and linoleic acid (C18:3). In conclusion, the study demonstrated that VF fed to HF bulls in the finishing phase improved the sensory attributes of meat, compared with RM. In turn, RM improved carcass conformation and the quality of fat contained in the LL muscle, compared with VF. Funded by the Minister of Science under the “Regional Initiative of Excellence” Program.

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Poster 14

Impact of individual milk free fatty acid concentrations on the extent of bulk milk lipolysis during storage

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Elevated free fatty acid (FFA) content in milk can impair milk foaming properties and cause undesirable flavours. Milk fat lipolysis is influenced by farm management factors, but an important variability also exists between individual cows. We investigated how milks from individual cows interact with regard to lipolysis during storage. We hypothesized that the incorporation of milk with a greater basal FFA concentration would amplify the concentration of FFA in the reservoir during storage beyond what would be expected based on the arithmetic average. Individual milk samples were collected from three cows. A second milk sample was collected from each individual and pooled to constitute a fourth sample. These four milk samples were stirred at 4°C for 72 h. The procedure was repeated eight times for a total of 32 milk samples. Milk FFA concentration was determined according to the copper-soap method at 0, 48 and 72 h of storage for each milk sample. Pre-planned contrasts were (i) individual milks vs. pooled milk, and (ii) milk with the greatest basal FFA concentration vs. pooled milk, and (iii) their interactions with time. Regardless of treatment, the increase in milk FFA content over time followed a quadratic pattern; the rate of increase being greater during the first 48 h ($P = 0.04$). Throughout storage, the calculated FFA concentration average of individual milks was not different from pooled milk ($P = 0.54$). On the contrary, the FFA content of the most lipolyzed individual milk was greater than the pooled milk, and the increase over time was also greater ($P = 0.05$). We conclude that a more lipolyzed milk does not contaminate a reservoir to its own level during storage. The milk reservoir is more likely to represent the average FFA content of the cows that contributed. Hence, dilution of lipolyzed milk can be used to mitigate the final FFA concentration.

A data driven approach for the evaluation of the long term productivity of dairy cows

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The challenge of increasing the economic and environmental sustainability of the dairy cattle sector involves several aspects and among these, the milk production throughout the career of a cow, is perhaps the parameter that all farmers would like to know for a more efficient planning of entries and exits. In fact, if on one hand numerous researchers are studying the problem selecting most efficient animals, on the other hand, few studies have focused on the definition of tools forecasting the productivity of dairy cows in the future lactations starting from the data collected in past lactations. This aspect has a particular importance in the first years of a cow since, as well known, first lactation usually has lower production than subsequent lactations. For a farmer it is important to know, as soon as possible, if a specific animal will have on a long term, lactations with high, medium or low milk productivity. In this study, firstly two supervised learning methods, i.e., Super Vector Machine and K-Nearest Neighbors, have been applied to a large dataset of 720 complete lactations, with the object to train machine learning tools able to classify and separate first and second lactation. The two classification algorithms have been applied to the raw dataset and after the application of a dimensionality reduction method. Four different dimensionality reduction methods (i.e., ISOMAP, UMAP, MDS and t-SNE) have been tested to evaluate the most efficient for this application. Finally, the same two classification algorithms have been used for the attribution of the productivity level of the second lactation starting from data of the first lactation. The two classification methods reached very encouraging accuracy values, ranging from 70% to 73%, indicating that selected predictors despite their simplicity look very promising and entail for the definition of enhanced future models. In fact, the method is particularly interesting for practical applications, as it represents a viable support-to-decision tool for selecting the most productive animals.

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The impact of the genetic variant of beta-casein in milk from Simmental cows on the physicochemical properties, sensory quality, and microbiological characteristics of various types of fermented milk

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A2 milk, which contains exclusively A2A2 β -casein, has gained significant interest in recent years among both scientists and consumers due to its potential health. From the perspective of dairy producers interested in introducing A2 milk products to the market, understanding the impact of casein polymorphism on the technological properties of milk is also crucial. The present study aimed to evaluate the effect β -casein (β -CN) genetic variant in Simmental cow milk on selected physicochemical characteristics, as well as sensory and microbiological quality of the selective types of fermented milks. Probiotic yoghurt (ABT-yoghurt), kefir and cultured milk were produced from A2A2 β -CN milk of Simmental cows and compared with those produced from A1A1 β -CN milk. The comparison included analyses of basic chemical composition, pH, titratable acidity, antioxidant capacity, sensory quality (assessed using five-point test for colour, appearance, consistency, odour, and taste), instrumental texture profile, and the number of starter microbial cultures. All samples were analysed when fresh and after three weeks of cold storage. The results were subjected to the statistical analysis using t-test or two-way ANOVA. The β -CN genetic variant had no significant influence on the basic chemical composition, acidity, anti-radical scavenging activity against DPPH radical, ferric reducing antioxidant power (FRAP), sensory quality, or the number of starter microorganisms in produced fermented milks during cold storage. On the contrary, A2 mesophilic fermented milks (kefir, cultured milk) exhibited better textural properties, including higher hardness, adhesiveness, and gumminess compared to the corresponding A1 milk-based products. These findings suggest that A2 milk from Simmental cows serves as an excellent raw material for the production of fermented milks.

Amino acid content in milk of Simmental cows depending on the β -casein variant A1A1 and A2A2I. Radkowska¹, D. Najgebauer-Lejko², A. Radkowski³¹ National Research Institute of Animal Production, Department of Cattle Breeding, Krakowska 1, 32-083 Balice n. Krakow, Poland, ² University of Agriculture, Department of the Animal Product Technology, Balicka 122, 30-149 Krakow, Poland, ³ University of Agriculture, Department of Agroecology and Plant Production, Mickiewicza 21, 31-120 Krakow, Poland

Dairy products, particularly those derived from cow's milk, constitute a fundamental component of global humans diets. Milk is valued for its beneficial nutritional properties, serving as a source of proteins, lipids, vitamins, minerals, and biologically active. Among these components, β -casein has recently garnered significant attention. Research indicates that β -casomorphin-7, a peptide formed during the digestion of A1 β -casein, exhibits strong opioid activity and may contribute to food intolerance or influence the development of certain human diseases. While milk is a rich source of amino acids, limited research has evaluated the impact of CSN2 β -casein genotypes (A1A1 and A2A2) on the amino acid composition of milk proteins. Consequently, this study aimed to assess the effect of β -casein variants on amino acid profiles in milk from Simmental cows. Milk samples were collected from 60 cows pre-identified as homozygous for either the A1A1 or A2A2 genotype. Amino acid analysis was conducted via reverse-phase liquid chromatography using the Waters ACCQ Tag analytical kit (USA). Results revealed that β -casein genotypes influenced amino acid content. Milk from A2A2 cows exhibited higher concentrations of histidine, cysteine, tyrosine, and phenylalanine ($p \leq 0.05$), whereas A1A1 milk contained elevated levels of proline and lysine ($p \leq 0.05$). Differences in other amino acids were not statistically significant. These findings align with existing evidence that milk protein composition is strongly linked to genetic factors, confirming that genotype can modulate amino acid profiles. This suggests that selective breeding programmes targeting specific β -casein traits could enhance milk quality and dairy product attributes. However, given the limited number of studies in this field and the relatively small sample size of this investigation, further research is warranted to comprehensively establish the relationship between genotype and milk amino acid composition.

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The UK Hoof Health Registry: creating and analysing a national hoof lesion database to better understand the landscape of dairy cow lameness

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Hoof trimmers (i.e. anyone who regularly monitors the condition of dairy cows' hooves), generate detailed records that can offer valuable insights into the types and frequency of infectious and non-infectious hoof lesions. The aim of this project was to collate and utilize these records at a national level across the UK, with minimal inconvenience to farmers/hoof trimmers. This in turn will provide insights into lameness leading to improved management practices and better animal welfare. Dairy farmers and hoof trimmers were recruited through a range of approaches: trade events, local farmer meetings, communications from their milk purchaser and the farming press. Anyone that recorded their hoof trimming data electronically via All4Feet, DairyComp, Vet Impress or Uniform were eligible to participate in the project. Data were collected retrospectively; where the hoof trimming data were recorded by management ID, cows were matched via herd ID and/or the farm's milk recording ID to the full eartag number. This allowed data including parity and calving date to be accessed via records kept by the Agriculture and Horticulture Development Board. Data were analysed using R, and erroneous records were removed (including those with missing or nonsensical IDs). Over 400 farms consented to share data with the project (farm recruitment is ongoing). Preliminary data from 361 farms over more than 9 years recorded 499,325 cow-trimmer-visits for 138,003 cows with a mean of 3.62 trimmer visits per cow. Data on 25 causes of lameness were recorded with 73,574 observations of sole ulcer. Parity and calving date gave further details on the occurrence of specific lesions during the lactation cycle and the lifespan of the cows which will be shared at the conference. In conclusion, we have successfully created a UK-wide hoof health database to support future lameness reduction programmes, including improved genetic evaluations to produce animals that are more resilient to lameness causing lesions. These records provide an opportunity to monitor trends, identify common pathologies, and assess the impact of interventions.

How much do you know about milk as a source of minerals?

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The rise of plant-based beverages consumption in Western countries in detriment of milk could compromise the adequate mineral intake. This study considered the impact of milk consumption on consumers' awareness of milk as a dietary source of energy, nutrients, and minerals (Ca, P, K, Mg, Fe, and I) through an international survey. The questionnaire included 7-point Likert scale questions on milk energy, nutrients and minerals content, and socio-demographic and consumption habits questions. Data were analyzed through a multiple regression fixed effects model which included consumer class (CON, milk consumers; N-CON, non-milk consumers), geographical area, education, gender, age, and first order interactions with milk consumer class as fixed effects. The sample, consisting of both CON (85%) and N-CON (15%), included 4,700 respondents from 16 countries. Sociodemographic characteristics were comparable between CON and N-CON; they were mainly young (CON, 55%; N-CON, 54%), women (CON, 63%; N-CON, 70%), and with university studies (CON, 45%; N-CON, 52%). Results revealed that consumer class only modified knowledge on milk as a source of energy content, with significantly more positive awareness for CON than N-CON. However, significant awareness differences aroused across geographical regions and education for milk as a source of energy, nutrients, and minerals. Overall, participants stated a strong association between Ca and milk, but many minerals received scores close to neutrality. In conclusion, this survey demonstrates that milk consumption improves the perception of milk as a source of energy, however, awareness of milk as an important mineral dietary source beyond Ca is scarce, highlighting a general lack of knowledge about critical health-related elements in milk. There is a need to promote the role of milk as a mineral dietary source to contribute to informed nutritional choices.

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A Field Evaluation of a Blend of Essential Oils, Tannins, and Bioflavonoids to Improve Milk Production in High-Producing Dairy Cows

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The study aimed to evaluate the effect of a blend of Essential Oils, Tannins, and Bioflavonoids (EOTB, ANAV-RIN®, VetosEurope, Lugano, Switzerland) on milk production in high-producing dairy cows. The trial was conducted on a commercial farm (Explotaciones Ferreiro, Baleira, Spain) using a Holstein herd (47.4 L/cow/day, 58% primiparous) housed in four pens with six milking robots. Pens were randomly assigned to two treatments: Control diet or Control + EOTB (3 g/cow/day). Individual daily milk production was recorded (n = 38,121), along with pen-level data on total milk, fat, protein, milk urea nitrogen, and dry matter intake (DMI) (n = 325). Milk production from primiparous cows was adjusted to adult equivalents ($\times 1.2$). Data were analyzed using SAS OnDemand for Academics (SAS Institute Inc., 2025). Individual milk production was compared between treatments, nesting cows within treatments. Pen-level data underwent Principal Component Analysis (PCA) by treatment, including daily corrected milk yield, fat, protein, urea yields, DMI, and days in milk. ANOVA on PCA scores assessed treatment effects. Cows supplemented with EOTB showed higher corrected milk yield than controls (Control = 43.7 L vs. EOTB = 44.3 L; SEM = 1.739, P = 0.048). PCA identified two principal components (PC1 and PC2) explaining 56.5% of variance. PC1 was positively related to fat and protein yields and negatively to DMI, while PC2 was positively associated with milk yield and negatively with days in milk. EOTB supplementation significantly influenced both components (P < 0.001), clearly separating Control and EOTB groups. ANOVA confirmed significant treatment effects (P < 0.001), with EOTB associated with increased milk, fat, and protein yields and reduced DMI. The results suggest that EOTB positively impacts milk production, potentially by enhancing nutrient use or improving resilience to production-related stress. Further studies on digestion and stress-related metabolites are recommended. Keywords: Dairy cows, essential oils, tannins, bioflavonoids, milk production, PCA, feed efficiency.

Incorporating the pre-slaughter phase into chicken welfare monitoring and labelling schemes

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Ideally, animal welfare labelling results in better informing stakeholders and improving the quality of the animals' entire lifespan, including the last phase when they are being depopulated, transported and eventually slaughtered. In the poultry industry, this pre-slaughter phase is critical for both animal welfare and for preventing production losses (due to mortality, weight loss and carcass rejections). Recent and ongoing research will be presented to document which welfare problems occur during which part of the pre-slaughter phase, what the risk factors are for these welfare problems, and the pros and cons of different catching & crating methods (inverted manual, upright manual vs mechanical catching). Examples will be provided of cost-efficient manual or automated tools for large-scale monitoring of bird welfare during pre-slaughter. Monitoring data can subsequently be used to drive improvements in poultry welfare by a) providing feed-back (e.g. benchmarking) to responsible parties (farmer, catching team, transporter, slaughterhouse), b) a punishment or reward system depending on whether or not target values are met, and c) incorporating this information into a welfare label.

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Theatre 2

The effect of pre-transport respiratory health status, breed, and sex on post-transport respiratory health status measured by thoracic ultrasonography and clinical respiratory scoring

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Many factors may influence the predisposition of unweaned calves to developing respiratory disease post transport. These include their management, pre-transport health status, breed and sex. This study aimed to assess the effect of predisposing factors (pre-transport health, breed, sex) on the post-transport respiratory disease development of calves transported from Ireland to The Netherlands. The respiratory status of calves (n = 65) was assessed by thoracic ultrasound (TUS; score 0 (healthy) to 3 (severe pathology)) and clinical respiratory score (CRS; score 0 (healthy) to 7 (severe symptoms)) recorded pre-transport at the dairy farm or livestock market of origin, and again on arrival, and 1-, 2-, and 3-weeks post arrival at the veal farm in The Netherlands. Post-transport respiratory disease status was recorded as the highest TUS (TUSpost), or highest CRS (CRSpost) between arrival and 3 weeks post arrival. Linear mixed models were used to analyse the effect of predisposing factors (pre-transport TUS (TUSpre), pre-transport CRS (CRSpre), breed, and sex) on post-transport health (TUSpost or CRSpost). TUSpost was more severe for calves with a TUSpre of 2 than for calves with a TUSpre of 0 ($p < 0.01$) and tended to be more severe for calves with a TUSpre of 1 than for calves with a TUSpre of 0 ($p = 0.07$). CRSpre did not affect TUSpost. Friesian calves had more severe TUSpost than Beef*Friesian calves, and female calves had more severe TUSpost than male calves (both $p = 0.02$). CRSpost was not affected by TUSpre, CRSpre, or sex, but Friesian calves had more severe CRSpost than Beef*Friesian calves ($p = 0.01$). In conclusion, calves with more severe TUSpre showed more severe scores post-transport, and Friesian calves were at higher risk of developing respiratory disease diagnosed by TUS or CRS post-transport, while sex had a minor but clear effect on post-transport TUS development.

Lying behaviour of 20-25 kg pigs during 23-hour transport: Impact of deck height, time since departure, and in-vehicle temperature

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Annually, about 33 million pigs, most weighing around 30 kg, cross the borders of EU member states. Despite welfare concerns, behaviour during transportation has received limited scientific attention. Our project studied the impact of deck heights (60 vs 80 cm and 70 vs 90 cm) on lying behaviour during long-distance transport of 20–25 kg pigs, along with time since departure and in-vehicle temperatures. Lying behaviour in 27–31 pigs during 16 journeys was evaluated using 15-min interval instantaneous scan sampling from departure to arrival, based on video recordings of vehicle compartments (~0.2 m²/pig). The impact of average and change of average in-vehicle temperatures since last 15-min interval on the proportion of lying pigs was analysed in interaction with time since departure in a mixed effects model. The median temperature was 17°C (2-29°C), and temperature changes varied from -3 to 2°C. In the first hour, the median percentage of lying pigs was 10% (0-93%). By the 4th hour since departure, the median percentage of lying pigs exceeded 60%, reaching above 90% from hours 11 to 20 (night-time). In the remaining hours until arrival, the percentage decreased to 66-78%. There was no effect of deck height on the odds of lying. In general, less pigs were lying with higher temperatures, particularly in the first 3 hours and to a lesser extent in the final hours, compared to the middle part of the journey. Higher temperature changes generally led to less lying except in the first and last hours of the journey in the lorry. In conclusion, temperature and time since departure, rather than deck height, affected the lying behaviour during transport.

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The effect of rest stop duration on the behavioural and demeanour responses of *Bos indicus* cross cows after long distance road transport

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Long distance cattle transport is common in Australia. This study determined how long road journeys affect cattle behaviour and demeanour, and if these differ with rest periods. Cattle (n = 952) were assigned to one of three rest treatments; short (15-18 h), medium (39-42 h), and long (61-66 h), after traveling 2200 km by road across four trials. Two observers scored posture and behaviour from video taken at the rest depot (~90 min). Principal component analysis (PCA) was applied to demeanour scores from one observer at 30 and 60 min. Individual animal drinking events, based on rumen loggers were assessed. REML linear analyses were conducted on ethogram, demeanour PC1 and PC2, and number of drinking events, at i) the first morning after arrival at the depot (ARR), and ii) the last morning prior to leaving the depot (Pre-DEP). Results indicated that rest period duration had a significant effect on drinking (p = 0.016), eating (p = 0.003), and PC1 (activity) (p = 0.009), with fewer cattle drinking from the medium compared to long group on PreDEP, and less cattle eating in the short compared to medium and long groups. Cattle in the short group were described as more 'active/alert/agitated' than those in the medium group who were described as more 'settled'. Cattle from all groups drank significantly more during hour 1 and 2 after arrival at rest depot compared to all other hours. Cattle in the medium group drank less than cattle in the short group on PreDEP (p < 0.001). Despite some differences between treatments, the very low incidences of drinking, eating, and lying suggests that the effects of the long journey may have overridden any effects of rest durations. The high incidences of drinking at hour 1 and 2 after arrival suggests that cattle prioritised drinking after arrival. Overall, the experiences of cattle undergoing transport are complex, and while rest duration is important, the quality of rest requires further research.

Welfare at control posts: an Italian scenario

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Long journey is an animal welfare concern, causing possible health problems or death. For this reason, in Europe, Council Regulation (EC) 1/2005 regulates journey conditions for different species. After the maximum journey duration, animals must be unloaded for resting, watering, and feeding at control posts (CPs) for at least 24 hours. At CPs, animals must be checked by Official Veterinarians (OVs) to ensure their welfare conditions and evaluate their fitness for further transport (FFT). This study documented the activity of a CP in Southern Italy from 2019 to 2024 analyzing Journey Logs and inspection reports filled by the OVs. A total of 97 trucks stopped at the CP, transporting 24,119 animals. The number of journeys increased progressively during the studied period up to 32 journeys in 2024. Transported species were mainly sheep (78.4%), followed by cattle (17.5%) and pigs (4.1%). Trucks came from France (71.1%) and the Czech Republic (14.4%), which were also the commonest nationalities of the journey organizers, and went to Greece (70.1%), Malta (15.5%), and Italy (10.3%). Trucks had a single stop (92.4%) before reaching the CP, with an average duration of 1.2 hours, for watering (76.7%) or driver's rest (15.6%). A maximum stop duration of 9 hours was recorded in one case, due to a truck breakdown. The average space allowance was always compliant with EC 1/2005, with an average stocking density of 0.34 and 2.4 m²/animal for sheep and cattle, respectively, and of 77.5 kg/m² for pigs. The transport documents were almost always in order, with only 6 cases (6.2%) showing issues related to driver license mismatches. The journey duration and the resting time at the CP were always compliant with European standards. Cases of mortality were reported in 4/97 trucks, and 10 dead on arrival were recorded in a truck transporting piglets weighing less than 25 kg, which also had transport issues and incomplete documentation. All other animals were judged FFT. Our data describes journeys stopping at a CP in Southern Italy, highlighting the main welfare issues and the positive aspects of stakeholder's work.

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Theatre 6

In-vehicle microclimatic conditions during livestock transport - using technology and weather data to improve animal welfare

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Transport of live animals is crucial for the livestock production chain. However, exposure to adverse microclimatic conditions during transit challenges animal welfare. Other environmental conditions than temperature influence heat load during transport, e.g. high humidity, thermal radiation and wind speed. So far, though, research and governance on in-vehicle conditions have focused on temperature. In a recently started research project funded by EFSA, we aim to use newest technology to develop and validate a comprehensive algorithm that describes relationship between weather forecasting data (temperature, humidity, solar radiation), space allowance, type of vehicle ventilation and effective temperature (temperature and humidity) within compartments of vehicles transporting weaned cattle in EU. The project involves development of a model based on computational fluid dynamic (CFD) simulations, which will be followed by a sensitivity analysis, leading to the final outcome: a user-friendly and fast microclimate evaluation tool with the potential to be used as part of risk management. The study involves freshly weaned beef cattle that is transported to the fattening unit, and fattening cattle that are transported to the slaughterhouse as these categories are the most frequently transported within the category of weaned cattle, especially during summer months with the increased risk of heat stress. The type of vehicles that will be modelled will constitute a foundational case for future developments. Livestock vehicles for transport of weaned cattle have multiple decks and more than one compartment per deck. The latter provides an environment of intermediate complexity and integrates a flexibility for generalizing models towards other livestock vehicles in the future. Model calibration and validation will be carried out by collection of data from consignments transporting weaned cattle in Spain, taking warmer and colder seasons and animal density into consideration.

Semi-mobile slaughter units for cattle: a benefit for animal welfare?

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Pre-slaughter transport and handling can cause stress, negatively affecting animal welfare. Special slaughter-units for on-farm slaughtering can present an alternative avoiding transport and stress. This study, carried out in the MuD-project “StronGeR”, aims to evaluate different semi-mobile slaughter units (MSU) for cattle with regard to their impact on animal welfare. Three different MSU were evaluated: Two of them with captive bolt stunning and one with gunshot method. Forty-five cattle were included in the study. Beef cattle breeds of all ages and sexes were involved. Animal behaviour was assessed via single animal scoring concerning stress indicated behaviours such as vocalisation, defensive movements or attacking during driving and fixation on the day of slaughter. The stunning effectiveness was assessed directly after stunning and during bleeding. Out of the animals with longer driving duration (> 2 minutes), 78.6% showed stress behaviour. With head-fixation durations of > 1 minute and inadequate design of driveway (e.g. steps), 100.0% of the animals showed stress behaviour. Suitable driveways did not lead to any stress behaviour. Isolation of the animals resulted in a stress reaction in 40.0% of cases. With both captive bolt stunning and gunshot method, 100.0% of the animals collapsed following the first shot. However, 10.0% of the captive bolt stunning cases and 6.7 % of the gunshot cases required an additional shot due to questionable stunning effectiveness. Animal behaviour was affected by human behaviour during driving and stunning, as well as environmental impacts such as driveway design, isolation from the herd, driving duration or fixation time. Short-time stressors had a low impact on stress behaviour of the examined cattle. Pre-slaughter handling is crucial for animal welfare. Using MSU can improve cattle welfare at slaughter. Further studies are needed to evaluate the practical use and the effects of MSU in detail.

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Theatre 8

Effects of reduced stun failures at slaughter on animal welfare and slaughterhouses' economic outcomes

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Stunning before bleeding is essential to ensure the humane slaughter of animals for consumption. The stunning process is complex, with high demands on equipment and properly trained staff. Failures in the stunning process can lead to unnecessary suffering for the animals, compromised worker safety, increased stress for workers as well as animals, and reduced production efficiency. From the slaughterhouses' perspective, stunning strategies are also an economic issue; this implies that a thorough understanding of the economic consequences of implementing strategies to avoid stun failures is crucial for grasping slaughterhouses' incentives to adopt such strategies. This study assessed the economic impact of investing in new stunning equipment and improving routines to reduce the occurrence of stun failures, using data from Swedish cattle and pig slaughter. A stochastic partial budgeting approach was used to analyse economic outcomes while considering the risk of individual components of the model and to assess the most important model component driving the outcome variable. By identifying key economic information to improve stun quality, this study will provide essential information for decision-makers at both the slaughterhouse and societal levels. The results will contribute to enhancing stun quality – and consequently, animal welfare – of cattle and pigs at slaughter while supporting slaughterhouses in making informed decisions that align with best practices and assessing their economic impact. We argue that these insights could be used as a basis for discussions on refining stun quality protocols and encouraging slaughterhouses to implement measures that ensure compliance with welfare standards. It will also bring clarity on whether policy incentives may be needed to support slaughterhouses' use of proper stunning techniques.

Carbon dioxide stunning of pigs induces the expression of anxiety associated genes in the amygdala

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When pigs are stunned with high concentrations of carbon dioxide (CO₂) prior to slaughter, aversions can be observed until loss of consciousness. These observations were shown to be less frequent when pigs were stunned with inert gases. In humans the inhalation of 35% CO₂ can cause panic attacks. It is known that different emotional states induce different transcriptional profiles in several brain areas. As the amygdala is known to be involved in processing fear, we hypothesized to find differences in the amygdala transcriptome depending on the gas used for stunning of pigs. Therefore, 27 male and female crossbreed slaughter pigs were stunned with either argon (Ar), a mixture of nitrogen and Ar, both with a residual oxygen (O₂) concentration < 1% or CO₂ (< 2% O₂). These 27 pigs were a subset of 1300 pigs, which were part of the project for “Testing inert gases in order to establish replacements for high concentration CO₂ stunning for pigs at the time of slaughter (TIGER)”. From each pig, amygdala samples were collected, total RNA isolated, and sequenced. Comparison of the amygdala transcriptomes showed that stunning with CO₂ induced different transcriptional profiles than stunning with inert gases. For example, gene set enrichment analysis revealed that G protein-coupled serotonin receptor activity was affected only in the comparisons of inert gases with CO₂. Within this gene set, the serotonin receptors HTR1A and HTR2A were downregulated in pigs stunned with CO₂. Both are described to be linked to psychological disorders and fear processing in humans and mice. Our findings support that pigs exposed to high concentrations of CO₂ during stunning may experience fear-like emotions.

Session 32

Theatre 10

Welfare of pigs in end-of-life emergency situations: A Scoping Review of current research and gaps in knowledge

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Deaths of pigs are inherent in meat production. Most are slaughtered, while others are euthanized, die from disease or injury, or are culled in mass depopulation programs due to disease outbreaks. A smaller proportion die or are killed or euthanized in emergency situations such as accidents or disasters. This study focuses on pigs dying in emergencies, including overturned pig transport vehicles, barn fires, and natural disasters. Extensive EU legislation exists for the killing of pigs for slaughter and on-farm, with procedures to minimize compromised welfare. However, there is a need for more understanding of how unconsciousness and death are assessed when pigs die or are killed outside slaughterhouses. To protect the welfare of pigs and involved people as much as possible during killing in emergencies, the aim of this study was to create an overview of existing knowledge on assessing unconsciousness and death in these situations. A scoping review was conducted in the WoS database, following the PRISMA ScR protocol, with search terms in 3 categories: “emergency,” “pigs,” and “death.” Of 816 hits, 30 met inclusion criteria, covering emergencies such as natural disasters, extreme weather and more general depopulation methods. The retrieved studies are geographically diverse, but predominantly North American. None mentioned transport accidents and there is a gap in knowledge of criteria for loss of consciousness and death in these situations. Eight papers addressed the human experience of killing animals in emergencies. These studies reported that it is mentally taxing (especially killing healthy pigs) and can include physical strain when large numbers of animals are killed. The current knowledge of welfare of pigs during each type of the end-of-life emergency situations is limited. Climate change, along with increasing intensive animal production and cross-border transport, suggests an increasing risk of such emergencies. Further research and protocol development are needed, especially in countries with extensive pig production, transport, and trade.

Effect of transport container space allowance, height, and thermal conditions on the physiological response and metabolism of rabbits

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The study simulated space restrictions and thermal conditions experienced by rabbits during 8-h truck transport. Rabbits (1.99±0.04kg) were deprived of food and water for 6 h to simulate commercial fasting before transport and placed in cages in a climatic chamber. The cages had space allowance of 182, 143 and 111 cm²/kg (S182, S143, S111), and heights of 20 and 35 cm (H20 and H35), creating six cage types, each replicated three times with five rabbits per cage. Nine sessions were conducted with 90 rabbits exposed to 3 thermal conditions: thermal-neutral (TN: 20°C, 70% HR), moderate (mHS: 25°C, 60% HR), and severe heat stress (sHS: 30°C, 55% HR). After each session, rectal temperature (RT) was measured in three rabbits per cage and then weighed. The remaining two were weighed, sedated, and blood samples were collected before euthanasia for haematocrit (HAE), glucose (GLU), lactate dehydrogenase (LDH), creatine kinase (CK), free fatty acids (NEFAs) and corticosterone (COR). Results showed that body weight loss (BWL) was similar between TN and mHS (51g vs 57g, respectively; *p*=0.45). However, BWL increased in sHS compared to TN (+23g, *p*<0.01) and between sHS and mHS (+17g; *p*=0.03). Rabbits in S111 had a higher BWL compared to S182 (+8g, *p*=0.03) and tended to have a higher BWL compared to S143 (+7g; *p*=0.07). Thermal treatment had no effect on RT, but S143 and S111 increased the TR compared to S183 (+0.4°C; *p*=0.01). Interactions resulted in an increase in RT in mHS-S111 compared to TN-S111 (+0.3°C; *p*=0.05) and in sHS-S111 compared to mHS-S111 (+0.4°C, *p*=0.02) and TN-S111 (+0.6°C, *p*<0.001). RT also increased in sHS-H35 compared to mHS-H35 (+0.4°C; *p*<0.01) and TN-H35 (+0.3°C; *p*=0.05). Cage height had no effect on BWL but H35 negatively affected RT due to piling (+0.4°C; *p*<0.01). HAE, LDH, NEFAs and CORT were stable across thermal conditions and cages. GLU and CK increased with reduced space allowance, while CK also increased in H20. Research is needed to replicate it in real transport scenarios.

Session 32

Poster 12

Enhancing Animal Welfare: Assessing Unconsciousness and Death in Cattle and Small Ruminants through Objective Measures and Protocols

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The European Partnership on Animal Health and Welfare (EUPAHW) is designed to improve animal health and welfare through a collaborative and data-driven approach. The SoA14 aims to improve the assessment of unconsciousness and death in livestock at slaughter with and without stunning, euthanasia, and emergency killing. Group 3 focuses on developing objective methods, tools and protocols to assess unconsciousness in cattle and small ruminants. The project is structured into four key tasks: (1) systematic reviews of unconsciousness assessment methods, (2) development of new protocols for euthanasia and emergency killing, (3) validation of animal-based measures (ABMs), and (4) communication and dissemination. Here we describe the results of the comprehensive review that was conducted to assess protocols, identify gaps, and improve methodologies using a standardized approach across partners. The review included experimental studies and reviews available in literature, focusing on indicators of unconsciousness such as body posture, muscle tone, eye-related reflexes (corneal, palpebral, pupillary), respiratory patterns, spontaneous blinking, and vocalization (stunning, at sticking, during bleeding). Body posture and movement, eye-related indicators, signs of pain or normal function at the key stages of slaughter have been generally reported as the most effective ABMs to check consciousness after stunning or neck cut. Evaluation of unconsciousness in non-stunned animals can be challenging due to the risk of return to consciousness. On the basis of the results from this review, future phases of the project will focus on developing and validating standardized protocols to validate unconsciousness at slaughter, integrating physiological and behavioural indicators. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Natural extract to reduce transportation stress in pigs before slaughter

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Pig transportation to the slaughterhouse is a source of stress, impacting their welfare and meat quality. Factors like grouping or vibrations can cause stress and aggressivity. For the slaughterhouse, this can induce injuries, bruises or Salmonella shedding[1]. Those issues linked with pigs' transportation must be dealt with for animal welfare and sustainability and safety of meat production. Natural solutions can solve this issue in a wide range of production without any withdrawal period. This study looks at the effect of lemon balm extract (*Melissa officinalis*) in association with magnesium in reducing the pigs' stress during transportation to the slaughterhouse. A total of 1093 pigs in six batches from the same farm were studied. Three batches have been supplemented with the product for at least 3 days, and the other three have not, as a control. The same truck brought pigs to the slaughterhouse and kept them in separate lairing pens to identify the groups. For both groups, a behavioral score was evaluated using the quality of the unloading process and the pigs' behavior one hour after unloading. Each parameter has been weighted according to its relevance to pigs' welfare in the literature to calculate a welfare score. After slaughter, the meat quality is recorded by a measure of the meat's pH and the proportion of abnormal pH values. Depending on the data distribution, all data have been processed by batch, with a T-test or a Mann-Whitney test. Regarding the welfare indicators, supplemented pigs have a better behavioral and welfare score than the control group (p-value < 0.05). At the unloading, fewer interventions were required (p-value < 0.05), and fewer pigs were sliding (p-value < 0.05). One hour after unloading, test pigs seem to have fewer fights (p-value < 0.1). Test groups had a lower boar taint prevalence but it was non-significant. Meat quality was not found to be significantly different between the two groups. Those results show a feed additive's impact on lowering transportation-related stress with a short duration of supplementation. Keywords: Pig, Transportation, Natural additive, *Melissa officinalis*, animal welfare [1] E. Lambooi (2007). Transport of pigs. CABI eBooks, pp.228–244. doi:<https://doi.org/10.1079/9781845932190.0228>.

Session 32

Poster 14

Mixing unfamiliar lambs upon arrival at the abattoir affects their social behavior and meat traits

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The aim of the study was to determine the effects of mixing unfamiliar lambs when they arrive at the abattoir 28-29h before slaughtering on social behavior and meat quality. Forty TexelxCorriedale male lambs were transported together in the same truck for 5 h to a slaughterhouse where they were separated into two experimental groups homogeneous according to their body weight: twenty lambs were allocated in a single resting pen (1.13 m²/animal) together with other ten familiar male lambs (CON group), while other twenty lambs were allocated together in a resting pen with similar characteristics, but mixed with ten resident unfamiliar male lambs (MIX group). Animals were kept in the resting pens for 28-29h before slaughter and were slaughtered on the same day following standard procedures. Lambs' behavior was recorded during the premortem period. The longissimus thoracis muscle pH, and temperature were recorded 45 min and 24h after slaughter. Meat traits were measured in 24h and 7-day-aged meat from the longissimus lumborum muscle. The MIX lambs displayed a greater number of both sexual behaviors and the sum of agonistic and sexual behaviors than the CON lambs (P<0.001 for all). The MIX lambs had lower carcass pH (P=0.04) and temperature (P<0.001), and meat was lighter (P=0.016), redder (higher a*values; P=0.003) and more tender (lower Warner Bratzler shear force values; P=0.048) than those from CON lambs. The MIX lambs tended to have more glycogen content than CON lambs (P=0.057). However, no differences were detected regarding sarcomere length or lactate residual content. Mixing unfamiliar male lambs during a 28-29h resting period before slaughter affected the lambs' social behavior and induced changes in meat quality. Lambs exposed to MIX treatment showed carcass with lower values of pH and temperature, meat lighter, redder, and more tender, with normal sarcomere lengths and residual lactate content.

Time series analysis of pig slaughterhouse data

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The aim of the present study was to model or predict the weekly number of pigs in slaughterhouses across Switzerland. Six distinct models, formed as an ensemble, were developed. These were primarily linear additive mixed models with the following model structure: $y_i = \beta_0 + f(\text{time}_i) + f(\text{seasonal}_i) + \text{holiday}_i + \text{predictors}_i + \varepsilon_i$. In this notation, y_i is the predictor value for week i , $f(\text{time})$ a term for capturing temporal dependencies, $f(\text{seasonal})$ a term accounting for seasonal variations, holiday a fixed factor for holidays, and predictors a set of fixed factors for lagged predictor variables. Model 1: Model including an autoregressive component (AR1, $f(\text{time})$), fixed factor for holidays, and random factor for calendar week ($f(\text{seasonal})$). Model 2: Extension of Model 1, incorporating lagged predictors: sold pig semen, number of piglets on the market, and animals registered in the animal traffic database. Model 3: Model with a smooth temporal effect (thin plate spline penalty, $f(\text{time})$), fixed factor for holidays, and a cyclic cubic regression spline for calendar week ($f(\text{seasonal})$). Model 4: Model 3, plus the aforementioned lagged predictors. Models 5 and 6: Variants of Models 2 and 4, excluding sold pig semen due to its significant time lag. For final weekly predictions, outputs from all six models were pooled. For forecasting multiple subsequent weeks, models were iteratively updated utilizing predictions from the preceding week. To evaluate the actual forecast results, we use the last twelve weeks of the data set as the test data set. The models are trained without this data. Overall, the actual movement is well recognized by all models. At the end of the period under consideration, larger deviations can be seen, but this is to be expected due to the distance to the last point in time with available data.

Session 33

Theatre 1

Selection for reduced methane emissions based on the genetic associations between abundances of microbial genes and metabolites in the rumen

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Short-chain fatty acids (SCFAs) are the primary energy source of ruminants and dietary interventions that alter the ratio of acetate-to-propionate are known to be related to methane (CH₄) emissions. However, there is a lack of knowledge of the role of animal genetics in these metabolisms. Additionally, the genetic associations between SCFAs metabolism and functional microbial genes identified using the KEGG database need to be studied, as they could enhance microbiome-driven breeding for reduced CH₄ emissions. Therefore, we tested the hypothesis that animal genomics influence the abundance of microbial genes favourably associated with propionate production and reduces CH₄ emissions. In a Bayesian analysis, we used data from 363 beef cattle genotyped with the 50k SNP chip, measured for CH₄ emissions in respiration chambers and analysed for ruminal whole metagenomics and SCFAs profiles. Heritability estimates for molar proportions of acetate and propionate were 0.22 (± 0.18) and 0.34 (± 0.24), respectively. These SCFAs showed genetic correlations with CH₄ production (g/day) at 0.51 (prob. $\neq 0$, Po=0.84) and -0.57 (Po=0.88), respectively. The microbial gene of anaerobic sulphite reductase (asr) subunit C exhibited strong genetic correlations with acetate (-0.74, Po=0.95) and propionate (0.87, Po=0.99) concentration and was negatively genetically correlated with CH₄ production. These findings indicate that including the asr gene abundances as an additional trait in microbiome-driven breeding would contribute to reduce CH₄ emissions by shifting ruminal metabolism toward higher propionate production relative to acetate. Further analyses will explore whether other identified microbial genes are associated with both SCFAs and reduction in CH₄ emissions to improve our understanding of how animal genomics is linked to functional microbial regulation of SCFAs metabolism. Overall, our results provide evidence supporting the hypothesis that there are animal genetic effects on the abundances of microbial KEGG genes affecting the concentration of SCFAs in the rumen, which are genetically correlated with CH₄ production.

Dynamism of Calves' Microbiomes: Stability, Resilience, and Patterns in Health and Disease

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To uncover the intricate relationship between the microbiome and host in early life, we monitored the temporal dynamics of the airways and gut microbiomes and their associations with immune and clinical markers in 30 veal calves over their first five months. Our findings reveal that microbiome stability and individuality are distinct and specific to each body site. Notably, the fecal microbiome demonstrated greater diversity, bacterial biomass, stability, and resilience as the animals matured, underscoring its essential complexity and functional changes in the gut environment. In contrast, the nasal microbiome displayed a more skewed ecological structure and higher turnover, suggesting dynamic rearrangements due to microbial immigration and mucociliary clearance. Collectively, weather, diet, and antibiotics influenced the variation observed in both the fecal and airways microbiota compositions. Host regulatory mechanisms, particularly the immune system, also contributed to this variation. Rectal temperature emerged as a significant indicator of microbiota structure in the airways and fecal samples, more so than diarrhea and respiratory scores. Interestingly, as the calves aged, their microbiome dynamics appeared synchronized across different body sites, hinting at an overarching system influenced by the holobiont. This study offers a comprehensive view of multi-site microbial dynamics and their relationship with the host and the environment, providing valuable insights for optimizing management strategies during this critical developmental window.

Session 33

Theatre 3

Microbiome dynamics across the lifespan of Australian Angus cattle

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Understanding the intricate nexus between cattle welfare and microbiome dynamics holds profound implications for enhancing animal's health, productivity, and animal welfare. In cattle production, animals undergo a series of production boundaries inducing stress and potentially increasing their susceptibility to infectious diseases. In this regard, immune competence (IC), corresponding to a combined measure of cell-mediated (cell-IR) and antibody-mediated (Ab-IR) immune responses, is a useful disease resistance trait that can be used in conjunction to production traits to select for resilient and fast-growing cattle. In this study, we characterised the microbiome dynamics of a cohort of 67 steers with known genotype and IC across multiple time points including marking, weaning, pre-feedlot and during feedlot. Metagenomics libraries were prepared using the Nextera DNA flex kit and sequenced on the NovaSeq Illumina platform. In total, we recovered 87, 348 and 1532 medium to high-quality dereplicated MAGs from nasal mucus, saliva and feces samples respectively. Microbial community composition and function differed between sample types and timepoints revealing important microbial dynamics across the lifespan of cattle. Metaproteomics also highlighted key microbial functional pathways being expressed at specific time points. By linking microbial functionality and traits such as IC and growth, we hope to better understand the interplay between the cattle and their associated microbes to further improve immune resilience in beef cattle.

Shotgun metagenomics reveals microbial impact on feed efficiency and methane emission in transition dairy cows

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Rumen microbiome composition can directly impact feed efficiency and methanogenesis, making it a key factor in sustainable dairy production. We investigated the association of rumen microbiome with feed efficiency and methane emissions during the transition and lactation peak periods using a deep shotgun metagenomics approach. Feed efficiency was measured as residual feed intake (RFI) and methane production (MP) was assessed by GreenFeed; Primiparous and multiparous cows had their RFI and MP classified as low or high (below or above the mean) between 56 to 210 DIM. Ruminal content from 64 cows was sampled at -21, 23, and 65 days relative to parturition. Total DNA was extracted, sequenced (50M reads) and quality-controlled. Taxonomy classification was performed using Sourmash with GenBank database. Final microbial counts were normalized using centered-log ratio. Microbial abundances were analyzed by fitting distinct mixed models that included the main effects of sample day (DAY) and RFI or MP, their possible interaction (i.e., DAYxRFI or DAYxMP) and the random effect of sire. A total of 3,616 microbial species were identified. Among them, 208 species showed significant DAYxRFI ($p < 0.05$), from which 23 belonged to the genus *Prevotella* and were more abundant in high-efficiency cows after calving. Likewise, 286 species showed significant DAYxMP ($p < 0.05$), including 33 from *Lachnospiraceae* genus, predominantly found in low-methane emission cows after calving. Additionally, the abundance of 82 and 141 species showed significant differences for RFI and MP, respectively, with no interactions with DAY. Notably, three *Methanobrevibacter* species were consistently more abundant in less feed efficient and higher methane emitting cows. These results highlight key microbial species associated with RFI and MP, providing insights that could guide efforts to improve feed efficiency and mitigate methane emissions in dairy.

Session 33

Theatre 5

Comparative analysis of the cattle rumen microbiome across three countries using long-read metagenomic sequencing for methane reduction

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The rumen microbiome plays a role in ruminant health and methane emissions, yet country-specific differences in the rumen microbiome remain largely unexplored using modern sequencing techniques. This study used long-read metagenomic sequencing to analyze the rumen microbiome of dairy cattle in The Netherlands (NL, $n=961$ Holstein) and Spain (ES, $n=445$ Holstein) and the oral microbiome of beef cattle from Australia (AU $n=218$ Brahman/Brahman-cross) and compared them. Methane emission (measured via Sniffer in NL/ES or GreenFeed in AU) and genotype data were integrated to explore potential methane mitigation strategies useful across countries. We assessed microbial diversity across locations, to quantify intra- and inter-country variation and explored the association between microbial taxa or gene abundances, with methane output. Genotype data further enabled estimates of heritability of microbial abundances and cross-country genetic correlations. Preliminary results indicate country-specific microbiome differences, suggesting that environmental and farm management factors may shape microbial communities. Moreover, we identified microbial taxa and genes correlating with methane emissions and heritability estimates suggest a genetic influence on microbial composition. This study provides novel insights into geographical and genetic drivers of the rumen microbiome and its impact on methane emissions, supporting breeding and management strategies to reduce cattle's environmental impact. This work received funding from: the EU's Horizon Europe research and innovation program under the grant agreement No 01059609 (Re-Livestock), the Knowledge and Innovation Agenda of the Ministry of Agriculture, Fisheries, Food Security and Nature, the COST Action CA22112- European Network on Livestock Phenomics and the Meat and Livestock Australia MDC project P.PSH.2010.

Co-occurring Bacterial Guilds in Dairy Cows: Diversity, Composition and Influence on Host Performance
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In the framework of the EU HoloRuminant project, we applied non-negative matrix factorization (NMF) to decompose the rumen bacterial microbiota into bacterial guilds, termed Ruminosignatures (RS), and explored the associations of RS with methane (CH₄) and CO₂ emissions. NMF was initially applied to a discovery metataxonomic dataset of 816 Holstein and 200 Nordic Red cows from seven farms located in UK, Italy, Sweden, and Finland. Furthermore, we employed as validation set data from 120 Holstein cows from France. The host genetic control of RS abundance was estimated using the discovery cohort. Our results suggest that the ruminal microbiota can be accurately described by combinations of at least seven RS, which capture 98% of the variance in the bacterial ecosystem. Furthermore, host genetics seems to play a deterministic role in RS assembly, with heritability values between 0.12 and 0.44. Based on their prevalence across both datasets, four RS driven by *Prevotella* (RS-Prev), *UBA2810* (RS-UBA2), *Fibrobacter* (RS-Fibr), and *RF16* (RS-RF16) seem to be core components of the dairy cows ruminal microbiota. In the discovery set, we detected a positive association of RS-RF16 with CH₄ and CO₂ emissions, whereas RS-Fibr and RS-UBA2 exhibited a negative relationship. Notably, despite variations in environmental conditions, farm management practices, and procedures for measuring CH₄ emission, the association patterns of RS-UBA2 and RS-RF16 with CH₄ and CO₂ emissions were consistently replicated in the independent French dataset. These findings highlight the potential of the RS concept as a tool for assessing the functional characteristics of the rumen microbiota that could be used for developing strategies to reduce greenhouse gas emissions in ruminants.

Session 33

Theatre 7

Milk shotgun metagenomics upon subclinical intramammary infection in Holstein cattle
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Milk harbors microorganisms whose interactions are key to mammary and host health. Understanding these dynamics is of particular interest during mastitis, giving information about its pathogenesis, development, and outcomes. This study characterizes the milk metagenome of dairy cattle with subclinical intramammary infection (sIMI) over time. In detail, single-quarter milk samples from 200 mastitis-resistant and 200 mastitis-susceptible Holstein cows underwent initial microbiological screening (T0). Results classified animals into two groups: those with at least one quarter positive to *Staphylococcus aureus*, *Streptococcus uberis*, or *Streptococcus dysgalactiae* (Pos), and those negative for all quarters (Neg). A subset of 86 animals was sampled again 2 (T1) and 5 weeks (T2) after T0. Shotgun sequencing on Illumina Novaseq X Plus platform was performed on 56 individual milk samples: 26 from T1 (9 Neg, 17 Pos) and 30 from T2 (11 Neg, 19 Pos). A total of 16 animals were evaluated both at T1 and T2. Sequencing generated 150-base paired-end reads, with an overall mean of 90,216,959 reads/sample, GC content of 50.05 %, and Q30 of 85.84 %. After raw data trimming and host DNA depletion, sequences will be analyzed to profile the composition of microbial communities using MetaPhlAn 2.0 while their metabolic potential will be assessed using HUMAnN2 tool. Finally, analysis of beta-diversity and differential abundant taxa among different microbiological and resistance/susceptibility conditions will be performed. The findings may highlight significant changes in milk microbiome dynamics over time in the presence of sIMI, as well as specific patterns associated with increased susceptibility/resistance to infection. Identifying these microbial features could support early disease detection and prevention efforts. Funded by the European Union – NextGenerationEU under the National Recovery and Resilience Plan (PNRR) – Mission 4 Education and research – Component 2 From research to business – Investment 1.1 Notice PRIN 2022 PNRR, “MASTITOMIC” project, proposal code P2022C74R9 – CUP C53D23009640001

Exploring Milk Somatic Cell Metagenomics: Links to Feed Efficiency in Holstein Dairy Cattle

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This study investigated differences in the milk somatic cell (MSC) metagenome between divergent feed efficiency (FE) groups. Eighty-five first-lactation cows (60–150 days in milk) were ranked for FE based on their official Canadian genomic evaluation. The High-FE group included 30 cows, while the Medium-Low FE group included 20 cows representing an average FE. The MSC pellets were obtained after milk centrifugation, and DNA was extracted using the Qiagen DNeasy PowerFood Microbial® Kit. The Amplicon Sequence Variants (ASVs) and taxonomic assignments were inferred using DADA2, with phyla filtered by prevalence using phyloseq. Alpha diversity indices (Observed, Shannon, Simpson, Fisher; significant when $p < 0.05$) and beta diversity were calculated. Exclusive ASVs were identified using Venn diagrams and differential abundance was assessed with DESeq2 (FDR < 0.01). Functional prediction of MetaCyc pathways was performed using PICRUSt2 (FDR < 0.05). A total of 397 ASVs were identified across the dataset. No significant differences in alpha diversity indices were observed between FE groups ($p > 0.05$), indicating minimal impact on microbial richness or evenness. PCoA revealed partial overlap between groups, suggesting subtle association with MSC metagenome. Two exclusive ASVs were identified in the High-FE group (Bacteroidaceae and Anaerovoracaceae). Differential abundance analysis identified 32 significant ASVs, with 13 upregulated in High-FE and 19 in Medium-Low FE groups. ASV401 (Erysipelotrichaceae) and ASV93 (Paludibacteraceae) exhibited the greatest fold-change values, with log-fold changes of -2.39 and 2.53, respectively. Fourteen metabolic pathways differed significantly, with six enriched in High-FE and eight in Medium-Low FE groups. Significant differences highlighted pathways linked to amino acid metabolism. Overall, this study highlights microbial and metabolic differences in MSC between divergent FE groups, with distinct ASVs and metabolic pathways linked to FE in dairy cattle.

Session 33

Theatre 9

Distinct gut microbiota response to heat stress in two rabbit maternal lines

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This study uses different modelling approaches to explore the impact of heat stress on gut microbiome in two maternal rabbit lines (LP and A) with distinct longevity. It hypothesizes that heat stress differentially affects the gut microbiota of these genetic lines, reflecting differences in physiological responses and providing potential biomarkers for heat stress adaptation. Faeces samples were collected from high-longevity line LP ($n=101$) and standard-longevity line A ($n=98$) under heat stress (Temperature-Humidity Index, THI: 25.7 ± 1.2) and thermal comfort (THI: 17.5 ± 1.7). The analysis included microbiota diversity and genetic line classification (A and LP) under both thermal conditions, using Random Forest (RF), Partial Least Squares Discriminant Analysis (PLS-DA), and Bayesian Regression (BayesC). Heat stress increased Shannon index ($p=0.01$) and altered beta diversity (Aitchison $p < 0.001$). This is likely due to intestinal barrier disruption, which facilitates pathogen proliferation. Prediction accuracy and key selected taxa distinguishing lines A and LP varied across thermal conditions, showing distinct microbiome responses to heat stress. Area Under the Curve (AUC) exceeded 0.92 under heat stress and 0.87 in thermal comfort. Stress-associated taxa, such as *Erysipelatoclostridium* and *Monoglobus*, were more abundant in the low longevity line A. Concerning comparisons between models, PLS-DA provided higher predictive accuracy and captured a broader range of features, while RF selected fewer but more biologically and taxonomically relevant taxa. These results highlight line LP's longevity and expected resilience, as reflected in a higher abundance of potential heat stress-associated taxa in line A. Additionally, this study showed the value of combining modeling approaches, offering both high accuracy and insight into key taxa driving heat stress responses.

Gut microbiota as predictor of fatty acid composition in Iberian pig

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Intramuscular fat (IMF) and subcutaneous fat (SCF) are key determinants in the sensory and nutritional quality of Iberian pig meat, but their measurement is economically costly and limited to post-slaughter analysis, restricting their use in breeding programs. Advances in predictive modeling have introduced gut microbiota information as potential indicators of complex phenotypic traits in livestock. This study explores gut microbiota as early predictors of fatty acid (FA) composition in both fat tissues (IMF and SCF) in Iberian pigs, reducing reliance on terminal sampling. Microbial data were obtained from 16S rRNA sequencing of 554 faecal samples collected from 226 Iberian pigs at 140 days, 180 days, and the end of the fattening period (~365 days, EFP). Records of 15 FA traits and nutritional indices (PUFA, ω -6, ω -3, MUFA, SFA) from both fat tissues were collected at slaughter. A Bayesian mixed model predicted FA traits including microbial information, with a feature selection (FS) process using Random forest to identify key ASVs and enhance predictability. Results showed that FS consistently improved prediction accuracy, though its effect varied across traits, demonstrating that a reduced subset of microbial data improves model performance. Sampling time was critical: FA prediction accuracies in the SCF often peaked at EFP (e.g., C14:0, 0.73 ± 0.10), reflecting stronger microbial influence on subcutaneous fat, while IMF-FA were better predicted earlier (e.g., C18:3, 0.74 ± 0.09), highlighting early microbial signals. PUFA traits, particularly ω -6 and ω -3 PUFA, C18:2, and C18:3, were the most predictable across tissues, with equal or higher prediction accuracies at 140 and 180 days compared to EFP in both fat tissues, enabling early selection before slaughter. ASVs from Lactobacillaceae and Lachnospiraceae families were identified as key contributors. Our findings highlight the temporal dynamics of microbial influence on fat metabolism, and support the integration of microbiota-based predictors into breeding programs to enhance meat quality.

Session 33

Theatre 11

Interactions between the host genome and gut microbiome in pig feed efficiency: a recursive modelling approach

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The gut microbiome plays a crucial role in swine feed efficiency by influencing nutrient absorption and immunity. Understanding the interactions between the host genome and gut microbiome is essential for optimizing feed efficiency. This study examines the relationship between host genetics, gut microbiome, and feed efficiency in intensively raised Iberian pigs, with a focus on the microbiome's mediating role and its contribution to total heritability. We analysed feed conversion ratio (FCR) and residual feed intake (RFI) from 587 pigs, incorporating 16S rRNA faecal microbial abundance from 156 individuals at 140 and 180 days of age. Univariate analyses estimated heritabilities (h^2) and microbiabilities (m^2), with h^2 of 0.16 and 0.15 for FCR and RFI, respectively, and m^2 of 0.13 and 0.14. Model comparisons showed that incorporating microbiome data improved the goodness of fit. Next, we performed bivariate analyses between feed efficiency traits and each amplicon sequence variant (ASV), followed by a conversion to a recursive modelling approach. The parameters from recursive models allowed us to partition direct genetic effects ($G \rightarrow P$) from microbiome-mediated effects ($G \rightarrow M \rightarrow P$). Microbial heritabilities ranged from 0.09 to 0.85. The total heritability for both traits was slightly lower than direct heritability indicating that the microbiome has a negative influence on the total variability. We identified 14 ASV significantly affecting FCR and 16 ASV affecting RFI, with seven overlapping between the two traits. The recursive modelling approach facilitated the classification of ASV based on their causal effects and microbial heritability. Microbial taxa with low heritability but significant causal effects could potentially be modified through nutritional interventions, while those with higher heritability may be considered for selection programs. These findings highlight the importance of integrating microbiome data into livestock strategies to enhance feed efficiency and sustainability.

The culturable reproductive tract microbiota of chickens

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While the gut microbiota of chickens is well researched, the reproductive tract microbiota has only recently come into focus. The reproductive tract of chickens includes three main sections, the infundibulum into which a mature ovum ovulates to and where fertilization takes place, the magnum which secretes egg white, and the uterus which secretes the calcium shell. We and others have previously utilized 16S rRNA sequencing to show that bacterial DNA is present throughout the chicken reproductive tract. However, as egg white includes many antimicrobial peptides and proteins, it was possible that identified DNA represents dead bacteria. To determine if live bacteria are found in the reproductive tract of chickens, we cultured samples from the different parts of the reproductive tract. We then used 16S rRNA sequencing to identify what grew. We found that bacteria from diverse phylogenies were culturable from the different sections. Furthermore, less bacterial strains (amplicon sequence variants) were culturable from the magnum compared to the other two sections implying it was a selective environment. Future work is required to understand if the bacteria found in the reproductive tract of hens affect sperm motility or survival, fertilization itself, or the secretion of egg elements. Furthermore, these results imply that live bacteria are in the right place to integrate into the forming egg and perhaps reach progeny, and that chicken physiology in the magnum section perhaps regulates this process.

Session 33

Poster 13

The influence of host genetics on gut microbiota and association with body weight in pigs

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This study was conducted to elucidate the regulation of fecal microbiota by the host genome and its association with body weight in Woori black pigs. A total of 923 pigs were analyzed for single nucleotide polymorphisms (SNPs) using the Porcine 80K SNP chip and for fecal microbiota composition using 16S rRNA sequencing. Body weight was measured at five months of age. Quality control (QC) of 75,753 SNPs was performed using PLINK, resulting in the confirmation of 53,087 SNP genotypes. The relative abundance of 324 bacterial genera were estimated using QIIME2. To investigate host genetic regulation of the microbiome, microbiome genome-wide association study (GWAS) analysis was conducted using GCTA. Among the 324 genera, 43 genera with a sample prevalence of 30%-60% were treated as binary traits, while 79 genera with a prevalence of 60%-100% were log10-transformed to satisfy normality assumptions for modeling. Microbiome GWAS identified SNPs significantly associated with microbiota composition based on a Bonferroni-corrected threshold (p-value < 0.05). Notably, a SNP on chromosome 5 (position: 60,981,634) showed genotype-dependent variation in the relative abundance of 33 genera. Furthermore, linear regression analysis between host-genetically regulated genera and body weight at five months of age revealed three genera (*Intestinibacter*, *Lachnospiraceae_ND3007_group*, and *Lachnospiraceae_UCG-007*) with significant associations. These findings provide insights into host genome-driven regulation of gut microbiota and its influence on growth traits. This study contributes to the development of precision microbiota management strategies to enhance pig productivity by leveraging host-microbiome interactions.

The ruminal microbial metagenome and metatranscriptome of low and high methane intensity dairy cows
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Reducing enteric methane emissions is crucial for sustainable dairy farming. Cows with lower methane intensity offer an opportunity to mitigate environmental impact by reducing methane emissions per unit of milk produced, but the composition and metabolism of the rumen microbiome driving these differences remain unclear. The objective of this study was to elucidate differences in composition and metabolism of rumen microbiome in cows classified by methane intensity. A total of 16 Norwegian Red dairy cows (638 ± 86 kg BW; 9 primiparous and 7 multiparous) classified retrospectively as low or high based on methane intensity were used in a trial with a common diet based on grass silage and concentrate at a ratio of 83:17 on dry matter basis, consisting of an 11-day adaptation period followed by 6 days of sample collection, including milk, methane (measured using the GreenFeed system), and ruminal content (collected via esophageal tubing). The ruminal metagenome and metatranscriptome were obtained by sequencing DNA and RNA using an Oxford Nanopore PromethION and an Illumina NovaSeq instrument, respectively. Metagenomic data was analysed bioinformatically to create Metagenome Assembled Genomes (MAGs), which were also used as a reference database for metatranscriptome data analysis. No differences were found in the ruminal metagenome (424 high and medium quality MAGs), neither in alpha nor beta diversity between low and high methane intensity animals. At the metatranscriptome level, DESeq2 analysis identified the overexpression of 1,206 genes in high-methane-intensity cows. Subsequent enrichment analysis of these differentially expressed genes indicated an increase in carbohydrate metabolism, with deeper annotation of identifying starch and pectin associated CAZymes. Ongoing efforts will seek to attribute these functions to specific microbial populations to better understand their relevance towards rumen metabolism and methane emissions, which could help design future mitigation strategies.

Session 33

Poster 15

Cross-fostering as a tool to detect the drift of microbial species and to disentangle genetic effects for feed efficiency in rabbits

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We describe the zootechnical performances, the fatty volatile acids and the microbiota of 525 cross-fostered rabbits from the G10 line, which was selected for decreased residual feed intake for 10 generations, and the ancestral line 1001 used as control. The kits were adopted 48h after birth, and were housed individually after weaning. The animals were produced in 3 successive batches and slaughtered at 63 days of age. The batch impacted the performance of the animal during fattening, i.e. the feed intake (FI), the residual feed intake (RFI), the feed conversion ratio (FCR) and the average daily gain (ADG). The batch also impacted the microbiota. Rabbits of the selected line had lower FI, RFI, FCR and ADG. Rabbits of the selected line had also lower resting concentrations of propionate and butyrate by 19% and 30% respectively, as evidenced by cross-fostering to disentangle the impact of the genetic background from the impact of the fostering dam. A discriminant analysis of principal components (DAPC) was used to distinguish the rabbits according to their genetic type and that of their fostering mother. The first axis, explaining the largest part of the variance, discriminates between rabbits according to their respective mother's genotype. The second axis discriminates between rabbits according to their own genotype. The genetic background of the fattening rabbit impacted 20 OTUs while the genetic of the fostering dam impacted 112 OTUs. These results show that the composition of the rabbits' microbiota is more influenced by the adoptive mother's genotype than by the rabbit's genotype

Synchronizing Feeding with Natural Rhythms Improves Health, Sleep Quality, and Longevity in Laying Hens G. Levkovich¹, D. Sagi¹, R. Shmulevitch², I. Halachmi²

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The poultry industry faces constant pressure to enhance efficiency and extend the laying cycles of hens, as chicken eggs remain the most affordable source of animal protein. Our data demonstrated for the first time that synchronizing feed availability with the hens' natural rhythm allowing unrestricted access to feed only during the daytime, significantly increased egg production and health span. Moreover, until now, no suitable model has existed to study the direct effects of nighttime eating on health and reproductive outcomes. Laying hens provide an ideal model due to their daily egg-laying cycle, enabling us to closely monitor the impact of nighttime eating on reproduction and overall healthspan. In our study, we divided hens into two groups: one with synchronized feeding, where access to food was restricted from 7 PM to 7 AM using Arduino-controlled covers, and a control group with unrestricted, ad libitum feeding. We monitored egg production, body weight, and overall health over an extended period. The results indicated that synchronized feeding significantly enhanced laying efficiency and improved health, particularly in older age. Our data indicate that aligning feed availability with the natural sleep-wake rhythm boosts egg production in older hens by more than fivefold, rising from below 15% in control groups to approximately 80% in synchronised hens ($p < 0.001$), enhances sleep quality threefold ($p = 0.015$), and reduces mortality, thereby extending healthspan. These findings have significant implications for the poultry industry, which aims to prolong the laying period, enhance sustainability, and reduce animal waste while improving welfare and economic efficiency. Aligning feeding schedules with natural circadian rhythms may be a novel strategy to optimize egg production and promote long-term poultry health.

Session 34

Theatre 2

Myo-inositol metabolism in two contrasting high-yielding laying hen strains

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In recent years, myo-inositol (MI) has increasingly gained attention in poultry research due to its possible beneficial effects on the metabolism. MI is derived from intestinal degradation of phytate by phytases, especially under low dietary phosphorus, and from endogenous cellular synthesis. This study aimed to characterize the effects of MI in Lohmann LB-Classic (LB) and Lohmann LSL-Classic (LSL) laying hens with equal high laying performance. Methods This study is part of the DFG-funded PFOWL research unit (<https://p-fowl.uni-hohenheim.de>). MI availability was varied in 2 experiments by either MI supplementation (E1) or by reduction of dietary phosphorus (E2) in two different production periods in LB and LSL hens. Blood plasma and tissue samples were collected at week 30 (E1) and 19 and 24 (E2), respectively. MI concentrations in liver, breast muscle and kidney were measured spectrophotometrically; inositol monophosphatase 1 (IMPase1) and MI oxygenase (MIOX) concentrations by ELISA. MI concentrations in plasma and chyme were analyzed using gas chromatography/mass spectrometry after derivatization. Metabolite profiles in plasma were measured using the Absolute-IDQ™ p180 kit. All data were analysed by using Metaboanalyst 6.0. Interesting metabolites were analysed in a 2- or 3-factorial analysis of variance using the MIXED procedure of SAS software. The level of significance was set at $P < 0.05$. Results In E1, MI concentrations in plasma, duodenum+jejunum (D+J) and ileum were increased by MI supplementation. However, in E2 the MI concentrations in D+J and ileum were not different between diet groups. LB hens had higher MI concentrations in D+J (E1) and in ileum in week 19 (E2) than LSL hens. MI and IMPase1 concentrations in muscle (E1) and liver (E2) were higher in LB hens than in LSL hens. Furthermore, LB hens had a higher MIOX concentration in kidney than LSL hens (E1). Conclusions Higher concentrations of MI, IMPase1 and MIOX could indicate an enhanced MI turnover due to an increased pro-inflammatory and pro-oxidative condition. It might be concluded that LB hens are in a more tensed metabolic condition than LSL hens.

Egg quality of laying hens fed hemp seeds and bred according to the agroecological principles of rational grazing

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The aim of the study is part of the HappyHens project that support the use of alternative protein for poultry nutrition in a socio-cultural context where egg-laying hens are bred according to the agroecological principles of rational grazing. The study involves four target breeds, whose eggs are being sampled over a ten-month period. Hens are allocated in mobile hen houses equipped with covered tunnels considering their breed (n.30): Lohmann Brown, Livornese, Milanino and Marans. The 2% of the hemp seed (HS) was included in the basal diet. Quality parameters (whole egg, yolk, albumen and shell weight, shape index, shell thickness and yolk colour) are determined monthly, functional properties are evaluated every three months. Yolks were subjected to the freeze-drying process (1 sample of 2 yolks from each breed, n=10). Diets and eggs yolks chemical analysis were performed according to AOAC. Lyophilized yolks were in vitro digested following the Infogest protocol. At the end of the digestion, the samples were used for the evaluation of total phenolic content (TPC) and antioxidant activity with the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Data were analysed through GLM procedure of SAS accounting for breed and time. The preliminary results obtained after three-month period monitoring showed that yolk colour, shape index, egg weight components and chemical parameters are significantly conditioned by breed, time and their interaction ($p < 0.05$). TPC and ABTS demonstrated a valuable functional profile of egg yolks. For the first time, to the best of our knowledge, the present project evidences the importance of feeding HS in laying hens breed raised according to the agroecological principles of rational grazing supporting the research related to the use of alternative and sustainable ingredients in animal nutrition. Research supported by the University of Milan "My first SEED Grant 2023", project n.23-UNIMI-MFSG-066.

Session 34

Theatre 4

Microbiota and metabolome in vaccine-compatible programming of gut ecosystem through probiotic and prebiotic application

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The developmental trajectory of the poultry gut microbiome provides a critical window for intervention. This study employs advanced multi-omics to characterize the functional consequences of in ovo administration of bioactives. Embryonated eggs (n=928, 4 groups) were injected in ovo at day 18.5 with: IBD vaccine alone or vaccine plus *Bifidobacterium lactis* (probiotic; 10^6 CFU), *Astragalus polysaccharides* prebiotic (AP, prebiotic; 1 mg), or synbiotic (10^3 CFU *Bifidobacterium* + 1mg AP). Feces (day 14) and ceca samples (day 42) were subjected to 16S rRNA gene sequencing (n=6/gr.) and the genera representations were compared. Metabolomics on gut content was performed using Biocrates MxP® Quant 500 kit via LC-MS/MS and FIA-MS/MS, with multivariate (PCA, PLS-DA) and univariate (ANOVA, t-test) analyses to identify differences in metabolite classes ($p < 0.05$). In feces, the synbiotic increased *Lactobacillus* spp. abundance while reducing abundance of potential pathogens, e.g. *Staphylococcus* and *Streptococcus*. However, the abundance of some commensals such as *Faecalibacterium* were also reduced. The prebiotic increased abundance of promising next-generation probiotic candidates *Oscillospira* spp. Metabolically, the probiotic enhanced cysteine and anserine synthesis pathways potentially improving antioxidant capacity, while prebiotic treatment increased carnosine synthesis, involved in muscle tissue function. The synbiotic enriched fatty acid metabolism and ornithine-related pathways which are linked to beneficial metabolic flexibility, enhanced urea cycle function, improved protein utilization, and immune system support. These metabolic shifts corresponded with bacterial community changes, suggesting functional alterations benefiting host metabolism and immunity, particularly in synbiotic-treated animals, along with the vaccination programme. Funded by grant No. 2019/35/B/NZ9/03186 (OVOBIO) NCN Poland.

Integrating machine learning to explore the structure-activity relationship of saponins in *Yucca schidigera*
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Integrating machine learning to explore the structure-activity relationship of saponins in *Yucca schidigera* This study aims to evaluate the bioactivity of *Yucca schidigera* saponins as a case study, linking their chemical structure to antimicrobial activity versus *C. perfringens*, using machine learning techniques. Three yucca extracts were obtained using 100% MeOH (M100), 50% MeOH (M50), and H₂O (M0). They were characterized by high-resolution mass spectrometry (LC-MS), identifying 15 saponins (yield:48.19%), 13 (yield:25.63%), and 11 (yield:26.18%), for M100, M50, and M0, respectively. GNPS software and PLS-DA method were used to analyze the saponins composition of the extracts, revealing two different clusters: one for M100 and the other for both M50 and M0. Antimicrobial tests were done using a microdilution method with *C. perfringens* ATCC13124 strain incubated with M100, M50, and M0 for 24h. Minimal inhibitory concentration (MIC) was considered the lowest dose giving null absorbance, being 750 ppm for M100 and M50, being upper than 2000 ppm for M0. To establish a structure-activity correlation, machine learning techniques (ridge regression and correlation matrix analysis) were applied to the M100 after the column chromatography fractionation. The obtained fractions were characterized by LC-MS and tested for antimicrobial activity. Ridge regression and correlation matrix analysis identified key bioactive saponins, like schidigerasaponin D3/D4, sarsasapogenin, smilagenin, hexogenin, and schidigerasgenin C, revealing a positive correlation between antimicrobial activity versus *C. perfringens* and chemical composition. These findings emphasize the potential of *Y. schidigera* saponins as natural antimicrobials against *C. perfringens* and showcase machine learning as a powerful tool for structure-activity analysis, enabling bioactivity prediction and reducing laboratory screening tests.

Session 34

Theatre 6

Accounting for phosphorus needs in precision feeding of gestating sows over three gestations

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A single diet for gestating sows fails to fit the daily and individual variability of nutrient needs. It may lead to long-term mineral imbalances and impaired performance, with welfare, environmental and economic impacts. A study was carried out to evaluate a new precision feeding (PF) strategy on productive performance, environment, health, and mineral status of sows over three reproductive cycles. The PF strategy involved daily adjustments of dietary energy, lysine (standardized ileal digestible, SID-Lys), and phosphorus (apparent total tract digestibility, ATTD-P). Two batches of 28 Landrace × Large White sows were allocated to PF or standard feeding (SF, single diet) strategy based on their parity, body weight, and backfat thickness. Daily feed allowance was adjusted for energy requirement with iso-net energy diets (9.0 MJ/kg). The PF sows received a daily blend of two diets, one poor (3.3 g/kg) and one rich (8.5 g/kg) in SID-Lys but with the same ATTD-P content (2.6 g/kg), from day 25 to day 80 of gestation. From day 80 to farrowing, a third diet (4.0 g/kg ATTD-P, 8.5 g/kg SID-Lys) was introduced to meet phosphorus requirements. The SF sows received a single diet throughout gestation (2.6 g/kg ATTD-P, 4.7 g/kg SID-Lys). Urinary C-terminal telopeptide of type I collagen (CTX) and calcium to phosphorus ratio monitored mineral status. Over the three cycles, SID-Lys intake was 18% lower for PF than SF sows ($P < 0.001$) without compromising the characteristics of the sows or their litters at farrowing ($P > 0.10$). Adjusting P intake to requirements only during the last month of gestation allowed a greater number of sows to be fed to P requirements, with a better regulation of mineral status, but the improvement of savings on feed costs was not significant (i.e., slight reduction of 4 €/T). Accounting for bone mobilised during the previous lactation would help starting nutrient assessment earlier, and adjustment of supplies throughout gestation could increase the savings on feed costs, reduce P discharges, and potentially benefit for sow longevity, significantly.

Exploring the Quality Traits of Pig Bones: Relationships and Implications

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Bone mineralization and bone strength is important to avoid lameness. Various technologies and methodologies have been used to assess bone characteristics, but they evaluate these traits in different ways. This study aimed to evaluate the relationship between different bone characteristics, including bone density distribution (low, medium and high) determined with computed tomography (CT), bone strength determined with a texture analyser, and chemical composition (Ca, P and ashes). For this purpose, 48 entire males were fed using two feeds: C (control, with recommended nutritional requirements) and R (restricted, providing 60% of the digestible phosphorus requirement). These diets were applied in 4 feeding strategies over 3 phases: CCC, RCC, RRC and RRR. Pigs were slaughtered at an average body weight of 133.80 ± 17.94 kg. At 24 h postmortem, the first (1R) and fourth (4R) last ribs, as well as the third (M3) and fourth (M4) metacarpals, were collected from left half carcasses. These bones were scanned using the Philips Brilliance 16 CT scanner. A bending test was then carried out with a texture analyser (TA.HD.plus Texture Analyser, Stable Micro Systems, UK). After that, ashes were obtained and chemically analysed to determine Ca and P levels of 4R and M4 bones. Results showed high negative correlation between proportion of low-density bone and bone strength within bone, while a strong positive correlation was observed between the proportion of high-density bone and bone strength. Within individual bones, strength was correlated with their ash content (4R=0.41; M4=0.61) but not with P and Ca levels. Additionally, ash content was also negatively correlated with the proportion of low-density bones (-0.85 both for 4R and M4) and positively correlated with the proportion of medium- and high-density bones (0.58 vs 0.78 for 4R and 0.64 vs 0.85 for M4). It can be concluded that, within similar types of bones, there is a relationship among the different bone quality measures, despite these measurements reflect different properties. This research is part of the SUSPORK project (PID2021-124423OR-100), financed by MICIU/AIE/10.13039/501100011033 and co-financed by FEDER, UE.

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Theatre 8

Growth performance and body composition of pigs under dietary protein, amino acid and phosphorus restriction

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Minimizing dietary protein and phosphorus (P) in growing pig diets helps reduce nitrogen (N) and P surpluses in agricultural nutrient cycles. However, consequences of excessive restriction of related nutrients namely digestible (dig) amino acids (AA) and dig P need clarification. This study investigated effects of essential AA and dig P restriction through soybean meal and mineral P removal on growth performance and body composition of 48 Swiss Large White pigs (12 blocks of 4 female or castrated littermates, 24.6 kg). Four iso-energetic (13.7 dig MJ/kg) diets were fed ad libitum. Control (C) grower (8.3 dig lys., 3.0 dig P g/kg) and finisher (6.3 dig lys., 2.2 dig P g/kg) diets met requirements for 40 and 80 kg pigs, respectively. Compared to C, other diets included: -20% crude protein, dig AA (N-); no mineral P with -15% (incl. phytase) and -47% (no phytase) dig P in grower and finisher diets, respectively (P-), and a combination of N- and P- (NP-). Final body composition was assessed by dual X-ray absorptiometry and computer tomography. Statistics included effects of sex, N, P and interactions. Effects of N and P restriction were independent ($P > 0.05$). N- impaired ($P < 0.001$) body weight gain (-10%) and feed conversion ratio, while P- had no impact on growth. Per kg weight, N- decreased ($P < 0.001$) lean tissue (-3%) and increased fat tissue (+14%) contents, without affecting backfat thickness. P- reduced ($P < 0.001$) bone mineral content (-15%), volume (-10%) and density (-4%). N- lead to lower ($P < 0.001$) blood serum protein and urea levels, while P- increased ($P < 0.001$) calcitriol without affecting serum P. Findings indicate that protein and AA restriction deteriorates growth and leads to fatter pigs, whereas P restriction solely limits bone mineralization. Funded by the Horizon 2020 Pigweb.

Impact of protein and phosphorus restriction on carcass composition and meat quality in pork

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Pig production faces challenges in nitrogen (N) and phosphorus (P) nutrition: environmental concerns related to resource utilization, manure composition and feedstuff costs. This study investigated the effects of restricted protein, essential amino acids, and mineral P intake on carcass composition and meat quality in pigs, as part of the Pigweb project. Swiss Large White pigs (24 females, 24 castrated males) were assigned to one of four dietary treatments: a control diet (C), a nitrogen-restricted diet (N-) without soybean meal and low in crude protein and essential amino acids (-20%), a phosphorus-restricted diet (P-) excluding mineral phosphate and containing 15% (with phytase) and 47% (without phytase) less digestible P in the grower and finisher phases, respectively, and a combined N- and P- restricted diet (NP-). Carcass and cuts composition was assessed by DXA, CT, NMRI and dissection. No interactions between N and P restrictions were observed ($P > 0.05$). The N restriction reduced carcass weight by 8% ($P = 0.003$) along with ham, loin, and shoulder cut weights ($P < 0.01$). Muscle cut weights were also lower ($P < 0.01$), and carcass lean tissue weight decreased by 6 to 12%, depending on the measurement method ($P < 0.001$). Fat and bone weights of cuts and meat quality were not affected by diet except loin luminosity ($P = 0.044$). The P restriction had no effects on carcass, cut traits, nor on lean or fat tissue weights. However, P restriction reduced carcass and cut bone mineral density by 12% ($P < 0.001$) and shoulder bone weight by 3% ($P = 0.032$). These findings highlight the unfavourable effects of protein and essential amino acid restrictions on muscle deposition while demonstrating that eliminating dietary mineral phosphate seems feasible with limited impact on carcass composition.

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Theatre 10

Effects of low-protein diets partially balanced in essential amino acids and pen cleaning frequency on growth, protein efficiency and carcass characteristics in growing-finishing pigs

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Reducing dietary crude protein (CP) in pig production can improve sustainability by increasing nitrogen (N) utilization, but low-protein (LP) diets must be fully balanced in essential amino acids and fed under stress-free conditions to maintain pig growth and carcass quality, otherwise performance may suffer. Therefore, forty-eight Swiss Large White female pigs (20 ± 2.2 kg BW) were reared in pens cleaned daily (D) or twice a week (W) and fed standard (ST) or LP diets according to a 2×2 factorial design. The ST grower and finisher diets were formulated to be isocaloric and contain 16.4 and 12.5% CP, respectively. The LP diets were obtained by reducing CP and digestible Lys, Met, Thr, Trp, and Val levels by about 20% compared to the respective ST diets. Weekly BW, feed intake (FI), and carcass composition by dual-energy x-ray absorptiometry were assessed for each pig. Data were analysed by linear mixed models (R, v.4.4.1), with diet, pen cleaning protocol and the interaction as fixed effects, and the litter of origin as random effect. The pen cleaning protocol influenced average daily feed intake and average daily gain. Compared to D, W pigs ate 8.4% more feed and grew 8.7% faster ($p < 0.01$), but total FI was similar as D pigs spent 5.9 more days on feed ($p < 0.01$), resulting in comparable feed efficiency. Regardless of the pen cleaning status, LP pigs were 7.5% less feed efficient, consuming 9.1% more feed and growing 4.9% less per day ($p \leq 0.03$). Carcass protein deposition efficiency was 5.4% lower in W than D pigs and 10.4% higher in LP than ST pigs ($p \leq 0.02$). Carcass fat content was 6.3 and 10.7% higher in W and LP pigs compared to D and ST pigs, respectively ($p \leq 0.04$). In conclusion, partially balanced LP diets improved protein efficiency but impaired growth and carcass characteristics. Despite growing faster, W pigs had fatter carcasses and lower protein deposition efficiency which tended to be more evident when pigs were fed partially balanced LP diets.

Standardized ileal digestible Lysine needs based on growth performance, carcass and ham quality of pigs for dry-cured ham production

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This study aimed to assess the standardised ileal digestible lysine (SID-Lys) needs of ad libitum-fed pigs for dry-cured ham production. The trial included 96 gilts and barrows [45 ± 5 kg initial body weight (BW) at 90 days of age], divided into 4 treatment groups (2 pens/group) receiving feeds with different SID-Lys levels: Low (L), Medium-Low (ML), Medium-High (MH) and High (H). In each treatment, dietary SID-Lys decreased with age in growing (90-118 d), early finishing (119-132 d) and late finishing (133-233 d) periods. The SID-Lys contents (g/kg) were: 6.9, 6.0, 4.2 for the L group; 7.8, 6.9, 5.2 for the ML; 8.3, 7.8, 6.0 for the MH; and 9.3, 8.3, 6.9 for H. Individual feed intake was measured with electronic feeders. BW and backfat thickness were recorded every two weeks. Pigs were slaughtered at 251 d (190 ± 15 kg BW). Growth, feed intake, gain-to-feed ratio, carcass and ham traits were analysed using a mixed model with treatment, sex and their interaction as fixed effects and pen within treatment as a random effect. No differences in growth performance, carcass and ham traits were found between ML and groups with higher SID-Lys (MH, H). However, the L pigs had lower final BW (197 vs 175 kg; $p = 0.016$), daily gain (985 vs 859 g/d, $p = 0.013$), gain:feed ratio (0.316 vs 0.283, $p = 0.004$), carcass weight (164 vs 146 kg, $p = 0.017$) fat cuts yield (234 vs 219 g/kg carcass, $p = 0.028$), and trimmed ham weight (14.8 vs 13.4 kg, $p = 0.030$) than ML pigs. Except for trimmed ham weight, no differences in ham quality traits were found. These findings indicate that the ML dietary SID-Lys levels optimize growth, feed efficiency and increase the carcass and ham weights, with minimal impact on carcass and ham quality traits. Research supported by 1) Agritech National Research Center, funded by the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4-D.D. 1032 17/06/2022, CN00000022). 2) European Union, Next Generation EU, Mission 4, Component 1, CUP: C53D23005470006, Progetti di Rilevante Interesse Nazionale (PRIN), title project: NUTR-PIG grant.

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Theatre 12

Genetic insights into backfat thickness and fatty acid composition in Krškopolje pig: Differential gene expression linked to genetic variants

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Key words: Local pig breed, adipose tissue, shallow DNA-sequencing, RNA-sequencing This study aimed to associate gene expression of the traits underlying backfat deposition, as well as palmitic and stearic acid content by linking RNA-sequencing with genetic variants. From a group of 15 Krškopolje pigs reared under the same conditions, extreme phenotypes ($n = 2 \times 5$) were chosen for backfat, palmitic and stearic acid content. DNA ($n = 142$) and RNA ($n = 2 \times 5$) were extracted and shallow DNA and RNA sequencing were performed to identify genetic variants and differentially expressed genes (DEG). DEG analysis highlighted 4 upregulated (e.g. PVAFEF) and 5 downregulated genes (e.g. heat shock proteins, and ACSM5 involved in fatty acid synthesis) in pigs differing in backfat thickness. Pigs differing in palmitic acid content revealed 4 upregulated (e.g. LPN2 involved in lipid transport) and 5 downregulated genes (e.g. PAPP2 being local regulator of IGF1 bioavailability, CYP26B1 involved in synthesis of cholesterol and lipids). DEG analysis of pigs diverging in stearic acid revealed 9 upregulated (e.g. PDK4 associated to fatty acid oxidation and glycolysis) and 10 downregulated genes (e.g. ABCD7 associated to energy expenditure). Using the WGS data, we identified variants in promoter regions, -1000 to +200 bp relative to transcription start site of genes that were differentially expressed in pigs with extreme phenotypes. Association analysis revealed a significant SNP in promoter region of PDK4 gene, associated with saturated fatty acid content. Given the PDK4 gene role in lipid metabolism, this variant may influence gene expression through transcriptional regulation. This preliminary study is the first to identify key genetic variants and genes associated with increased fat deposition and composition in the Krškopolje pig. Acknowledgement: Funding by Slovenian Agency of Research (grants P4-0133, P4-0220, J4-3094, Z4-60178) is acknowledged.

Effect of dietary mineral P renunciation on NaPi-IIb and TRPV6 protein expression in duodenal enterocytes of two high-performing laying hen strains before and after start of egg laying

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Phosphorus (P) and calcium (Ca) absorption in the intestine is mediated by specific transporters, including NaPi-IIb, an intestinal sodium-dependent phosphate (Pi) transporter, and TRPV6, a key calcium channel in duodenal brush border membranes. Their expression appears to be modulated by luminal Pi levels [1]. This study aimed to evaluate NaPi-IIb and TRPV6 protein expression in the duodenum of two laying hen strains at different production stages in response to P-restricted diet. **Methods.** The study was a part of the DFG-funded P-FOWL research unit (<https://p-fowl.uni-hohenheim.de>). The experiment followed 2×2×2 factorial design: hen strain (Lohmann Brown (LB) vs. Lohmann LSL (LSL)), period (weeks 19 vs. 24), and dietary mineral P supplementation (0 vs. 1 g P/kg feed). After 4 weeks of experimental feeding, at 19 and 24 weeks of age, 10 hens from each group were sacrificed. Duodenal mucosa samples were collected, and brush border membranes were enriched. Semi-quantification of NaPi-IIb and TRPV6 protein expression was conducted by Western Blotting [1]. Data were analysed using a 3-factorial ANOVA, some biologically meaningful correlations were built up [2]. **Results.** Dietary P levels did not affect protein expression of both, NaPi-IIb and TRPV6. However, NaPi-IIb expression increased from 19 to 24 weeks in LSL hens ($P = 0.012$), while LB hens showed no changes across age periods (interaction period*strain $P = 0.040$). TRPV6 expression exhibited no age-related differences in LSL hens, whereas LB hens tended to reduce its expression from 19 to 24 weeks (period*strain $P = 0.074$). **Conclusions.** LB and LSL hens exhibited distinct regulatory patterns for NaPi-IIb and TRPV6 expression across periods. Transporter protein abundance was more influenced by production stage than by dietary P content, reflecting shifts in mineral demand associated with egg laying. Correlations between the expression of NaPi-IIb and endocrine factors, as well as TRPV6 and intestinal P, reflected complex regulatory absorption mechanisms in laying hens. [1] Huber et al. 2015. *Poult. Sci.* 94:1009-1017 [2] Qasir et al. 2025. *BMC Genomics.* 26:129

Session 34

Poster 14

Eggshell Quality of Laying Hens in Free-Run System

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Eggshell Quality of Laying Hens in Free-Run System Quality of the eggshells is crucial for marketing of eggs. The weight of eggs increases by a longer duration of lay cycle. Because of that hens may get problems to provide enough calcium for shell. In the present study hens of Lohmann white and Lohmann brown were included. In each week from the 46th to the 50th week of life, 6 randomly selected eggs of both genetic were analyzed. The egg weight, shell weight, and eggshell thickness, length and width of the egg, height of egg whites and egg yolks, eggshell thickness at the sharp end, in the middle and the blunt end and calcium content of the eggshell were determined. Ca content was analysed by digestion by aqua regia and subsequent measurement with a flame photometer. All values were analysed by using SPSS. Univariate analysis of variance and correlation analysis were used. The weight of the eggs is $65.1\text{g} \pm 4.9$, the eggshell weighs $8.5\text{g} \pm 0.8$. The eggshell contains an average of $3.5\text{g} \pm 0.4$ Calcium, which corresponds to a percentage of 41.9%. The egg index is 75.5 ± 2.9 . The eggshell is thickest at the sharp end with $0.396\text{mm} \pm 0.045$. After that, the eggshell becomes thinner. It is $0.387\text{mm} \pm 0.032$ in the middle and $0.376\text{mm} \pm 0.057$ at the blunt end. Haugh Units range from 76 to 97 and average 88.6 ± 4.4 . All eggs are of a very good AA quality. For all parameters, there is no influence of genetics or the week of lay cycle. There are small significant correlations between the weight of the egg and the thickness of the eggshell at the sharp end ($r=0.028$) and at the blunt end ($r=0.274$). Higher significant correlations exist between the Ca content and the thickness of the eggshell at each of the three measurement points. The level of correlation decreases from the sharp end to the blunt end of the egg (0.517; 0.510; 0.348). The results are mostly confirmed by analyses by TUMOVA and GOUS (2012) on the egg index and Haugh Unit. The values on the thickness of the eggshell at various points tend to confirm the studies of SUN et al. (2012). However, the values according to the eggshell in the present study are lower than in studies by MOLNAR et al. (2017) and GUO et al. (2021).

Translational in vitro to in ovo model for evaluating probiotics and phytobiotic effects on intestinal barrier function and metabolic profiles

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In ovo technique has potential as a model for host-microbe interactions, enabling prenatal modulation of microbiome and immunity through administration of vaccines and bioactives. Earlier, we showed that an in ovo-administered synbiotic composed of a Bacillus probiotic and a vegetable protein hydrolysate (PH) increased post-hatch infectious bronchitis disease vaccine antibody titers. This study investigated functionality of the prebiotic (PH) and Bacillus probiotic in intestinal cells and the chicken intestine. Cells (Chic-8E11, Caco-2) were exposed to Bacillus (10² CFU: cells) or cell free supernatant. Embryonated eggs (n=928, 4 groups) were injected in ovo at day 18.5 with: IBD vaccine alone or supplemented with either Bacillus (10⁶ CFU), prebiotic (1mg PH), or synbiotic (10³ CFU Bacillus+1mg PH). Metabolomic analysis (MxP Quant 500 Biocrates kit with LC-MS/MS and FIA-MS/MS) was followed by multivariate (PCA, PLS-DA) and univariate statistics. The Bacillus exhibited selective adhesion to the chicken over human cells. IHC stains showed that Bacillus CFS enhanced tight junction proteins (ZO-1, occludin) in both cell types. There were cell-specific responses, with increased phosphatidylcholine, sphingolipid metabolism, and SCFA production in Chic-8E11. The in ovo analysis showed treatment-specific pathway enrichment: Bacillus enriched indole synthesis and antimicrobial defense; synbiotic uniquely enriched sarcosine, 3-indolepropionic acid, and GABA pathways, related to gut-brain axis communication; PH treatment enhanced serine, choline, and cysteine pathways, related to membrane integrity and antioxidant activity. In vivo, the bioactives showed diverse beneficial metabolic signatures, supporting applications based on specific health targets. 2019/35/B/NZ9/03186 NCN Poland-OVOBIOM

Session 34

Poster 16

Evaluation of different sources of oregano essential oil (OEO) on piglet performance in diets containing the EU authorized level of Zn

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Oregano essential oil (OEO) exhibits antimicrobial, antioxidative, anti-inflammatory and antiviral properties (Leyva-Lopez, 2017). Carvacrol and thymol have been proven to disturb the structure of pathogenic bacterial cell membranes such as E. coli and salmonella causing their death (Burt, 2004). This study compared the effects of 2 different sources of OEO in comparison to a control diet. PhytaBoost (Devenish Nutrition Ltd, Belfast, UK) is a highly concentrated OEO product with a minimum 18% content as per specification, the alternative OEO has a typical 5% content. 468 pigs were allocated at weaning into groups of 26 balanced for weight, parity and weaning age. Treatments: Control – standard creep diet (1.6% Lys, 15.8DE MJ/kg) and weaner ration (1.50% Lys, 15.5DE MJ/kg). PhytaBoost – standard creep diet and weaner ration+ 0.03% PhytaBoost. OEO – standard creep diet and weaner ration + 0.1% OEO. All diets contained 150ppm Zn. Pigs were weighed at weaning and at 32 days post weaning. Results were analysed using ANOVA with weaning weight applied as a covariate, pen used as a blocking factor and Tukey HSD post-hoc test. The inclusion of PhytaBoost significantly improved feed intake (515g/d vs 481 and 494) and growth rate (402g/d vs 370 and 388) in comparison to the control and the alternative OEO respectively. Greatest end weight was achieved with PhytaBoost (19.08 vs 18.07 and 18.70kg). This demonstrates that PhytaBoost optimises feed intake and nutrient absorption resulting in improved growth rate and resultant end weight in first stage piglets fed diets containing 150ppm Zn. The lower inclusion level of PhytaBoost in comparison to the alternative OEO would suggest an improvement in return on investment in the piglet rations.

The effect of D-vitamin sources on exterior traits and performance in grower-finisher pigs

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Alongside the correct levels of Ca and P, proper dietary levels of Vitamin D (Vit D) are essential for bone development in pigs. Vit D is involved in Ca and P blood homeostasis, immune modulation, muscle growth and reproduction. Vit D can be supplemented in pig diets as Vitamin D3 (cholecalciferol) or 25(OH)D3. The latter is the circulating form of Vit D, which is more bioavailable for pigs. As modern pigs become more feed-efficient and fast-growing, the right levels of vitamins and minerals in their diets are crucial. 25(OH)D3 is frequently included in sow diets because of its well-documented positive effects on reproduction and health. In contrast, due to cost considerations, nursery and grower-finisher diets more commonly utilize Vitamin D3 (Vit D3). This study compared the maximum supplementation (2000 IU per kg feed) of Vit D3 versus 25(OH)D3 on exterior development and performance in grower-finisher pigs. The trial was designed as a 2 x 2 factorial, and 160 pigs were supplemented with Vit D3 or 25(OH)D3 during the nursery and grower-finisher periods. This gave four dietary treatments, DD (only Vit D3), DH (Vit D3 + 25(OH)D3), HD (25(OH)D3 + Vit D3), and HH (only 25(OH)D3). Individual feed intake and weight were recorded daily throughout the grower-finisher period, and all pigs were exterior scored at trial start and trial end. Blood samples were taken from a selection of pigs at the trial start and end. Regardless of dietary treatment, results showed an average daily gain of 1255 ± 85 grams and FCR (calculated as kg gain per kg feed) of $2.25 \text{ kg} \pm 0.16$. Higher circulating levels of Vit D were found for the DH and HH group compared to the DD and HD group ($P < 0.0001$). Of the seven exterior traits scored (gait, knee position, four pastern positions and standing under), only the gait score was significantly affected by treatment, with HH having a better overall score than the DD group ($P < 0.05$). Farm records also indicated more lame pigs in the DD and HD groups compared to the DH and HH groups. Dietary treatments did not affect overall performance.

Session 34

Poster 18

Relationship between body composition of live pigs determined by imaging X-ray techniques and chemical analysis

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The reference methods for measuring body composition of live pigs include slaughtering, cutting, and dissection, or mincing and chemical analyses. By using non-destructive methods to measure body composition and bone mineralization of live pigs, the slaughter could be avoided. Some of these methods such as dual-energy X-ray absorptiometry (DXA) and computed tomography (CT) are X-ray based and enable to predict LEAN, FAT and BONE composition quickly, after adjusting raw data to accurately reflect the chemical values. The aim of this work was to carry out a joint evaluation of the body composition of pigs using DXA and CT and to see its relation to those determined chemically. For this purpose, 48 pigs (barrows and gilts) were fed diets with different levels of nitrogen and phosphorus to obtain a wide range in body composition and bone mineralization. Pigs were anesthetized and scanned with DXA and CT at around 160 days of age. Pigs were slaughtered the following day, minced, and later chemically analyzed for protein (PROT), water (WAT), lipid (LIP) and ASH content. Pearson correlations between predicted data from DXA, CT and chemical variables and a principal component analysis were carried out with XLStat. Results show that the correlations between LEAN and PROT or WAT and between FAT and LIP were very high (0.92-0.99) while those between BONE and ASH were lower (0.74-0.81). Correlations between DXA and CT raw variables were high for LEAN (0.93), FAT (0.98) and a bit lower for BONE (0.81). To conclude, despite differences in the absolute values of the raw measurements, there exists a strong correlation between DXA and CT compared to chemical analysis results. This robust relationship would allow the development of accurate calibration equations, enabling reliable conversion between the two methods. EU Horizon 2020 Research & Innovation program: PIGWEB grant #101004770.

Exploring local pig body morphology in relation to agroecological zones in Burkina Faso, West Africa
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This study was set up for the phenotypic characterization of pigs in different environments and production systems in Burkina Faso. The study was carried out in two agroecological zones, namely Sudano-Sahelian and Sudanian zones and both in peri-urban and rural areas of Burkina Faso. A total of 207 local adult animals (>12 months), were sampled in two agroecological zones namely Sudano-Sahelian and Sudanian zones in Burkina Faso, using FAO guidelines for phenotypic characterization of farm animal genetic resources featuring 13 qualitative traits and 20 quantitative traits. A general linear model was used to assess the effects of environmental factors influencing pigs' body shape while the distribution of individual pigs related to the environment was analyzed using Principal Component Analysis (PCA) and hierarchical clustering. The results indicated no significant ($p>0.05$) difference between local boars and sows within the same agroecological zone. However, Sudano-Sahelian sows and boars were significantly ($p<0.05$) larger than those from the Sudanian region. The bidimensional map constructed for local pigs sampled in the two areas showed two overlapping clusters, while the ascending hierarchical classification suggested they are morphologically structured into three groups with their linear body measurements significantly ($p<0.05$) increasing from cluster 1 to cluster 3. Animals from Cluster 1 with smaller body came in majority from the Sudanian areas while animal from cluster 2 and 3 with relative bigger size were predominantly from the Sudano-Sahelian zone. The findings of this study reveal the complex morphological patterns of pigs in Burkina Faso but indicated that similarly to other livestock species, individuals from humid Sudanian zones tend to have smaller body size compared to others from the Sahelian zones.

Session 34

Poster 20

Weaned piglets fed DevAmine exhibit increased concentration of metabolites that reduce oxidative stress compared to traditional sources

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Weaning is a period of multiple stressors for piglets, including oxidative stress. Oxidative stress negatively impacts the development and maintenance of the villi. This study examines the effects of different protein sources on metabolites that have antioxidant properties. 96 piglets ($14.5\text{kg}\pm 0.35$) were offered diets containing either vegetable protein (VP), synthetic amino acids (SYN), a nutritionally enhanced protein (DevAmine) or a combination of sources (COM). Blood samples were collected post-feeding and analysed using GC-MS, with ANOVA for statistical analysis. Results showed when piglets were fed DevAmine, in comparison to VP or SYN, there was a significant increase of 6 plasma amino acids which have antioxidant properties (Table 1). Table 1 Post-feeding blood metabolite levels Metabolites (umol/ml) VPSYN Devamine COMS.E.MP-Value Glycine $87.02\text{b}64.29\text{b}341.89\text{a}131.25\text{ab}60.70<0.05$ Serine $11.55\text{ab}4.81\text{b}28.03\text{a}13.22\text{ab}6.78<0.05$ Methionine $13.30\text{b}9.06\text{b}44.57\text{a}19.97\text{ab}9.65<0.05$ Glutamic Acid $19.43\text{b}14.48\text{b}80.25\text{a}25.89\text{b}13.08<0.05$ Tryptophan $1.28\text{b}2.56\text{b}66.74\text{a}6.85\text{b}15.13<0.05$ Cysteine $1.52\text{b}3.07\text{b}73.10\text{a}9.74\text{b}16.76<0.05$ Studies have shown that additional supplementation of methionine and tryptophan beyond the nutritional requirement results in improved intestinal physiology and antioxidant capacity. The DevAmine fed pigs had significantly more Methionine and Tryptophan, despite ratios being balanced across diets. The remaining metabolites identified are classified as non-essential and not typically supplemented in piglet diets. In weaned pigs, these are conditionally essential with research demonstrating improved intestinal function, antioxidant capacity and performance when piglets receive increased concentrations of these amino acids. Despite there being no supplementation of these amino acids to the diets, there was an increase in the concentration of these amino acids in the plasma of the DevAmine fed pigs. In conclusion, utilising DevAmine elevates amino acid concentrations, which can reduce oxidative stress, ensuring the intestinal physiology is maintained for optimal and efficient nutrient absorption.

Comparing metabolomic profiles among three Italian heavy pig breeds

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The phenotypic characteristics of an animal are the result of complex molecular interactions between different layers of biological complexity, including the genetic background and intermediate phenotypes. These intermediate phenotypes describe the molecular phenome, which includes the transcriptomic, proteomic and metabolomic profiles. The final objective of this study was to identify novel breed-specific markers by exploring the pig plasma metabolome. We applied untargeted metabolomics to characterize the plasma metabolomic profile of 168 animals from three Italian heavy pig breeds: Italian Large White (ILW; n. 56), Italian Duroc (IDU; n. 56) and Italian Landrace (ILA; n. 56). These animals were sib-tested pigs, included in the selection programs of Italian heavy pigs run by the Italian Pig Breeders Association (ANAS). The pigs were raised in the same performance station and slaughtered at nine months of age (approx. 160 kg live weight). The metabolomic profiles, which included ~700 metabolites, underwent quality checks and were analyzed using a machine learning approach (Random Forest) to identify metabolites that could discriminate between pairs of breeds. In total, we identified 84, 19 and 10 metabolites for the ILW-IDU, ILW-ILA and IDU-ILA comparisons, respectively. These metabolites clearly separated the pig breeds when tested in an unsupervised manner, reflecting the different genetic backgrounds of the three breeds. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).

Session 34

Poster 22

Effect of backfat thickness and growth rate on the reproductive performance of the hyperprolific sow at first service

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To determine the influence of growth rate (GR) and backfat thickness (BFT) on the performance of hyperprolific sows at first service, a prospective comparative observational study was conducted in northwestern Mexico. One hundred and seventy-eight PIC Camborough® piglets preselected as future breeders from birth were used. The piglets were classified into four groups according to their GR from birth to 148 d of age (≤ 0.400 , 0.401 to 0.500, 0.501 to 0.600 and ≥ 0.601 kg/d); in four GR groups from birth to first service (≤ 0.500 , 0.501 to 0.600, 0.601 to 0.700, and ≥ 0.701 kg/d) and in four BFT groups at first service (≤ 10 , 10.5 to 12.5, 13 to 15, and ≥ 15.5 mm). Reproductive performance data by GR and BFT were analyzed with ANOVA ($p \leq 0.05$). Sows with GR ≥ 0.501 kg/d from birth to 148 d of age presented puberty at a younger age (213 vs. 229 and 237 d) and at service (231 vs. 272 and 255 d) compared to those with GR between 0.401 to 0.500 and ≤ 0.400 kg/d ($p = 0.001$). Sows with a GR ≤ 0.500 kg/d from birth to service had puberty at an older age (255 vs. 231, 217, and 209 d; $p = 0.001$) and older age at service (309 vs. 263, 239, and 220 d; $p = 0.001$) than those with GR of 0.501 to 0.600, 0.601 to 0.700, and ≥ 0.701 kg/d. Sows with a GR ≤ 0.600 kg/d had lower BFT at service (13 vs. 15 and 15.5 mm) than those with GR of 0.601 to 0.700 and ≥ 0.701 kg/d. Sows with BFT ≥ 15.5 mm at service presented puberty at an earlier age (217 vs. 226, 227 and 239 d; $p = 0.03$), compared to those with 13 to 15, 10.5 to 12.5 and ≤ 10 mm. The observed results allow to conclude that first-time sows with GR less than 0.601 kg/d have lower BFT at service and present puberty and are served at a later age. Keywords: Gilt, growth rate, backfat thickness, puberty.

Factors influencing the time budget of dairy goats in intensive farming systems

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Aim of this study was to evaluate the factors that influence the activity time budget patterns of farmed dairy goats kept indoors. Dairy goats in three farms located in Northern Italy were continuously recorded from September 2021 to April 2022, and 20 days per farm were randomly selected for the analysis. Five categories of behaviours (i.e., resting, feeding, moving, standing, other behaviours) were assessed by a single assessor applying a scan sampling method at 30 min interval. Information on routines was collected (e.g., time of feed delivery, milking, cleaning, adding/removing bedding). Percentages of activities performed by goats were analysed by aligned rank transformation ANOVA to test the effects of Farm (F; F1, F2, F3), Time Band (TB; D: dark, L: light), Season (S; S1: cold season, T = 5.9±3.07 °C; S2: temperate season, T = 13.7±4.81 °C) and their interactions (i.e., F×TB and F×S). Our analysis showed that time budget of activities was significantly affected by both F×TB and F×S interactions (e.g., resting: F1-L: 31.8%, F2-L: 38.6%, F1-S1: 50.3%, F3-S1: 58.4%, P<0.05; feeding: F2-L: 34.4%, F3-L: 44.7%, F1-S2: 21.4%, F3-S2: 26.8%, P<0.05; moving: F2-L: 4.7%, F3-L: 8.1%, F2-S1: 2.5%, F3-S1: 4.1%, P<0.001). This suggests that management could influence the time budget of goats: for example, higher resting was found in F3 compared to F1 and F2 during D, possibly due to higher stocking density and amount of straw litter: 77.3% vs. 70.7% and 66.1%, respectively; P<0.001). Although preliminary, the results of this study show that routine and management can have a significant effect on time budget of goats. Furthermore, this study suggests the importance of including information on farm routines when planning behavioural observations, especially if used in welfare assessment protocol.

Session 35

Theatre 2

Development of a ClassyFarm protocol for on-farm animal welfare assessment in meat-producing sheep and goats

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In Italy, 36,273 sheep and goat farms are registered for meat production (vetinfo.it/j6_statistiche/#/report-pbi/29), with the highest prevalence in Lombardy (n = 5,461), Sicily (n = 5,385), and Piedmont (n = 3,949) regions. Of these, 60.2% are small-scale farms with herd size not exceeding 20 animals. The total livestock population includes 852,569 sheep and 270,194 goats, amounting to 1,122,763 animals. The ClassyFarm system is a national platform for risk categorization of farms and their protocols for on-farm animal welfare (AW) assessment are currently applied nationwide for self-assessment controls in major livestock species. These protocols adopt a multifactorial approach integrating management-based (MBIs), resource-based (RBIs), and animal-based indicators (ABIs). In 2024, IZSLER and IZSLT, with the contribution of experts from the University of Pisa and Milan, private and official veterinarians, as well as the outputs from the Animal Welfare Indicators (AWIN) project and the Welfaruminant project, developed a dedicated ClassyFarm AW protocol for meat-producing sheep and goats. It consists of 33 indicators, including 11 MBIs, 13 RBIs and 9 ABIs, such as: (i) familiar human approach test, (ii) presence of isolated or disoriented animals, (iii) body condition score (BCS), (iv) cleanliness, (v) skin lesions, (vi) prevalence of lameness, (vii) annual mortality of adult animals, (viii) annual mortality of lambs/kids, (ix) presence of mutilations. The protocol was tested in the field by 20 trained private veterinarians to assess its feasibility and data entry tool appropriateness. Since January 2025, this protocol has been publicly available, with results soon accessible via interactive dashboards displaying overall and partial AW scores on a 0-100% scale. Additionally, it can serve as a tool for AW risk assessment, also useful to funding agencies, supporting the improvement of farm management practices and promoting the enhancement of animal welfare standards.

Welfare Indicators for Lambs and Goat Kids from Dairy Farms: An Analysis Based on a Large-Scale Dataset

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Welfare assessment studies conducted on operating farms often rely on a limited selection of parties and thus bear the risk of sample bias. Dairy lambs and particularly goat kids have low economic value, potentially predisposing them to welfare concerns. This study aimed to survey stillbirth rates (<48 h post partum), mortality rates until 365 days of age, and animal movements of lambs and goat kids, from dairy farms using a large-scale dataset. Data were obtained from the official Swiss national animal identification, registration and movement database, operated by Identitas AG. Every lamb and dairy goat kid born in Switzerland must be registered by the farmer. If an animal dies, is slaughtered, or is transferred to another holding, all these events must be notified on a legal basis. Stillbirth and mortality rates were calculated for all lambs (n = 79,606) and goat kids (n = 160,544) born between January 2020 and December 2023 to an ewe or goat on a holding registered as 'dairy type of use'. Male dairy goat kids exhibited the highest stillbirth rate (7.5%) compared to female dairy goat kids (4.9%) and dairy lambs (male: 4.6%, female: 3.2%), while the mortality rate was 12.9% in dairy lambs and 11.3% in dairy goat kids, respectively. Furthermore, movement patterns were analysed for lambs (n = 25,049) and goat kids (n = 58,556) featuring a slaughter notification in the considered time period. While 51.9% of dairy lambs showed at least one movement from the birth holding to another (e.g., summering pasture), dairy goat kids remained mainly on their birth farm (83.1%) until slaughter. Notably, 51.8% of lamb mortality cases (n=5,080) were reported in lambs that had been moved to another holding. These findings indicate that the reasons for high stillbirth rate in male goat kids requires further investigation. Additionally, a more detailed analysis of lamb movements is necessary, as relocation to other holdings seem to be associated with increased mortality risks. Large-scale datasets provide valuable insights into potential welfare issues and should be further studied.

Session 35

Theatre 4

Cryptorchidism in German Mutton Merino Sheep

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The German Mutton Merino is a sheep breed derived from fine-wool Merino sheep at the turn of the 19th and 20th century. Although this endangered breed with around 5,000 breeding ewes is still of the fine-wool type, under today's German conditions the main emphasis in the breeding goal is on lamb and meat production. The presence of cryptorchidism in newborn ram lambs (N=8,009) has been documented for the population in the federal states of Saxony-Anhalt and Brandenburg since 2009. The affected status for cryptorchidism occurred in 5 large flocks with an average incidence of 5.74 %, specifically with one undescended testicle at a frequency of 0.96 % and a frequency of 4.78 % for or both testicles affected. The incidence does not vary between singletons and multiples, but across flocks and time. A model including the fixed effects of flock (5), lamb-year (2009-2020) and type of birth (1-2) with binary status of cryptorchidism as the dependent variable revealed that affected animals showed significantly less lifetime daily gain than healthy rams (LTDG_50: 261 vs 281 g/d or LTDG_100: 280 vs 298 g/d) in the field performance tests. Although carriers are excluded from breeding, cryptorchidism persists in the population, perhaps because environmental factors such as estrogen or nutrition are considered to be triggers. The genetic component of this malformation in sheep is to be investigated with the aid of pedigree analyses and population genetic calculations. As the accuracy of pedigree records in sheep is insufficient (182 rams), subgroups of phenotyped and clearly identified animals with cross-checked pedigrees with at least ram (S1: N=7,584, based on 41 rams with known and 65 rams with unknown sire) or ram and sire of ram (S2: N=4,516) information was selected. The subgroups represent the same or nearly the same incidence (5.4 % for S2), S2 is recruited from only 4 flocks. According to initial estimates on using a threshold sire-model (thr Gibbsf90 and post Gibbsf90 of the blupf90 family programs, Misztal et al., 2014), the heritability range between 0.65-0.83 (se=0.18) for S1 and 0.53-0.79 (se=0.26) for S2 by using flock and lamb-year as combined or separate fixed effects.

Biosecurity Checklist as a Prevention Tool for Toxoplasmosis Control in Sheep and Goat Farms in Southern Italy

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Animal health defense is fundamental in livestock farming. The introduction of diseases in herds can lead to significant economic losses and impact public health. Biosecurity is essential in preventing pathogen entry. Among the most relevant pathogens in small ruminants, *Toxoplasma gondii* poses a major zoonotic risk. This study assessed risk factors for toxoplasmosis in (no. = 113) sheep farms in Campania (Southern Italy) using the ClassyFarm biosecurity checklist, integrating specific indicators for *T. gondii* monitoring. Findings revealed that (n=112/113) farms do not submit biological samples for toxoplasmosis detection, despite 76% reporting abortions and 24% neonatal mortality. Additionally, 55% of farms practice continuous grazing, increasing exposure to *T. gondii* oocysts. A key risk factor is the lack of rodent control measures: 49% of farmers do not implement any control, while others use unofficial methods. Furthermore, 90% of farmers do not report health issues to veterinarians, nor do they submit fetuses, carcasses, or blood samples for analysis. This highlights a critical gap in disease prevention awareness. Regarding parasite control, 71% of farmers have partial knowledge of endo/ectoparasite management but fail to apply measures consistently. To address these gaps, tailored biosecurity manuals were provided, emphasizing the importance of laboratory diagnostics and structured biosecurity plans. The evaluation system proved to be a valuable tool, engaging farmers and veterinarians in a collaborative approach to risk mitigation and prioritization of interventions.

Session 35

Theatre 6

Effect of ewe body condition score (BCS) at mating and mid-pregnancy on ewe and lamb performance post-weaning

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Body condition score (BCS) is a vital indicator of ewe nutritional status impacting flock performance and the health and welfare of ewes within the flock. Ewe BCS is typically described on a scale of 0-5 in intervals of 0.25. Targets for BCS levels change throughout the year depending on the time of the production cycle with changes depending on the nutritional demands of the ewe. At farm level BCS is typically further simplified to three categories of 'thin' 'fit' and 'fat'. Data from 5500 mating BCS records were assessed from the Teagasc BETTER farm programme sheep flocks and the BCS of the ewes at mating were categorised into three levels <BCS 3.0 was considered 'Thin' (T), BCS 3.0 – 4.0 was considered 'Fit' (F) and BCS >4.0 was considered 'Fat' (FT). Within these records 13% of ewes were T, 83% of ewes were F and further 4% of ewes were FT. This indicated that of the ewes that were not F at mating time T ewes were the larger proportion of commercial sheep flocks than FT ewes. Following this data from six of the flocks were examined to investigate the effect of ewe BCS at mating (M) and mid-pregnancy (MP) on lamb performance, lamb mortality and ewe culling rate. Ewes had BCS assessed by one of two trained technicians. Lamb performance data was recorded using EID technology all lambs were linked to their dam at birth. All data was compiled, cleaned and analysed using SAS (V9.4). Litter performance from birth to weaning (14 weeks post-partum) of twin born and reared lambs from T ewes at MP was lower than twin born and reared lambs from F ewes at MP ($P<0.05$). Ewes that were T at MP were more likely to have a lamb die before weaning ($P<0.05$). Ewes in T category at both M and MP were also more likely to be culled from the flock before the following mating ($P<0.05$). Ewe age and flock were both significant factors in the model ($P<0.05$). This indicated that the structure and management of the flock are key factors influencing ewe BCS but also performance and health performance indicators. Thin ewes are a big source of variation in grass based ewe flocks and reduce flock performance compared to ewes in acceptable BCS at M and MP.

Comparative analysis of mediastinal lymph node transcriptomes in sheep with opposite SRLV infection status and different TMEM154 E35K genotypes

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Sheep susceptibility to SRLV is influenced by genetics, including the TMEM154 E35K polymorphism. It has been observed that KK homozygotes are less susceptible than EK/EE carriers, but the effect varies by breed and SRLV genotype, indicating that additional genetic and environmental factors also contribute to SRLV resistance. This study investigated mediastinal lymph node transcriptome differences in sheep with varying SRLV infection status and TMEM154 E35K genotype. Merinoland ewes (>3 years) were tested for SRLV and genotyped, forming three groups: (1) SRLV-positive, TMEM154 EK (posEK); (2) SRLV-positive, TMEM154 KK (posKK); (3) SRLV-negative, TMEM154 KK (negKK). RNAseq libraries were sequenced on Illumina NovaSeq 6000. Differential expression analysis was performed using DESeq2, with KEGG and GO enrichment via ShinyGo 0.82. The highest number of differentially expressed genes (DEGs, n=207) was found between posEK and negKK, enriched in GO terms and KEGG pathways related to natural killer cell chemotaxis, cytokine activity, viral protein interaction with cytokine and cytokine receptor, primary immunodeficiency, and chemokine signaling. Interestingly, 79 DEGs were identified between posEK and posKK. These DEGs were enriched in various pathways including those linked to chemokine receptor binding, toll-like receptor signaling, and viral life cycle (HIV-1), chemokine signaling pathway. These results contribute to a deeper understanding of the genetic factors for SRLV susceptibility in sheep; further analyses are in progress. Funding: National Science Centre, Poland (UMO-2020/39/1/NZ9/01304), German Research Foundation (DFG, LU 1439/4-1). Bioinformatics analysis: Poznań Supercomputing and Networking Center resources.

Session 35

Theatre 8

Genetic parameter estimations for somatic cell score, milk yield, and milk composition in Cyprus Damascus goats

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Despite the importance of milk somatic cell count (SCC) as an indicator of milk quality, udder health, and an animal's general health status, as described in sheep and cows, there has been limited research on SCC in dairy goats. Furthermore, for several goat breeds, including the Cyprus Damascus goats, the specific SCC threshold to distinguish between healthy and mastitis prone or infected animals remains undetermined. The objective of this retrospective longitudinal study was to define both genetic and environmental factors influencing the somatic cell score (SCS), total milk yield, fat, protein, lactose and total solids content in Cyprus Damascus goats. The data consisted of 1410 milk samples from 524 kiddings of 270 does collected between 2021 and 2024 from the Agricultural Research Institute breeding nucleus, located in Nicosia, Cyprus. Pedigree information was also available for all animals. The overall means and standard deviations of SCS, MY, fat, protein, lactose and total solids were 4.13±0.49, 170.0±86.5 litres, 4.3±0.7%, 3.6±0.4%, 4.5±0.3 and 12.9±1.0 %, respectively. Overall estimates of heritabilities (h²) and repeatabilities obtained using the Restricted Maximum Likelihood procedure in ASReml version 3 ranged from 0.13 to 0.37 and 0.22 to 0.66, respectively. SCS was moderately negatively correlated with milk yield (r=-0.10) and lactose content (r=-0.22) but positively correlated with protein (r=0.24). Thus, in an effort to examine SCC as a quality parameter in goat milk, we examined the associations of SCC with daily milk yield and composition and investigated how varying levels of SCC (400, 800, 1200 and 1600×10³/mL) are correlated with qualitative and quantitative properties of milk.

Parity-Based Variation and Heritability of Somatic Cell Score in Cyprus Chios Sheep

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The Somatic Cell Score (SCS) is an important indicator of udder and milk quality in dairy sheep. Knowledge about the difference of SCS among parities and its relationship to the main constituents of milk may be useful in genetic selection schemes of Cyprus Chios sheep. This study sought to identify key factors that influence selection and breeding programs, helping to improve decision-making for better animal traits, welfare, and productivity. The main aims of this study were: (a) To analyze variations in SCS measurements across different parities, (b) To explore the association between average SCS and major milk components (total solids, protein, fat, and lactose) as well as milk production, and (c) To ascertain the statistical significance of such associations and estimate relevant genetic parameters, such as heritability. Data for about 900 Cyprus Chios ewes with parities 1 to 6 were analyzed in this study. Three SCS readings per animal per parity and the average SCS of the flock per parity were recorded for parities 1 to 6. We also recorded the average value for each parameter per parity with the same methodology for milk yield, total solids, protein, fat, and lactose. We found that lactose and protein content significantly correlate with changes in SCS (p -values < 0.001). In addition, ANOVA showed significant differences in SCS ($F = 16.42$, $p < 0.001$), lactose ($F = 23.09$, $p < 0.001$), and protein ($F = 105.1$, $p < 0.001$) across parities. Tukey's post hoc tests revealed significant pairwise differences, particularly between early (1-3) and later parities (5-6). SCS differed significantly ($p \leq 0.001$) in most pairwise combinations, with notable differences for parities 5-2 ($p = 0.0003$), 6-2 ($p = 0.036$), and 5-3 ($p = 0.019$). Similar results were observed for protein ($p < 0.001$) and lactose ($p \leq 0.05$). These trends were investigated in greater detail to measure heritability and genetic merit using BLUP. A more extensive study will identify the genetic factors underlying these correlations and act as the foundation for implementing a breeding program to improve udder health and milk quality in Cyprus Chios sheep via genomic selection and improved management.

Session 35

Theatre 10

PathoProof: available mastitis detection in dairy goats

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Mastitis is primarily caused by the penetration of various microorganisms into the mammary gland through the teat canal. Somatic cell count (SCC) serves as a relatively good indicator of udder health in dairy cows, but there is still discussion about the importance of SCC in dairy goats, where it is often observed culture-negative samples with high SCC. The aim of the study was to use the commercially available PCR assay for detecting pathogens in half-udder milk samples (99 samples) collected from a conventional goat farm. Milk samples were aseptically collected for assessment of pathogen prevalence. Fossomatic 7 was used to determine SCC, and the PathoProof Complete-16 kit was used for the pathogen detection. Overall, more than 70% of half-udder milk samples had SCC more than 10^6 cells.mL⁻¹ and 22% below 0.5×10^6 cells.mL⁻¹ and even under 0.2×10^6 cells.mL⁻¹ only 10%. Of the total samples, in 92 (93%) samples there were detected one (71 samples) or two (21 samples) pathogens. *Staphylococcus* spp., particularly coagulase-negative staphylococci (CNS, 88 samples) were the most detected mastitis pathogens. The staphylococcal β -lactamase gene was detected in 49 samples containing *Staphylococcus* spp. only and in 21 samples containing *Staphylococcus* spp. together with other pathogens. Contagious pathogens such as *Prototheca* spp. were detected in 7 samples, *S. aureus* in 3 samples and *Mycoplasma* spp. in 2 samples. In conclusion, the high prevalence of positive samples could reflect a high number of samples with high SCC. On the other side, aseptic milk sampling in parlour could be some risk if PCR methods are used due to possible contamination from the environment. Moreover, the absence of conventional bacteriological culture limits the ability to assess pathogen viability and antimicrobial susceptibility, which are crucial for treatment decisions. Therefore, results obtained solely from DNA-based PCR methods should be interpreted with caution in both diagnostics and herd health management. Supported by: APVV-21-0134 and VEGA 1/0597/22

Impact of management practices on milk yield and quality traits in dairy goat farms

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In Italy, the dairy goat sector is still behind compared to the dairy cattle industry, with room for improvement in management and efficiency. The aims of this study were (i) to investigate the managerial practices of goat farming in the Veneto region through a survey and (ii) to evaluate their impact on individual milk yield and quality traits. A survey was conducted among goat farms to investigate aspects related to animal health, farm structure, and management. These data were matched with test-day records obtained through official routine milk recording tests. The available data included days in milk (DIM), parity, milk yield, as well as fat, protein, casein, and lactose content, somatic cell count (SCC), and urea concentration. The final dataset consisted of 16,259 records from 2,345 Alpine and Saanen goats reared in 29 farms. A linear mixed model was used for variance analysis, considering indicators collected through questionnaires, breed, parity and DIM as fixed effects, while goats, herd-test-day and residual as random effect. A stepwise regression was then conducted for each milk trait to discard non-significant effects ($p > 0.05$). Results provided an overview of dairy goat farms in northeast of Italy in relation to various practices e.g., milk destination, animal feeding, milking routines, and reproduction strategies. In particular, 48% of the farms supplied milk to dairy companies, 38% produced farm-made cheeses, and the remaining farms did both. Regarding feeding, 28% of the farms provided total mixed ration to the goat, while 72% supplied separate forages and concentrates. As for the type of milking apparatus, 66% of the farms were equipped with a pipeline, while 34% used buckets. Milk SCC were influenced by type of milking and milking routine. Milk yield, fat, protein and casein content were influenced by the type of feeding and access to pasture, the presence of ventilation systems, heating systems for drinking water, and environmental enrichments. In conclusion, the study indicates that milk production and milk quality can be improved by adopting specific managerial practices. This study received support from the project Vene-To-Goat (CSR 2023-2027, Regione Veneto, Italy)

Session 35

Theatre 12

Microbiota Shifts in Kid Goats: The Impact of Sugar Beet as a Sustainable Feed Alternative to Maize

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The MEDGOAT project (PRIMA 2022) aims to enhance the sustainability of goat farming in Mediterranean regions by integrating alternative, drought-resistant feed resources. Given the challenges of climate change and water scarcity, reducing reliance on conventional maize-based diets is essential. Sugar beet, with its lower water requirements and potential benefits for carbon sequestration, represents a promising alternative. This study evaluates the effects of replacing maize with sugar beet in the diet of fattening kid goats, focusing on microbiota composition and its implications for digestion, metabolism, and sustainability. Twenty-four five-month-old goat kids were assigned to three experimental groups ($n=8$ per group), receiving isoenergetic pelleted diets containing 18% maize (control), 9% sugar beet, or 18% sugar beet. The study assesses microbiota shifts in saliva, rumen, and faeces, focusing on nutrient absorption, digestion, and methane reduction. By analyzing ruminal methane producers, the research supports climate-smart feeding strategies for sustainability and animal health. It also explores links between microbiota, immune function, and disease resilience, identifying microbial markers for stress tolerance. Based on alpha diversity analysis, rumen samples showed significant microbiota shifts ($p < 0.05$), while faecal and salivary microbiota remained stable. These findings suggest that dietary modifications, such as the inclusion of sugar beets, influence ruminal microbial diversity, impacting fermentation and metabolic pathways.

Effect of Using Post-Day One Colostrum Supplementation on Health and Growth in Dairy Goat Kids

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This study assessed post-day one colostrum replacer (CR) supplementation on dairy goat kid health and growth. In January 2023, 143 female kids (≥ 2.1 kg) from a Dutch farm were separated at birth and given pooled goat colostrum enriched with CR up to 30% Brix within 24 hours. Were randomly assigned to one of four groups: 6%C2W (n=30), milk replacer (MR)+6% CR for 2 weeks; 6%C3W (n=43), 6% CR for 3 weeks; 10%C2W (n=39), 10% CR for 2 weeks; and CON (n=31), MR only. All kids were fed in an automated feeder that recorded daily milk intake. Growth performance was assessed through weekly body weight measurements and average daily gain (ADG) calculated weekly. Health monitoring occurred three times per week, recording incidences of diarrhea, respiratory disease, ecthyma, and lameness using a clinical scoring system, and all treatments and mortality events were documented. A negative binomial model was used to evaluate the number of treatments, logistic regression models assessed associations with individual diseases, and a repeated-measures linear regression model evaluated differences in ADG through week 7. Birth weight and colostrum intake were similar across groups ($P > 0.05$); however, kids in 6%C2W received their first colostrum feeding later than other groups ($P=0.02$) and this was controlled for in all models. Compared to CON, kids in 6%C2W (IRR: 0.49; 95% CI: 0.32–0.77), 6%C3W (IRR: 0.64; 95% CI: 0.43–0.96), and 10%C2W (IRR: 0.50; 95% CI: 0.33–0.76) had a lower incidence of treatments. Additionally, the odds of respiratory disease were lower in 6%C2W (OR: 0.10; 95% CI: 0.03–0.34) and 6%C3W (OR: 0.09; 95% CI: 0.03–0.28) compared to CON; however, kids in 6%C3W had a higher odds of diarrhea (OR: 37.36; 95% CI: 3.19–436.93). For ADG, 6%C2W had higher ADG in weeks 4–7, 6%C3W had higher ADG in weeks 2–3 and 6–7, and 10%C2W had higher ADG in weeks 6–7. These findings suggest that post-day one supplementation of colostrum replacer may reduce treatment incidence and improve growth performance in dairy goat kids. However, specific disease risks may vary by treatment duration.

Session 35

Poster 14

Continuous milking in goats: data from goat milking farms and aspects of practicability and breeding aspects

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Goats are very fertile animals giving birth to 1 to 2 kids each year, which must be raised and marketed. The marketing of male kids in particular continues to pose challenges for the entire goat dairy industry. Since the number of milking days has increased over the last 10 years (2004: 275 to 290 days, 2014: 458 to 481 days), it can be assumed that continuous milking will become more common. Milk collection data from two organic dairy goat farms are presented here. These two farms have been milking continuously for over 20 and 9 years, respectively. Data such as the number of milking days per lactation, milk (kg) per milking day, milk (kg) per lactation, protein (%), fat (%) and protein and fat (kg) are presented. The number of milking days varies between 45 and 2834 days in the first lactation, between 134 and 4203 days in the second lactation, between 269 and 3427 days in the third lactation and between 45 and 1398 days in the fourth lactation. The average daily milk yield is 2.1 kg in the first lactation, 2.6 kg in the second lactation 2.7 kg in the third lactation and 3.0 kg in the fourth lactation. Milk yield per lactation varied between 317 and 785 kg in the first lactation, between 979 and 3986 kg in the second lactation, between 746 and 5503 kg in the third lactation and between 1486 and 3925 kg in the fourth lactation. The average protein content was 3.0 % in the first lactation, 3.2 % in the second lactation and 3.1 % in both the third and the fourth lactation. The fat content was 3.2 %, 3.3 %, 3.2 % and 3.4 % in the first, second, third and fourth lactation, respectively. The fat and protein content was 32.4 kg, 161.4 kg, 150.6 kg and 145.8 kg in the first, second, third and fourth lactation, respectively. Besides the positive side of continuous milking (possible in Saanen goats, fewer kids, especially male kids; results in a relief of the peripartum metabolism because of missing high milk production during the peripartal period) the negative side has to be mentioned too and results in the competition with breeding work. If the lactation period exceeds 305 days, the question arises whether the sole consideration of 240 days' yield is sufficient for the breeding assessment and decision ?

Effects of dietary protein supplementation on the periparturient relaxation of immunity towards gastrointestinal nematodes in a mountain goat breed

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In ruminants, the periparturient relaxation of immunity (PPRI) leads to higher fecal egg counts (FEC) around parturition and has been linked to nutrient scarcity, resulting in limited resources for immune expression. We studied the effects of protein supplementation on parasite levels and animal responses in a mountain goat breed around parturition. Female goats (Styrian Pied; n = 11) were synchronically mated in October 2023 to allow kidding in late March 2024. All goats were stabled and naturally infected with mixed gastrointestinal nematodes during the previous grazing season: >70% of the infections corresponded to *Haemonchus contortus*. Two groups were formed 4 weeks before kidding (Day [D] -28) based on similar FEC and liveweight and were randomly allocated to either a: control diet (CD; n=5) covering 100% of the MP requirements; or protein-supplemented diet (PD; n=6) covering 140% of the MP requirements. The experimental feeding started on D-24 (before birth) until D39 (after birth), with kidding as D0. Faecal samples were taken twice per week to determine individual FEC. Blood was collected weekly to determine packed cell volume (PCV), biochemical parameters and anti-H. contortus IgG by ELISA. FEC were evaluated with generalised linear models. All other parameters were evaluated as repeated measures (linear mixed-effect models). FEC increased in both groups over the trial, but with a sharp rise in the CD group from D0 and higher EPG in control animals from D18 until the end of the trial ($P < 0.001$; D39 mean EPG $\pm$ SD CD = 4447 ± 3596 vs PD = 1974 ± 1656). PCV, serum albumin and protein were affected by time, diet and their interaction, with higher values in the PD group ($P < 0.05$). High variability of anti-*Haemonchus* IgG levels were detected within groups. These were affected by time but not by diet. However, animals with > 2000 EPG and fed different diets had a distinct shift in IgG from D25 until D39, with 53-62% higher IgG levels in high FEC-PD animals compared with high FEC-CD goats ($P < 0.05$). Dietary protein supplementation reduces the PPRI in goats.

Session 35

Poster 16

Effect of garlic inclusion on growth and feed conversion in Merino lambs

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The search for sustainable nutritional strategies in livestock production has promoted the use of agricultural by-products in animal feed. Garlic waste, derived from garlic cultivation, was used in this study due to its recognised antimicrobial, antioxidant and immunomodulatory properties, positioning it as a sustainable alternative for animal nutrition. The aim of this study was to evaluate the effect of including 4% garlic in the diet of Merino lambs on growth performance and feed efficiency. The trial was carried out at the CICYTEX experimental farm using 30 Merino lambs, divided into two groups of 15: a control group with no garlic inclusion and an experimental group receiving 4% garlic in the diet. Both diets were formulated to be isoproteic and isoenergetic. Lambs were weighed weekly and individual feed intakes were recorded daily. Parameters evaluated included weight gain, average daily gain (ADG), feed intake and feed conversion ratio (FCR). Statistical analyses were performed using SAS® to compare differences between groups, with a significance level of $P < 0.05$. Lambs fed the garlic-supplemented diet showed significantly higher weight gain ($p = 0.023$) and ADG ($p = 0.023$), with values 7.79% higher than the control group. Although no significant differences were found in feed intake ($p = 0.472$) or FCR ($p = 0.419$), a trend towards improved feed efficiency was observed. The beneficial effects of garlic on lamb growth may be attributed to its influence on the gut microbiota, potentially improving digestion and nutrient absorption. In addition, no rejection of the garlic-supplemented diet was observed, indicating good palatability and regular intake. These results suggest that the inclusion of 4% garlic in the diet of Merino lambs can improve growth performance without negatively affecting feed efficiency. This study highlights the potential of garlic by-products as a natural and sustainable feed additive in sheep production, supporting more environmentally friendly livestock systems. Acknowledgements: Project 0100_TID4AGRO_4_E is co-financed by the European Union through the INTERREG VI-A Spain-Portugal Programme (POCTEP) 2021-2027.

Using subcutaneous loggers for monitoring dairy goats: Preliminary information on physiological recording and activity peaks during milking and feeding

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Effective welfare assessment in livestock remains a challenge. Existing methods are often time-consuming, insufficiently informative, or not adapted for goats. This preliminary study evaluates the suitability of the Star-Oddi® subcutaneous logger for continuous monitoring in dairy goats. A logger was implanted subcutaneously in a Majorera dairy goat and programmed to record subcutaneous temperature (ST), activity levels, and heart function at 15-minute intervals over three days. Electrocardiograms were performed before and after implantation and prior to the logger removal. The ST stabilized 24 hours post-implantation, with an average of $27.54 \pm 1.69^\circ\text{C}$, exhibiting a circadian rhythm. Activity levels averaged 20.75 ± 30.13 mg, with peak values recorded at 08:00 (milking, 100–150 mg) and 12:00 (feeding, 100–127 mg). Mean heart rate (HR) after stabilization was 74.50 ± 15.07 bpm. The analysis of the heart rate variability (HRV) showed a Standard Deviation of NN intervals of 120.66 ± 77.58 ms and a Root Mean Square of Successive Differences of 177.01 ± 112.64 ms. These findings suggest that the Star-Oddi® subcutaneous logger is a viable tool for real-time monitoring in dairy goats, providing continuous physiological and behavioral assessments with minimal intervention. Further research is required to validate its long-term effectiveness across different management systems. Funded by Fundación CajaCanarias and Fundación Bancaria La Caixa (Ref. 2023DIG29).

Session 35

Poster 18

Dietary n-6 vs. n-3 PUFA. Effect on the resilience of dairy sheep to a mammary inflammatory challenge: 2) Clinical parameters

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In the current scenario of global instability, animal resilience plays a key role in livestock production. The immune response, essential for resilience against infections, might be affected by polyunsaturated fatty acids (PUFA), with n-6 PUFA exacerbating inflammation and n-3 PUFA resolving it. With the aim of evaluating the effect of dietary n-6 vs. n-3 PUFA on dairy sheep's resilience, 22 Assaf ewes were offered 2 supplemented TMR: 2% soybean oil (SO, rich in n-6 PUFA; n=11) and 2% linseed oil (LO, rich in n-3 PUFA; n=11). After adaptation to the diets (pre-challenge, 4 weeks), both groups underwent a challenge (10µg of *Escherichia coli* lipopolysaccharides (LPS) infused in each mammary gland) simulating an infectious mastitis. Rectal temperature and blood total leukocyte, lymphocyte and neutrophil counts were monitored before and after LPS infusion (-24, 0, 3, 6, 12, 24, 48, 72, and 144 h), and analysed with repeated-measures (SAS v9.4). Milk samples were collected weekly during the pre-challenge, and also at the above-mentioned times (except 3 h) and up to 3 weeks post-challenge for somatic cell counts (SCC). These SCC were analysed using a piecewise model (R v4.2.1), with 5 parameters: V1 (pre-challenge constant), V2 (challenge response, until 12 h post-infusion), V3 and V4 (linear and quadratic recovery, until 6d post-challenge), and V5 (stabilization, until the end of the post-challenge). Orthogonal contrasts compared V1 vs. V5. Only rectal temperature differed between diets, with higher values in LO ($P<0.05$). In both groups, temperature peaked at 6 h ($P<0.001$) and normalized by 24 h. Blood leukocyte, lymphocyte, and neutrophil counts declined until 24 h ($P<0.001$) reflecting tissue infiltration of neutrophils and lymph node shut-down, and recovered by 48 h. Regardless of diet, SCC increased with the challenge (V2; $P>0.10$), peaking at 12 h. Although LO and SO groups showed a similar recovery (V3 and V4; $P>0.10$), LO tended to maintain higher SCC during stabilization ($P<0.10$ for V1 vs. V5). Substitution of dietary soybean oil (n-6 PUFA) by linseed oil (n-3 PUFA) would not improve dairy ewes' resilience to this kind of inflammatory challenge. Acknowledgment: PID2023-146979OB-I00 and PRE2021-098235 (MCIN/AEI, Spain).

Preliminary results on the prevalence and treatment of *Cryptosporidium* infections in different sheep breeds in South Africa

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Cryptosporidiosis is an enteric disease caused by protozoan parasites of the genus *Cryptosporidium*. It commonly affects young ruminants and is linked to global economic losses. Symptoms include diarrhea, dehydration, delayed growth, and weight loss, often leading to death which raises welfare concerns due to veterinary costs and animal health. The aim of this study was to evaluate the prevalence of *Cryptosporidium* in lambs of different sheep breeds in South Africa. The study monitored 471 lambs (243 ewes and 228 rams) under 4 weeks old from 10 breeds during the autumn lambing season at Langgewens Research Farm in the Swartland area. Breeds included European-origin woolled types (Merino, SA Mutton Merino, Dohne Merino, Dormer, Ile de France) and African-origin haired types (Dorper, Meatmaster, Namaqua Afrikaner, Damara, Blackhead Persian). Ewes were kept in groups of 15 in 399 m² open field lambing camps, with litter left undisturbed, relying on sunlight for natural disinfection. *Cryptosporidium* parvum was positively confirmed by using Huvepharma Huve-Check® Calf Scours 5 test kits and subsequently affected lambs were identified through physical observation of yellowish, profuse diarrhea. Lambs were immediately treated with 0.25 ml Parofo® Crypto (140 mg/ml) per kilogram live weight for 7 days, along with Electroguard® NF Gel (0.3 ml/kg live weight) for 1–3 days as an immune booster. A significant breed difference ($P \leq 0.05$) was observed in susceptibility and morbidity. Sheep breeds from African-origin had significantly ($P \leq 0.05$) lower susceptibility (0.25 ± 0.04) and morbidity (0.42 ± 0.10) compared to breeds of European-origin (susceptibility of 0.53 ± 0.03 and morbidity of 1.25 ± 0.08). These findings suggest that African-origin sheep breeds have better resistance to *Cryptosporidium* infection compared to European-origin sheep breeds which may be due to genetic or environmental factors. However, further research is needed to better understand these differences and to investigate the factors behind breed susceptibility and develop better strategies for controlling *Cryptosporidium*.

Session 35

Poster 20

Identification of INDELs Affecting Alternative Splicing in Sheep Challenged with Gastrointestinal Nematode Infections

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Gastrointestinal nematode (GIN) infections pose significant challenges to the global sheep industry, causing economic losses and increasing concerns about animal health and welfare. Genetic variation influencing the host response to infection plays a vital role in resistance mechanisms. Insertions and deletions (INDELs) can disrupt gene function by altering splicing patterns. This study aimed to identify INDELs affecting alternative splicing (AS) in sheep with varying resistance to GIN infections. We used publicly available RNA-Seq data from the NCBI Gene Expression Omnibus database (Accession ID: GSE63547), consisting of 20 abomasal lymph node samples collected from the most resistant (R; n=10) and most susceptible (S; n=10) Scottish Blackface lambs exposed to *Teladorsagia circumcincta* nematode. Sequence quality control and alignment to the Oar_rambouillet_v.3 reference genome were performed using CLC Genomics Workbench v.20.0.4. INDELs discovery was conducted using the fixed ploidy variant detection tool to identify functional INDELs with splice site effects (SSE) uniquely fixed in R and S groups. These INDELs were further investigated for their potential association with phenotypic variation in response to GIN infections through AS. Genes associated with INDELs causing SSE and INDELs' functional consequences were identified using Ensembl VEP, while functional analysis was conducted using REACTOME. We identified 62 and 61 insertions with SSE uniquely fixed in R and S groups, respectively, while 24 and 25 deletions with SSE uniquely fixed in each group. Functional enrichment analysis revealed that genes affected by insertions in R group were significantly enriched in immune pathways, including interleukin-12 signaling ($p < 0.05$). In contrast, genes affected by deletions in R group were primarily linked to basic cellular physiological functions ($p < 0.05$). These findings provide insights into the genetic mechanisms underlying parasite resistance in sheep and support breeding strategies to improve resilience against GIN infections.

Thermoregulatory response to winter shearing in rams sheared six or twelve months before

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Wool ewes and rams are typically sheared once a year simultaneously. Since winter shearing of pregnant ewes increases lamb survival, rams are often sheared simultaneously for easier field management. However, shearing rams in summer, before the breeding season, enhances their reproductive performance. Consequently, these rams undergo shearing twice yearly -once in summer and again in winter alongside ewes. Rams sheared every six months are expected to adapt better to environmental conditions after a winter shearing than those sheared 12 months before. The aim of this study was to compare rectal and tympanic temperatures (RT and TT) after winter shearing in rams previously sheared either 6 or 12 months earlier. Nineteen adult Corriedale rams were last sheared either six months before (n=8, group Sh6) or 12 months before (n=11, group Sh12) and were all sheared again in August (mid-winter in the Southern Hemisphere, Hour 0). RT and TT were recorded every 6 hours from Hour -24 to Hour 60, every 12 hours from Hour 72 to Hour 156, and at Hours 240 and 252 (10 days post-shearing). Data were analysed using a mixed model, with treatment, time, and their interaction as fixed effects. RT was greater in Sh6 than in Sh12 rams (38.67 ± 0.03 °C vs 38.57 ± 0.03 °C, $P = 0.028$), while TT was lower (35.79 ± 0.05 °C vs 35.99 ± 0.04 °C ($P = 0.002$)). No significant treatment-time interaction was observed for either variable. Rams sheared six months before winter were more effective in reducing heat loss than Sh12 rams, maintaining a more stable core temperature. In addition to the reproductive benefits of summer shearing, these findings suggest that rams sheared biannually experience less cold in winter, improving their overall welfare compared to those sheared annually.

Identification of selection signatures and candidate genes related to skin-photosensitization in Leccese sheep

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Photosensitivity in animals refers to an intense dermatitis triggered by an increased sensitivity of skin cells and surrounding tissues to sunlight, occurring after the ingestion or direct contact with UV-reactive secondary compounds from plants. The Leccese sheep is an endangered Italian breed raised in Salento, a traditional breeding site characterised by the presence in the pastures of *Hypericum crispum*, a plant containing hypericin, a compound known to induce photosensitivity in grazing animals upon ingestion. Starting from local populations, the breed has been specifically selected to tolerate skin photosensitivity. In this study, genotyping data and two complementary selection signatures approaches (Rsb and iHS) were used in comparative analyses to identify putative genomic regions related to dermatitis by skin photosensitization in sheep. After quality control, a total of 46,827 markers from the Illumina OvineSNP50 BeadChip and 48 animals were retained and used for the analyses. We found four congruent signals between iHS and Rsb tests that could potentially explain the mechanism underlying the considered trait in sheep. The strongest signal was found on chromosome 10 (OAR10: 39.93-40.63 Mb). In this region, the only protein coding gene is PCDH9, associated with atopic dermatitis in human, and known to be under selection in sheep, with an important role in local adaptation. In addition, we identified other candidate genes related to immune response and coat color, that could potentially explain the mechanism underlying photosensitization in sheep. The findings of our study improve the understanding of the genetic basis of the sheep photosensitization process and provide a reference to study the mechanisms underlying livestock adaptation to local environmental pressures.

The stress responsiveness of habituated and non-habituated Merino-type sheep during electro-ejaculation and routine handling procedures

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To assess the behavioral and physiological stress responsiveness of habituated and non-habituated Merino-type rams subjected to electro-ejaculation (EE) with either a 3-second or 10-second rest interval or a routine handling procedure. Sixty (n = 60) mature rams were assigned to six groups of ten each using a 2 x 3 factorial design. The procedures were repeated over three days, at the same time each morning (6 am). Behavior (nostril flaring, struggling, eye closure, or vocalization) was video graphed, scale-scored, and statistically analyzed using the Chi-Square test of independence. Blood was collected post-treatment at different time intervals (0, 30, 90, and 150 minutes) and analyzed for cortisol concentration. The data was analyzed for repeated measures over time using the GLM procedure. Differences were indicated at a 5% probability level. Habituation had no significant effect on animal behavior or cortisol concentration. There were no significant differences for most behavior parameters except nostril flaring. There was a significant relationship between variables on all three respective days $X_2Day1 = 19.26857$; $X_2Day2 = 19.56158$; $X_2Day3 = 22.19258$, $p\ Day1 = 0.001713$; $p\ Day2 = 0.00151$; $p\ Day3 = 0.000481$. Nostril flaring was hardly present for the oral dose treatment, irrespective of whether animals were habituated or not. Nostril flaring was significantly present for rams that received EE. The presence of nostril flaring indicates the animal's behavior in reaction to pain or discomfort. Rams subjected to EE with a 10-second rest interval had lower ($p < 0.05$) cortisol concentrations 28.21, 24.84 and 23.83 ng/ml on all three days compared to rams subjected to EE with a 3-second rest interval 37.11, 30.01 and 31.20 ng/ml. Therefore, EE with a 10-second rest interval can be recommended over EE with a 3-second rest interval. Animals that received an oral dose of water had an equal stress response in terms of cortisol concentration compared to EE averages. Habituation does not facilitate a positive behavioral response and does not suppress cortisol secretion.

Session 35

Poster 24

Physiological responses to social stress in lambs born to ewes sheared during gestation

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The aim of this study was to compare the physiological response to social isolation in a novel area of weaned male lambs born to ewes that were sheared or not during mid-gestation. On day 80 of gestation, 12 multiparous Australian Merino ewes carrying a single male fetus were sheared, and the remaining 9 ewes remained unshorn as a control group. Ewes were sheared during winter with the Tally-Hi procedure. Lambs remained with their mothers from birth until abrupt weaning (90 days old). When lambs were 105 days old, each one was individually isolated for 5 min in a novel area (2.0 m x 2.0 m x 4 m), alternating lambs from each group over two days. After the test, the lambs were moved to a sampling pen (4.0 m x 4.0 m), located approximately 5 m from the test pen, avoiding visual or chemical communication with the lambs that were still not tested. During the sampling period, another lamb from the same group was housed with the tested one to avoid prolonging the social isolation period. Blood samples were collected by jugular venipuncture 5 min before the test (-5 min), immediately after the test ended (time 0), and 15 and 60 min after the test. Serum total protein, albumin, and plasma glucose concentrations were measured in all samples. Cortisol concentration was measured in all samples, and serum triiodothyronine concentration in samples collected -5 min, 15, and 60 min after the test, using commercial radioimmunoassay kits. Data were analyzed with a mixed model, including the treatment, the time, and their interaction as the main factors in the model, and the day of the test as a random effect. Triiodothyronine concentration tended to be greater in lambs born to ewes sheared during mid-gestation than those born to control ewes (1.74 ± 0.18 nmol/L vs 1.50 ± 0.19 nmol/L; $P = 0.098$). All the blood stress biomarkers changed over time ($P \leq 0.045$), indicating that social isolation was stressful, with no effects of treatments. In conclusion, pre-lambing shearing did not affect the blood stress biomarkers in lambs subjected to social isolation.

Lambs born to ewes sheared during gestation reacted with less fear and more exploratory activity when isolated in a novel environment

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Pre-lambing shearing modifies the ewes' metabolic status, inducing greater fetal growth and lamb birth weight. However, shearing is a long-term stressful event for the mother, altering her offspring's behavioral reaction to stressful situations. The aim of this study was to compare the behavioral response to social isolation in a novel environment in lambs born to ewes that were sheared or not during mid-gestation. Twelve multiparous Merino ewes carrying a single male fetus were sheared on day 80 of gestation (winter), while 9 were left unshorn. Lambs remained with their mothers from birth until abrupt weaning (90 days old). At 105 days old, lambs were individually isolated for 5 min in a novel area (4.0m²), alternating lambs from each group over two days. The novel area had white walls, roof, and a floor with 16 squares. The tests were video recorded, and the number of lines crossed, vocalizations, urinations, defecations, escape attempts, sniffs, and freezing were determined using the Behavioral Observation Research Interaction Software. The proportion of lines crossed, vocalizations, freezing and sniffs in relation to the maximum value of that behavior in the group was calculated for each animal, and a fear index was determined as the sum of this value of lines crossed, vocalizations, freezing minus that of sniffs. Lines crossed, vocalizations, sniffs, freezing and the fear index were compared with a mixed model, considering the treatment as the main effect, and the day of the test as a random effect. The remaining behaviors were compared with the Mann-Whitney test. The number of sniffs was greater in lambs born to ewes sheared during mid-gestation than those born to control ewes (16.5±1.3 vs 9.2±1.5; P=0.001). The other behaviors did not differ between groups. The fear index was reduced in lambs born to sheared ewes from 1.2±0.5 to 0.6±0.5 (P=0.01). Lambs born to sheared ewes exhibited reduced fear and increased exploration during social isolation, suggesting they cope better with social stress.

Session 35

Poster 26

Novel Technologies for Monitoring Small Ruminant Welfare

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This work is a critical overview on the use of novel technologies for monitoring small ruminant welfare, focusing on flock protection, maternal care, nutrition, health, heat stress, and behavior. Technologies like GPS tracking collars can track animal movements and identify risks such as predation, but accuracy can be affected by obstacles or signal interference. Accelerometers can track animal activity levels and detect imminent lambing, but accuracy may vary based on sensor placement and individual behavior. Sensors can monitor grazing patterns and nutrient intake, detect changes in gait indicating lameness and identify pain in sheep through facial expression analysis. Machine learning algorithms can help analyze sensor data and predict nutritional requirements. Infrared thermography can measure skin temperature to indicate heat stress, while drones can capture behavior footage for analysis. It's crucial to carefully consider the limitations of these technologies and validate their accuracy and effectiveness in real-world scenarios before widespread adoption. Some authors report on the lack of practical applicability of the systems, either because the systems are too complex and still require a manual retrieval of data, or they fail to make a general evaluation of the individuals. The variability of sensors and algorithms only creates further complications in statistical validation, since comparison between sensors in a single work would be a massive endeavour. Research is also not evenly distributed, with most studies focusing on females. Around 60% of the presented research focused on females, with some of the analysed manuscripts not providing any information on the gender of the animals. There is also a lack of cost analysis of the practical applications of the researched technologies which should also be a priority since productivity of the farms depends on the correct investments. This work was supported by the projects UI/00772 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

From Barn to Barcode: Welfare Labelling in Turkish Lamb Meat through Integrated Precision Livestock Farming

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This study explored the integration of animal welfare labelling in lamb meat production systems in Turkey, focusing on intensive farms that adopted Precision Livestock Farming (PLF) technologies and full supply chain management. As global consumers increasingly demanded ethically produced, traceable, and residue-free meat, several Turkish initiatives aligned lamb production with international welfare and quality standards. Pilot programs used PLF tools such as camera-integrated automatic sorting and weighing units, enabling animals to be classified by health, weight gain, or behavior with minimal stress. Software-based flock management systems recorded productivity metrics, tracked disease, treatments, and withdrawal periods. These tools allowed centralized veterinary oversight and real-time monitoring, reducing medication overuse and supporting compliance with food safety and export rules. A key innovation was the use of computer vision to assess welfare indicators like resting time, feeding, and social interactions. These behaviors were captured via ceiling-mounted cameras and analyzed using AI, providing continuous, objective evaluation without human presence. All data from behavior and weight monitoring to treatment logs were integrated into a central platform supporting traceability, early disease detection, and transparent reporting for welfare certification. This enhanced both animal well-being and farm efficiency, while building consumer trust. Welfare-labelled lamb products from intensive but high-welfare systems were positioned in premium retail segments that prioritize sustainability and welfare. Vertical integration of production, processing, and distribution ensured consistent welfare protocol application and streamlined certification. Though still emerging, this tech-driven model offered a scalable approach to welfare-focused lamb production. With stakeholder collaboration and national support, Turkey showed strong potential to become a leader in digitally verified, welfare-certified lamb meat.

Session 35

Poster 28

The role of herd functional diversity on Mediterranean agropastoral dairy systems' performance in a changing climate

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Despite their flexibility, Mediterranean agropastoral livestock farming systems (APLFS) are now challenged by climate change. Their feeding system based on semi-natural resources constitutes both a strength and a weakness, and climate-driven fluctuations in the productivity and quality of these resources across seasons and years lead to a risk of feed shortages. This may negatively impact herd performance, but the animal diversity inherent to APLFS could be an asset in reducing their sensitivity. In particular, functional diversity – where animal entities are defined by their different biological functions or responses to perturbations – can both enhance the level of herd performance and reduce its inter-annual variability. This study aims to investigate the role of functional diversity among females in shaping herd-level performance. To address this objective, we developed an individual-based model to simulate the functioning of a small ruminant dairy APLFS. We used different female profiles based on milk production curves, body reserve dynamics, and fertility to calibrate an individual animal model, which was then used as the building block of a herd simulator. This approach enabled the virtual representation of functional diversity within the herd. We explored various herd configurations in terms of number and relative abundance of female profiles, as well as their degree of dissimilarity. We simulated these configurations under different dietary challenges reflecting potential climate change impacts. We analyzed herd performance over ten years, including total milk production, female longevity, and reproductive performance. Our findings demonstrate that functional diversity mitigates the impacts of dietary challenges on herd performance level and variability. The magnitude of this buffering effect depends on the degree of dissimilarity and the relative importance of different profiles within the herd. These results offer new insights for designing adaptation strategies for APLFS in the face of climate change, leveraging functional diversity within the herd.

Development of decision-support algorithms for environmental management and welfare assessment in dairy sheep

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This research aims to create innovative decision-support algorithms that combine environmental parameters with animal biomarkers to address environmental challenges and welfare issues in dairy sheep farms. Dairy sheep industry is vital to the agricultural sector in Greece and other Mediterranean countries; however, gathering and processing environmental and welfare-related data in intensive, zero-grazing farms presents significant challenges, such as insufficient facilities for continuous monitoring and variation on the overall management and, genotypes resulting in different physiological and behavioral responses. Therefore, an interdisciplinary approach that combines sensor-based monitoring devices, physical recordings, and data analytics is proposed. A range of sensors installed in intensive dairy sheep enterprises will be used to measure important environmental parameters, including ambient temperature, air humidity, CO₂, CH₄, and NH₃ concentrations. To determine the animals' reaction to various environmental conditions, relevant markers including serum cortisol concentration, respiratory rate, body temperature, body condition score, and daily activity levels are being collected and analyzed in parallel. Following the preprocessing and cleaning of data meaningful trends and features have been evaluated through robust analytical methods, while appropriate modelling techniques, have been used to investigate potential correlations between environmental conditions and welfare issues. Ultimately, a decision-support system will be developed to promote resource-use efficiency, reduce stress and -related production losses, and enhance the overall well-being of animals and sustainability of farms. This will enable a proactive approach towards evidence-based environmental management and animal-friendly, dairy sheep husbandry systems.

The frequency of distribution of somatic cell count in selected farms of Lacaune breed in Slovakia

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Somatic cell count (SCC) can be used in the diagnosis of subclinical mastitis in ewes, although in practice this method is used to a much lesser extent than in dairy cows. The aim of our study was to assess udder health using SCC in ewes' milk as part of milk recording on selected Lacaune dairy ewes' farms during 2023. The study was performed at seven dairy farms. Totally 4041 milk samples were analysed as a part of milk recording service. Milk samples were divided according to SCC into the following groups: SCC1 $\leq 200 \times 10^3$ cells/ml; SCC2 $> 200 \leq 400 \times 10^3$ cells/ml; SCC3 $> 400 \leq 600 \times 10^3$ cells/ml; SCC4 $> 600 \leq 1000 \times 10^3$ cells/ml; and SCC5 $> 1000 \times 10^3$ cells/ml. MS Excel (Office 365) was used to mathematically evaluate the percentages of milk samples in each SCC group. SCC was determined using a Somacount 150 (Bentley Czech, USA). The frequency of distribution of milk samples in SCC1 ranged from 26.65% (farm 3) to 76.74% (farm 4). The representation of milk samples with SCC $\leq 400 \times 10^3$ cells/ml ranged from 38.99% (farm 3) to 86.81% (farm 4). The frequency of distribution of milk samples in SCC5 ranged from 6.24% (farm 4) to 37.89% (farm 3). On selected farms we observed a fairly constant frequency of distribution of milk samples in SCC3 and SCC4, however, on farms that had a low frequency of distribution of milk samples in SCC1 and SCC2 we observed a high distribution of milk samples in SCC5, indicating a high prevalence of subclinical mastitis on these farms. Since several studies point to the importance of monitoring SCC also in ewes. Regular milk recording could help identified ewes with high SCC and subclinical mastitis and improve health status of udder in herds. Supported by the APVV-21-0134 "Subclinical mastitis in ewes and goats: somatic cells and udder morphology".

How are local and endangered livestock breeds linked to the provision of ecosystem services and the management of specific landscapes?

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Globally and within individual countries, livestock breeds contribute to a wide range of functions, services, and environmental interactions. In regions such as Europe, growing recognition of these contributions—along with the increasing threats that put certain breeds at risk of extinction—has led to institutional and financial support for the most vulnerable breeds. Using data from the Domestic Animal Diversity Information System (DAD-IS), we examine how the geographical distribution and endangerment status of national breed populations relate to their role in the delivery of ecosystem services. Our analysis indicates that local breeds are reported to be associated with a significantly higher number of ecosystem services than transboundary breeds (5.3 vs. 3.8 on average), particularly in provisioning, and regulating and maintenance services. We also found that breeds classified as at risk are linked to fewer reported ecosystem services than those not at risk (4.0 vs. 5.9 on average). Additionally, we present preliminary findings on the relationship between the geographical distribution of local and endangered breeds and environmental factors such as elevation and land cover, highlighting their crucial role in the management of specific landscapes.

Can local breeds be a lever to develop organic and agro ecological livestock farming? Questions raised from exploratory interviews in Guadeloupe

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Local breeds are often considered as original genetic resources with adaptation aptitudes that could be useful in agroecological transition. Producing situated knowledge about their actual role in the development of agroecological farming systems, in various cases, is a way to produce more generic knowledge about the links between local breeds and agroecology. The core question of the REGALDOM project is: what contribution can local animal genetic resources have to the development of organic mixed crop-livestock systems in the French overseas departments? To contribute to answer it, the obstacles to and levers for the use of local breeds should be better characterized. Nine exploratory interviews were conducted with stakeholders of livestock farming in Guadeloupe. The main highlights were discussed during a working session gathering the authors of this communication, based on their expertise on farming systems and animal breeding of local breeds in the region. We underlined the need to qualify precisely the transitions considered, as logics underpinning either agroecology or organic farming can be different, and farmers' motives to go towards such systems can be diverse. We also question how knowledge produced about local breeds' characteristics could be valorized. Indeed, stakeholders perceived a diversity of specificities of local breeds, but some of them also perceived a lack of operational references concerning local breeds. We third highlight the question of how to add value to local breeds' products. We identified several individual projects to add value to food products. Some rested upon local breeds, or would see an interest in using them. However, we identified possible difficulties to the development of such initiatives. Finally, we question how to connect dynamics at farm scale and dynamics at collective, and territorial levels.

A multidisciplinary approach to add value to endangered livestock breeds: The Case of the Indigenous Cattle Breed of Cyprus

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The indigenous cattle breed of Cyprus is the island's only native cattle breed, shaped by millennia of adaptation to local environmental conditions and rural traditions. Following a trend similar to other native breeds within the Mediterranean Basin, the indigenous cattle breed of Cyprus is currently endangered. To highlight its diverse value and promote its conservation, the ARETI project employed a multidisciplinary research methodology, integrating zooarchaeology, history, genetics, archival research, ethnography, and folklore studies. This holistic approach confirmed the breed's long-term—and possibly continuous—presence from the 1st millennium BCE to the present day, highlighting the integral role of cattle to the island's cultural life. Genomic analysis for a DNA sample from cattle bones, dating to the Iron Age, indicates that the animal of origin was genetically influenced by both *Bos taurus* and *Bos indicus* genes. High-throughput (800K) DNA microarray data from 96 samples from the contemporary local Cyprus cattle breed and neighboring populations from Greece and Turkey were generated and further enriched with publicly available genotypes from commercial breeds. Findings highlight the breed's significant genetic distinctiveness from other geographically adjacent or commercial breeds. This multidisciplinary approach deepened our understanding of the breed's unique genetic traits and connection to people and local environments, further reinforcing the breed's historical significance. Beyond its scientific objectives, ARETI fosters community engagement through educational initiatives for younger audiences, seminars for breeders, and a documentary aimed at raising public awareness. This project's conceptual framework and key findings could serve as a paradigm for holistic characterization and valorization of other endangered breeds in Cyprus, the broader Mediterranean and beyond.

Session 36

Theatre 4

A third breed? Suri llama farming in the Peruvian Andes

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In Peru, llama farming has been practiced for thousands of years and is still of great importance to many, mainly poor, farmers. In 1997 the Peruvian government officially recognized 2 breeds, namely Chaku (wool type) and the Q'ara (meat type). For over 2 decades a small group of farmers have been breeding and trying to conserve what they believe to be a third breed, the Suri llama. The animals in this variety exhibit distinct phenotypic characteristics, the most notable being their long, lustrous and slick locks that lie parallel to the body. The specific traits could make the Suri llama a promising option for fibre production. Llamas thrive in extensive systems, even more so than alpacas, and their temperament makes ideal for tourism purposes such as carrying bags along the famous Inca Trail. While some suggest the Suri llama is just a crossbreed between a Suri alpaca and a Chaku llama, others believe this is an ancient breed. They point to historical evidence such as ceramic figurines and mummies from pre-hispanic civilizations that resemble the Suri llamas that are alive today. Acknowledgement that these animals are a separate breed would not only help farmers bring their animals and their products to market but could also provide a significant opportunity for conserving a breed that is now only represented by a few hundred individuals. This study documents the Suri llama's history, management practices, and genetic strategies for conservation, exploring the possibility of its future recognition as a distinct llama breed. Interviews with farmers and stakeholders, including researchers from the Peruvian Institute of Agrarian Innovation (INIA), were conducted, and the collected information was translated for analysis. By combining results from the field with studies on the Suri llama's genetic variation, a case can be made for its recognition as an official llama breed.

Local breeds and landscape genomics: A powerful combination for detecting adaptive genes

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Since the introduction of *Gallus gallus domesticus* to the European continent the species has differentiated into numerous local breeds specific to regions. While, local chicken breeds exhibit a wide array of morphological phenotypes resulting from human driven isolation and selection, it has been hypothesized that these breeds also adapted to their environment in terms of both climate and breeding practices. In the current context of global warming, these local breeds could appear as valuable animal genetic resources to operate the necessary agroecological transition. Consequently, the aim of this study was to identify the underlying genes contributing to adaptation to local breed environmental conditions. The samples consisted in 1337 individuals from 27 breeds spanning over a large climatic area encompassing six countries (Egypt, France, Iceland, Ivory Coast, Norway, Russia) and genotyped for 57K SNP Chip. Twenty-three climate variables were extracted from the WorldClim database for each breeds origin and were reduced into five principal components. We used landscape genomics with two different approaches to evaluate Genomic-Environment Association (GEA) using both Redundancy analysis (RDA) and latent factor mixed model (LFMM). To account for the genetic structure, we computed admixture proportions and used them as a covariate in both analyses. We identified 547 significant SNPs (p -value < 0.05) with RDA and 49 significant SNPs (q -value < 0.1) with LFMM. Gene identification found 396 and 39 genes for the significant SNPs identified in RDA and LFMM respectively. Gene ontology and enrichment for both RDA and LFMM results highlighted significant increase (p -value < 0.05) in genes related to retinal metabolic function in human databases. RDA gene ontology also identified functions related to thermoregulation, immunology and vascular development. These results highlight the value of local breeds and landscape genomics for identifying genomic regions associated to climate adaptation that can be used to inform future breeding efforts. Keywords: Adaptation ; poultry ; landscape genomics Funding: GERO NIMO (H2020 GA No 101000236)

Session 36

Theatre 6

Placing Varzese-Ottonese-Tortonese dual-purpose cattle breed in the genetic landscape of cattle genetic resources

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The Varzese, also known as Ottonese or Tortonese, is an indigenous cattle breed originated in Lombardy and is primarily raised in the mountainous regions of the Appennino Lombardo. Over time, its population has significantly declined due to competition from agricultural mechanization and the dominance of higher-yielding cosmopolitan breeds. By the 1990s, the breed had reached critically low numbers. However, biodiversity conservation efforts and the recognition of its high-quality meat and milk products have contributed to its revival. This study aimed to analyse the population structure and identify selection signatures in the Varzese breed ($n = 82$), comparing it with two closely related Italian autochthonous cattle breeds: Reggiana ($n = 196$) and Modenese ($n = 92$). Using high-density single-nucleotide polymorphism (SNP) chip data, we performed population structure analyses, including multidimensional scaling, genetic admixture analysis, and linkage disequilibrium decay. Selection signatures were detected using multiple approaches, such as runs of homozygosity (ROH), fixation index (FST), integrated haplotype score (iHS), and cross-population extended haplotype homozygosity (XP-EHH). Our results indicate a strong genetic relationship between the Varzese and Reggiana breeds, with notable selection sweeps along the Varzese genome. The annotation of these selected regions revealed genes linked to key traits relevant to dairy and beef production, underscoring potential avenues for genetic improvement and conservation. This study provides a comprehensive genomic characterisation that can guide future breeding and conservation strategies for this endangered cattle breed. This research was funded by the PSRN (Programma di Sviluppo Rurale Nazionale) 2014–2020 and 2020–2023, measure 10.2, Dual Breeding and Dual Breeding 2 projects.

Genetic diversity and conservation of local goat breeds in northern Belgium (Flanders)

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Genetic diversity screening is a crucial initial step in evaluating livestock populations, essential for breed characterization, management and genomic assessment. This study analysed genetic diversity in three local goat breeds in northern Belgium (Flanders): Kempische Geit, Vlaamse Geit, and Belgische Hertegeit. Pedigree analysis revealed inbreeding coefficients of 10.3%, 7.4%, and 9.0% and effective population sizes of 17, 28, and 43, respectively. Despite the critically small population sizes, with as few as 33 active dams in Kempische Geit, breeding numbers have increased by up to 153% (Kempische Geit), and registered breeders have grown by up to 90% (Vlaamse Geit) since 2017. Genomic analysis was conducted on 280 goats (88 to 97 per breed) using the GGP Caprine SNP array ($\pm 67,000$ SNPs). Inbreeding degrees estimated via runs of homozygosity (ROH) ranged from 8% to 15%, with a maximum of 39%. Several ROH islands were identified, for example Vlaamse Geit showed overlap of two ROH islands on chromosomes 10 and 13 with several other international sheep breeds. Effective population size estimates based on linkage disequilibrium were equal to 21 or 22, indicating endangered status for the three local goat breeds in northern Belgium. The F_{st} analysis showed substantial differentiation ($F_{st}'s \pm 0.12$) between breeds, confirmed by principal component analysis. This study represents the first combined pedigree and genomic assessment of goat diversity in northern Belgium, providing valuable insights for the genetic management of these endangered breeds. Additionally, the newly acquired genotypes offer opportunities to explore genetic links with other international goat breeds.

Session 36

Theatre 8

Preliminary analysis: unravelling the genomic background of West African sanga cattle using signals of local ancestry

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African cattle underwent extensive admixture for centuries giving rise to different Sanga cattle populations. Local ancestry analysis could provide insights into their demographic history and genetic ability for adaptation. However, such analyses are dependent on the populations used as reference and reaching a consensus among local ancestry comparisons can be challenging. This study aims at analyzing the zebu- and taurine-derived ancestral components in Sanga cattle. Two previously genotyped datasets, including 253 West African cattle were available. After removing mendelian errors, loci having $MAF < 0.01$ and pruning for linkage disequilibrium, a total of 35,387 autosomal SNPs were retained. Local ancestry was inferred for 61 sanga individuals performing 16 different pairwise comparisons combining four taurine and four zebu cattle populations. A novel framework coupling beta regression modelling with a consensus approach was applied to identify consistent signals of local ancestry across comparisons. Results highlighted a higher African zebu introgression into sanga cattle. Resampling was performed to identify consensus SNPs with significant local ancestry signals based on a majority rule approach. A total of 1,867 consensus SNPs were retained, mainly located on BTA1 and BTA2. Consensus SNPs were summarized into 15 (2,996 kb; 10 chromosomes) and 10 (2,729 kb; 6 chromosomes) candidate regions with taurine and zebu ancestry, respectively. The taurine-derived regions intercepted genes involved in vascular development, angiogenesis, and immune responses, potentially linked to resistance against bovine trypanosomiasis and Plasmodium infections. Zebu-derived regions included genes associated with key zebu traits such as horn development, heat stress resistance, and muscle growth. We confirm that using complementary statistical approaches is essential to accurately identify consensus SNPs having stronger signals of local ancestry when comparing target populations with different sets of reference populations.

Assessment of the Effectiveness of the National Breeding Nucleus in the Lithuanian Blackface Sheep Population

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The Lithuanian Blackface (LBF) sheep breed was officially recognized in 1961 as a semi-fine-wooled, dual-purpose meat–wool breed. Breed monitoring data indicate that the Lithuanian Blackface sheep population remains stable, with 7,523 purebred individuals recorded in the main section of the studbook and 220 registered breeders. One of the largest farms housing this breed is the national sheep breeding nucleus, established in 1963, which has played a crucial role in advancing research and reinforcing the breed's status as a valuable genetic resource for Lithuania. The national sheep breeding nucleus is supported by the Ministry of Agriculture of the Republic of Lithuania under the national program for the conservation of endangered farm animal genetic resources. In 2023, an assessment of the Lithuanian Blackface sheep population was conducted to evaluate the effectiveness of this breeding nucleus and its contribution to overall population sustainability, with a focus on the effective population size (N_e). To assess the impact of the state-supported breeding nucleus, a pedigree analysis was performed on registered purebred sheep from the studbook. The LBF population was stratified into two subpopulations: individuals within the national breeding nucleus and those maintained by private breeders. Using the statistical software PopReport, the effective population size was estimated based on the rate of inbreeding (ΔF). The results demonstrated that the effective population size ($N_e\Delta F$) for the entire breed exceeds 200, primarily due to the contribution of the national breeding nucleus, which comprises 24% of the total population. In 2022, the $N_e\Delta F$ for the national breeding nucleus was 282, whereas in private farms, it was 86. These findings underscore the importance of population structure assessment and effective population size estimation in breed conservation and breeding program evaluations. Maintaining sustainable genetic diversity is essential for ensuring the long-term viability of the Lithuanian Blackface breed.

Session 36

Theatre 10

Phylogenetic position of Hungarian Grey cattle breed based on total representation sample

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The Hungarian Grey (HG) cattle was almost extinct after WW2; only 200 cows and 6 bulls survived in Hortobágy. Therefore, it was possible to use a complete representative "founder" sample ($n=110$), which had not been used before, for phylogenetic analysis of the mitochondrial control region. 777 bp-long fragments of CR mtDNA were used (BRS 15764-201 on Bovine Reference Sequence). The number of haplotypes in the sample was 47. The haplotype diversity (H_d) was 0.941 ($SD=0.016$). The nucleotide diversity (π) was 0.00482, ($SD=0.00033$). The average number of nucleotide differences (k) was 3.661. Our results indicate that the HG breed retains the original taurine (T) CR haplotype groups (haplogroup-n: T1'2'3-3, T1-4, T2-5, T3-98), but does not carry any genetic traces of hybridisation with European aurochs' haplogroups (P, Q). This is presumably because HG was never tried to be used for anything other than extensive beef production, and therefore the taurine stock of eastern origin was not crossed with local, European cattle. The original breeding purpose of the Podolian cattle was for beef production. In the original Podolian breeds, this has been preserved and not changed, so they have not been crossed with cattle carrying mitochondrial aurochs haplogroups. On this basis, the Podolian cattle can be divided into two groups, the original 'primitive' Podolian breeds bred exclusively as extensive beef cattle and the crossbred Podolian cattle, which were crossbred with high production breeds as intensive beef or dual purpose or dairy cattle.

Milk, meat, robustness, pasture and fight: genetic and genomic analysis of new and classical phenotypes depicting the genetic, cultural and ecological value of the Alpine Valdostana cattle

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Aosta Red Pied (PR) and Aosta Black Pied-Chestnut (CN), also known as “Valdostana”, are local cattle of the western Alps, providing important ecosystem services to the territory where they live. Extensive breeding and summer grazing contribute to land protection, while production is aimed at typical products, such as PDO Fontina. CN adds to the dual purpose of milk and meat a peculiar aptitude to fight, expressed in traditional competitions attracting farmers, valley dwellers and tourists. New phenotypes (NP) have been recorded since 2017 including some reflecting typical features of the breeds like social dominance in herd (DOM), aptitude to pasture (PAS), and general desirability of a cow (DES), in addition to body condition score, mungibility, aggressiveness, all linearly scored in primiparous cows. The study aimed to estimate genetic parameters, correlations and selection response in the NP and in current productive and functional traits including milk, fat and protein (MY), linear type traits for muscularity (MUS), fighting ability (FIG), somatic cell score, fertility and longevity. Genomic information was also included where available to estimate genomic breeding values and associate genes and traits. NP of nearly 16000 cows were available after editing, and about 7240 genotypes. Heritabilities ranged from 0.03 for aggressiveness and fertility to 0.17 for DOM in herd and muscularity. Adverse genetic correlations (r) were found among DOM and FIG on one side, MY on another, and fertility, reflecting the complex relationships among different aptitudes and fitness. PAS was positively related with MY in PR. DES showed positive r with DOM and FIG in CN, and opposite r with MY in both breeds, reflecting the main breeds' aptitudes. Some associations were found between genes and traits. Results portray the peculiar features of Valdostana breeds, offering insights for breeding management of local populations.

Session 36

Poster 12

Exploring genomic diversity in rainbow and brown trout populations in Slovakia – Pilot study

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Rainbow and brown trout represent key species in the Slovak aquaculture sector, with rainbow trout accounting for the largest volume of the country's farmed fish production. The aim of this study was to realize a pilot study assessing the state of genomic diversity of both species represented by a sample of animals from one of the largest fish farms in Slovakia. It was collected 50 samples of rainbow trout and 15 samples of brown trout that were genotyped by the GGP Rainbow trout 20k. After standard SNP pruning, the genomic database included a total of 14274 informative SNP markers. The level of genomic diversity within populations was quantified by standard population diversity indices: observed (H_o) and expected (H_e) heterozygosities, and genomic-based indicators of inbreeding (FHOM and FROH). The population structure and genetic patterns were then tested by PCA and a neighbour-joining tree derived from relationship and genetic distance matrices. Rainbow trout showed a higher proportion of heterozygosity ($H_o=0.36\pm0.16$, $H_e=0.35\pm0.14$) compared to the brown trout ($H_o=0.10\pm0.29$, $H_e=0.06\pm0.15$). This was also confirmed by the FHOM (-0.02 in rainbow and 0.49 in brown trout). Despite the relatively low F_{ST} value (0.38) between them, both species formed clearly differentiated genetic clusters. A comparison of genetic pattern variability across both species confirmed relatively high genetic uniformity of the brown trout group. The obtained results provide an initial insight into the genetic diversity and population structure of farmed trout populations in Slovakia, which may serve as a valuable baseline for further genomic monitoring. The observed higher genetic uniformity in brown trout highlights the need for careful management strategies to preserve its genetic variability in aquaculture stocks. Future research will extend sampling across more farms to gain a broader insight into the genetic diversity of both species, supporting sustainable breeding and conservation strategies in Slovak aquaculture. This study was supported by grant no. APVV-17-0060 and APVV-20-0161.

Assessing structural variants in DSN cattle and their impact on genomic features

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German Black Pied (DSN) is an endangered cattle breed valued for its genetic diversity, robustness, and high milk fat and protein content. Previous studies using short-read sequencing data primarily identified SNPs and small insertions/deletions (indels). However, structural variants (SVs), which include large deletions, duplications, inversions, and translocations, have remained largely unexplored. This study utilizes long-read sequencing technology (PacBio Sequel II HiFi and PacBio Revio HiFi) to assess SVs in 13 DSN cattle including two parent-offspring trios. SVs were identified using sniffles2 v.2.5.3 and functionally annotated using the Ensembl Variant Effect Predictor. Additionally, previously identified QTLs for milk and meat traits in DSN were screened for locally relevant SVs. We identified a total of 56,820 SVs, with large indels being the predominant type. Genotype concordance in trios was 84.9% for PacBio Sequel II and improved to 97.3% with PacBio Revio HiFi, highlighting the higher data quality of the latter. Functional annotation of SVs revealed 1 start lost, 57 stop lost, and 130 frameshift SVs across the whole genome of the investigated cattle. In one of the previously identified meat QTLs for birth weight on chromosome 5, we discovered a 7,504 bp deletion that resulted in the loss of exons 14-21 (out of 21) in the WC1.3 (WC1.3 molecule) gene, including the stop codon. This deletion likely disrupts WC1.3, a gene involved in cargo receptor activity. Another 126 bp long insertion, found in a QTL for body weight at 7 months on chromosome 10, caused a frameshift in exon 35 (out of 82) of the gene VPS13C (vacuolar protein sorting 13 homolog C), which is necessary for proper mitochondrial function and lipid transport. Both SVs had no linkage ($r^2 \leq 0.05$) to the top variants of the respective QTLs, but contribute to local genetic variation. This study provides novel insights into structural variations in cattle. Further investigations are necessary to evaluate the functional impact of these SVs on economically important traits and their potential role in breeding strategies.

Session 36

Poster 14

Twinning in Maremmana cattle: Do polymorphisms in the GDF9 and BMP15 genes influence trait expression?

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Twinning in cattle is considered an undesirable trait, due to multiple negative effects such as abortion, dystocia, stillbirth, retained placenta, metabolic disorders, displaced abomasum, and ketosis. Furthermore there is the effect on future calving because twinning increases the calving interval and the time period between calving and conception. Nevertheless, several studies have shown that the production of twin calves offers the opportunity to increase economic efficiency in beef cattle farms. Twinning rate is affected by genetic and environmental parameters. In cattle, both candidate gene studies (involved in folliculogenesis) and GWAS have been conducted, but no real gene related with the trait has been discovered, leading to the conclusion that the twinning trait is extremely complicated and appears to involve a large number of genes. The objective of this study was to evaluate the influence of growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) genes on twinning in cattle. In this study we analyzed the Maremmana cattle reared at farm of CREA-ZA (Monterotondo, Roma). From 2012 management of this population was changed with the scope to increase twinning rate. Three different regions of GDF9 (I and II exon, 3'UTR) and II exon of BMP15 were amplified by PCR and sequenced using DNA extracted by blood samples of 111 cows. The sequencing of these fragments revealed: 1) GDF9: one SNPs in the first exon (g.44445268G>C) and one deletion in 3'UTR (g.44442671_44442672del); 2) BMP15: two deletions (g.88810168_88810171del, g.88810174_88810177del). GDF9 mutations were present only in cow with single birth, where g.44445268G>C causes AA substitution (p.Q26H) in GDF9 proregion with no influence on 3D folding, and g.44442671_44442672del doesn't influence any regulatory sites in 3'UTR. BMP15 deletions were present in both twin and single-birth cows, and both resulted in a frameshift variation and a premature stop codon. This mutation could affect the function of the BMP15 protein, resulting in a possible explanation of twin birth in Maremmana cattle. In a sustainable livestock system, an increase in twinning in beef cows could be a chance to boost profitability.

Uncovering the architecture of production-driven introgression in Cinisara cattle breed

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Local livestock breeds play a pivotal role in agricultural sustainability, biodiversity conservation, and cultural heritage. These breeds possess unique genetic traits adapted to their specific environments, such as rusticity, heat tolerance, and digestive efficiency. The Cinisara is a dual-purpose local cattle breed primarily farmed in western Sicily, Italy, and represents an integral part of Sicilian agriculture. However, reports suggest crossbreeding with cosmopolitan breeds to enhance meat or milk yield. To assess the conservation status and selective pressures on the Cinisara, we investigated the extent of introgression from cosmopolitan breeds using local ancestry inference (LAI). We genotyped 71 unrelated Cinisara cattle at 65k SNPs and compared them with publicly available genotype data from cosmopolitan breeds (Holstein, Brown Swiss, Limousine, Modicana, Podolica). Cinisara showed moderate heterozygosity (0.359) and low inbreeding levels (0.040). Global ancestry and Treemix analyses confirmed its Podolian origin while highlighting significant introgression from Holstein and Brown Swiss. Local genomic ancestry was inferred using three reference populations: Podolica (ancestral origin), Holstein, and Brown Swiss. We identified 262 introgressed SNPs from Holstein and 261 from Brown Swiss, with 57 candidate genes within these regions. These genomic regions showed high to complete substitution by cosmopolitan genomes and overlapped mainly with QTLs associated with milk production traits. Our findings align with the historical use of cosmopolitan breeds to enhance local breed productivity while raising significant concerns regarding the conservation of genetic diversity, cultural value, and economic significance of the local population. These results highlight the need for breeding strategies that balance production improvements with the preservation of genetic heritage.

Session 36

Poster 16

Deep pedigree verification using mtDNA and Y-chromosome data: advancing MaGELLan 2.1

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Low-coverage whole genome sequencing (lcWGS) has become powerful and cost-effective approach in population genomics and genome-wide association studies (GWAS). Here we present the utilization of lcWGS data to test a new version of the MaGELLan 2.1 software. The main aim and capability of the previous version was to analyse maternal lineage using mitogenome polymorphism from the whole mitogenome sequence and the GG-P100K SNP array, while it has now been extended to analyse paternal lineage using Y-chromosome markers. In addition, the software has been updated with a new graphical user interface, keeping the existing modules into the command line interface. Computational cost of the sampling procedure, which has been greatly reduced through the implementation of two efficient algorithms, has been tested on large Holstein cattle pedigree using retrieved mtDNA and Y chromosome markers from lcWGS. With the new visualisation module, we were able to check the insight into important parts of pedigrees. The software is fully functional under Windows and Linux operating systems. This research was supported by grant HRZZ-IP-2022-10-6914. Key words: software, pedigree, lcWGS, mtDNA markers, Y-chromosome markers

Enteroids as species-specific in vitro models for the intestinal epithelium

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In 2009 Sato et al. described the ability of intestinal stem cells to form crypt-villus-like structures mimicking the gut epithelium [1]. These so-called “mini guts”, or enteroids, have significantly advanced the field of gastrointestinal epithelial research. While early studies primarily focused on murine and human enteroids, a growing number of species have since been brought „to the dish“ [2]. Enteroids consist of multiple epithelial cell types that form a 3D structure and resemble the in vivo situation more closely compared to immortalized cell lines. This allows to investigate species-specific (patho-)physiological mechanisms in vitro. However, for a biologically relevant result, it is essential that enteroids not only proliferate but also differentiate to mirror the in vivo situation. This requires optimized cultivation protocols and thorough characterization to select the most suitable model for each research question. While the isolated cultivation of intestinal epithelia enables the investigation of molecular mechanisms at a single-cell level, interactions between different tissues, particularly epithelium and immune system, must not be neglected. Thus, the next step toward replicating in vivo conditions more accurately is the development of coculture models within microphysiological systems. Therefore, we aim to establish a coculture system using equine colon enteroid derived epithelial monolayers and peripheral blood mononuclear cells. This system may be used to test, e.g., the effects of probiotics on epithelial-immune cell interactions. [1] Sato T et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009 459, 262–265. doi: 10.1038/nature07935 [2] Seeger B. Farm Animal-derived Models of the Intestinal Epithelium: Recent Advances and Future Applications of Intestinal Organoids. *Altern Lab Anim.* 2020 Sep-Nov;48(5-6):215-233. doi: 10.1177/0261192920974026

Session 37

Theatre 2

Development of immune-organoid co-culture to study gut health in pigs

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In vitro models, such as intestinal organoids, have emerged as powerful tools for studying gut health. Here, we developed a two-dimensional co-culture model of pig intestinal organoid-derived epithelial cells and pig blood-derived macrophages. We cultured monocytes in transwells either as a monoculture or in co-culture with organoids. To investigate the influence of different culture conditions, we compared organoid culture medium with or without macrophage-colony stimulating factor (M-CSF). Immune cells were characterized by flow cytometry, focusing on the macrophage pro-inflammatory marker CD80 and anti-inflammatory marker CD163. Additionally, co-culture functionality was assessed through barrier permeability and LPS response. CD80 expression increased and CD163 expression decreased in immune cells upon LPS stimulation, with these effects more pronounced in monoculture and moderated in co-culture with organoids. However, M-CSF treatment had no effect on CD80 and CD163 expression in immune cells, with or without co-cultured organoids. These findings suggest that immune cells remain viable in co-culture with organoids and exhibit a pro-inflammatory shift in response to LPS. As these results are preliminary, additional repetitions are needed to ensure statistical robustness. Additionally, cytokine levels will be analyzed to further assess immune cell responses. In conclusion, this pig-specific in vitro system demonstrates a pro-inflammatory response to LPS, highlighting the potential of pig-based organoid models for studying intestinal immune responses and gut health. This model may provide deeper insights into immune system-feed efficiency interactions and could serve as a platform for studying nutritional interventions to improve gut health, ultimately contributing to more resilient livestock production.

An ovine intestinal organoid-macrophage co-culture model to test the effects of methionine and betaine on the intestinal barrier function

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The “intestinal barrier function” is a defensive system developed to block the passage of antigens, toxins, and microbial byproducts, and also preserves the acquisition of tolerance against dietary antigens and the intestinal microbiota. In this sense, methionine (a sulphur-containing amino acid playing a role in protein synthesis) and betaine (a natural compound of the beet) supplements are commonplace in the feed industry in order to improve feed efficiency and health of livestock. Therefore, methionine and betaine supplementation might modulate the intestinal barrier function and immunity parameters. To test this hypothesis, a trial with ovine intestinal organoids and macrophages was attempted (three Rs principle). Duodenum of a 4-month-old male lamb (slaughterhouse) was collected to isolate adult stem cells and IntestiCult™ OGM Mouse Medium was used to culture for 7–10 days, with medium refreshed every 2–3 days and passaged organoids in 24-well plates. Blood was collected from a healthy 6-month-old lamb to isolate monocytes (magnetic cell separation using CD14 MicroBeads) that were differentiated into macrophages (10 days). Finally, a 12-well transwell system with 0.4 µm PET membranes was used to co-culture lamb intestinal organoids and macrophages, and methionine (1.8 µL, 2 mmol/L), betaine (1.8 µL, 2 mmol/L) and a mixture of both compounds (1.8 µL + 1.8 µL) diluted in monolayer medium were added to the wells. After 12 h of incubation (37°C with 5% CO₂), samples were collected from both chambers. The expression of genes encoding tight junction proteins (e.g., TJAP1 and OCLN) was assessed (RT-qPCR) on intestinal organoids whereas gene expression of others related to immunity response was measured in macrophages (e.g., TNFα, IL10 and TGF). The effects of treatments on immunity parameters are not conclusive, but methionine might contribute to improve the intestinal barrier function of organoids at the dose selected.

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Theatre 4

Strategies for coupling in vivo and in vitro methods in ruminant nutrition studies, benefits and limitations
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In recent years, there has been growing interest in fermenters to quantify ruminal microbiota functions as they enable their extensive characterisation using various substrates, under would-be hazardous conditions in vivo and in response to societal demand to reduce animal experimentation. Inoculating fermenters with rumen fluid collected through oral tubing paves the way for their coupling with in vivo methods on a larger number of animals in the same study (free from the constraints of surgical cannulation) while preserving individual variability (ex vivo) and in a variety of zootechnical contexts (e.g. growing animal). Three possible coupling strategies are exposed below. The most common coupling is the use of short-term, low-volume batch systems in studies of individual variability in zootechnical performance and production of fermentation end-products since quantifying ruminal fermentation in vivo is very restrictive. This strategy also makes it possible to define the ruminal metabolic pathways of pure molecules, such as polyunsaturated fatty acids in different ruminant species. However, the accumulation of end-products and pH decrease quickly alter microbial activities. A second strategy is to use long-term fermenters (mostly Rusitec and dual effluent continuous culture) to extensively characterise the ruminal metabolism (e.g. biomass synthesis) in animals without duodenal cannula and to adapt the microbiota to experimental treatments, despite possible ciliates relative rarefaction and poorly controlled solid turn-over. To be fully operational, this coupling requires the use of a fermentation device both adapted to the smaller volume of inoculum collected from oral tubing than from cannula, and made up of a large number of units. The most complete strategy is based on the collection of zootechnical and digestive data along with in vitro batch and semi-continuous fermenter data on the same group of animals. This dual coupling, performed in our research unit to study rumen microbiota-host interrelationship, takes advantage of the potential of the three experimental set-ups by compensating for their specific limitations.

Simulation of rumen hyperthermia (heat stress) in in-vitro rumen fermentation

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Rumen fermentation produces ~15 % of heat and plays a significant role in managing heat stress in cattle. In-vitro rumen fermentation is widely used to simulate rumen fermentation and study the microbiome and metabolite profiling. However, understanding of the interplay between rumen hypothermia, fermentation kinetics, and metabolic profiles in-vitro rumen fermentation is scarce. To fill the knowledge gap, we established an in-vitro method to test the effect of rumen hyperthermia on rumen fermentation and fermentation products (pH, CH₄, short-chain fatty acids). To define the variation in rumen temperature to be represented during the in-vitro fermentation, one year (07/2023-07/2024) of rumino-reticulum bolus data (SmaXtec, Graz, Austria) was recorded from 171 cows in Denmark. Five temperatures, 39.5, 40.5, 41.5, 42.5, and 43.5 °C, were selected for in-vitro rumen fermentations with maize silage as the substrate, monitoring total gas production (TGP), methane (CH₄) yield (ml CH₄ per g DM at standard temperature and pressure), and volatile fatty acid (VFA) production for 48 h in each fermentation. There was no significant effect of fermentation temperature on the pH, total gas production (TGP), and CH₄ yield. We, however, found a significant ($P < 0.05$) effect of temperature on acetic acid, propionic acid, butyric acid, and total VFA production. The temperature of 43.5 °C had the lowest acetic acid (24.6 mMol/L), propionic acid (8.59 mMol/L), butyric acid (6.28 mMol/L), and total VFA (8.90 mMol/L) when compared to the other examined temperatures. In contrast, the 39.5 °C (Control) resulted in 26.9 mMol/L of acetic acid, 9.78 mMol/L of propionic acid, 7.55 mMol/L of butyric acid, and 45.5 mMol/L of total VFA. A linear effect ($P < 0.05$) was observed on concentrations of propionic acid, butyric acid, and total VFA, while temperature exhibited both linear and quadratic effects on acetic acid production. This study demonstrates that in-vitro rumen hyperthermia influences fermentation and fermentation end-products.

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Theatre 6

An interlaboratory test with the rumen simulation technique (RUSITEC)

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For ruminant nutrition studies the semi-continuous culture method RUSITEC (Rumen Simulation Technique), developed in the 1970s (Czerkawski & Breckenridge 1977), can be a useful tool. The system commonly consists of up to eight fermentation vessels, which are inoculated with fresh rumen contents from cannulated donor animals. Besides the number and sizes of fermentation vessels, deviations between research institutions include ruminant species of the donor animals, their diets, artificial saliva composition and flow rate, pore size of the nylon feed bags and several more. So far, no comparative studies including different laboratories have been carried out. Therefore, two trials with nine laboratories from Germany and Switzerland were initiated. In the first trial all nine labs received the same feedstuffs (a concentrate mixture and hay) to be incubated according to their commonly used protocols and analytical procedures. In the second trial a uniform operating protocol was used by each lab with the donor animals as the only variable factor. Substantial variations in operating procedures were found in the first trial. While pH could be kept relatively constant among groups and anaerobic conditions were maintained, the concentrations of NH₃ ($10.2 \text{ mmol/l} \pm 2.9 \text{ SD}$) and short chain fatty acids ($63.4 \text{ mmol/l} \pm 20.6 \text{ SD}$) varied considerably. Likewise, the degradability of DM ($53\% \pm 10 \text{ SD}$) and NDF ($41\% \pm 18 \text{ SD}$) varied considerably. Harmonisation of the RUSITEC protocol decreased variation of most determined parameters. Across all fermentation parameters and both feed types, the variation in the second trial was 14.1% lower and for the nutrient degradability it was more than 30% lower than in the first trial. Accordingly, in order to guarantee a better degree of comparability between labs a standardized protocol is recommended. Czerkawski J., Breckenridge C. 1977. Br. J. Nutr. 38, 371-384.

Exploring rumen fermentation and microbiome of freshly killed steers

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Freshly killed animals can be used as an alternative to live animals to obtain rumen fluid (RF) to estimate in vitro degradation of numerous feeds. This non-invasive approach may replace the need to use surgically modified animals that are costly and ethically undesirable. However, obtaining RF from killed animals at an abattoir might be challenging due to the logistics and diversity of animals that are routinely killed for human food. Thus, this completely randomized study explored firstly the fermentation features of 6 pooled RF from 12 steers of multiple breeds and ages (500-820 days) post-killing on 6 occasions at an abattoir. Secondly, RF of only 2 steers (Steer A, B) of the same breed, age, location and diet were extracted for DNA by DNAzol reagents. The DNA samples were processed for shotgun metagenomic analysis with Next Generation Sequencing and Bioinformatics by Novogene Ltd. The data sets were either numerically or visually assessed for similarities or variations in RF. The pH (5.89-8.48) and total volatile fatty acid (59-117mM/L) values varied more than the ammonia (185-202 mg/L) contents reflecting the variations in the pre-killing features of the steers. Metagenomic analysis revealed comparable total scaftig (>500bp) number (317,986 vs 326,979) and average length (949 vs 936 bp) for Steers A and B respectively. While RF in both steers had 60% identical genes, each steer also had 20% unique genes. The steers compared well for the abundance of bacterial phylum, family, genus and species. The phylum data showed good correlation ($r=0.6$) and the alpha diversity indices compared well for both steers. The phylum abundance (45-47%) for the top five bacteria also compared well for Bacteroidetes, Firmicutes, Fibrobacteres, Proteobacteria, Lentisphaerae in these steers. Despite natural differences in fermentation and microbiome, RF from freshly killed steers can be used as an alternative to live animals to evaluate ruminant feeds. However, coordinated efforts are needed to minimize complex logistics and variations between animals that are killed at an abattoir before their use to replace live animals to promote 3Rs.

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Theatre 8

Characterizing the nutritional value of high-latitude pastures for dairy cows using an in vitro technique

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The study followed the Grazing Living Lab methodology within SustAnimal—a knowledge centre focusing on the production animals' future role in Sweden. Our objective was to characterize the nutritional value of pasture for dairy cows in high-latitude areas by assessing volatile fatty acids (VFA), ammonia nitrogen (NH₃-N), and utilisable crude protein (uCP) using an in vitro technique. Pasture samples, primarily composed of timothy, other grasses, and red and white clover, were collected from 12 field spots at seven dairy farms in Västerbotten, Sweden, across three months during summer (June-August) in the years 2021-2022. After excluding dead material, the samples were dried, milled, and then grouped by farm, month, and year, totalling 34 samples. Samples were incubated for 28 hours in three replicates, using rumen fluid as an inoculum. Fluid samples (0.6mL) were collected at 4, 8, 20, and 28 hours post-incubation for NH₃-N analysis and estimation of uCP at 16 hours of retention time. Additional fluid samples were taken at the 28-hour time-point for VFA determination. The data were analysed using ANOVA in PROC GLM in SAS, with farm, month, and year, along with their interactions, included as fixed effects in the model. Results showed significant variation in the parameters measured: NH₃-N (50-255 mg/L), uCP16 (119-274 g/kg DM), total VFA (2.39-3.90 mmol), and individual VFAs as a proportion of total VFA—acetate (598-68.0 mmol/mol), propionate (235-307 mmol/mol), and butyrate (71.3-95.9 mmol/mol). For all parameters except for butyrate, there was a significant effect for farm, month and year. uCP showed interaction between month and year with the highest value presented in June 2022 (221 g/kg DM). The study highlights the high variability in the nutrient content of pastures among farms, across the summer months, and between years. The in vitro technique used provides a reliable method for assessing protein quality and rumen fermentation, which, when combined with chemical composition data, can help farmers optimise pasture use during the summer.

Using faeces as an alternative to rumen fluid for gas production in batch culture incubations – a systematic comparison

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In vitro gas production using rumen fluid as an inoculum is widely employed to assess the nutritional value of ruminant feedstuffs. However, ethical concerns regarding fistulated animals have driven interest in alternative inocula and reliable evaluation methods. This study, integrating eight experiments with 359 treatments, investigated in vitro gas production using rumen inoculum (RI) and faecal inoculum (FI) to assess the feasibility of replacing RI with FI and to identify factors influencing differences in gas production between the two. Gas production at later incubation time points with FI showed greater accuracy and precision in predicting RI gas production at 24 hours (72 h > 48 h > 24 h). FI concentration (g DM/mL), substrate type, and incubation time were identified as significant factors affecting gas production differences between RI and FI ($P < 0.001$). These findings suggest that inoculum preparation, particularly the initial microbial biomass, is the primary determinant of FI's suitability as a substitute for RI. A higher faecal dry matter concentration reduced the difference in gas production between FI and RI incubations. Future research should explore methods to maintain a high initial microbial biomass in FI and assess whether the predictive model remains robust across different feed substrates.

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Theatre 10

The protective effects of curcumin loaded silica nanoparticles on obesity; In vitro and In vivo studies

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Obesity is a serious health issue in humans and companion animals, associated with metabolic disorders, premature mortality, and various diseases across species. Curcumin, derived from the rhizomes of turmeric (*Curcuma longa*), has been shown to have a protective effect on obesity and metabolic diseases. Additionally, nano-encapsulated curcumin offers better bioavailability, stability, and absorption, ensuring improved therapeutic effects over normal curcumin. In the present study, we have synthesized and characterized curcumin-loaded silica nanoparticles (CLSN) and examined their effects against obesity using in vitro and in vivo models. CLSN had a uniform 200 nm particle size with a -15.36 mV zeta potential. UV-Vis, FTIR, and XRD confirmed the silica-curcumin composition, characteristic absorption peaks, and dual amorphous-crystalline nature. Further, CLSN's anti-obesity effects were assessed using oleic acid (OA)-induced HepG2 cells and high-fat diet (HFD)-fed mice. The results revealed that CLSN reduced lipid accumulation in OA-induced HepG2 cells and curbed body weight gain, decreased liver and adipose tissues weight, and lowered triglyceride and total cholesterol levels in HFD-fed mice. Collectively, our findings suggest that CLSN supplementation may help prevent obesity, highlighting its potential for future clinical investigations. Keywords; Anti-obesity effects, Curcumin, HFD-fed mice, nanoparticles

Comparing traditional forms of supplemental copper to a novel monovalent copper oxide on bacteria control and piglet performance after weaning

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Copper (Cu) is widely used in animal production for its antimicrobial properties and growth-promoting effects, but different sources vary in bioavailability and antibacterial efficacy. *Streptococcus suis* is a significant pathogen causing severe post-weaning infections in piglets, leading to reduced growth and increased mortality. This study compared Cu sources on bacterial growth and piglet performance. Cu sources included copper sulfate (Manica Cobre, Spain), chelated Cu (AvailaCu®, Zinpro, USA), tribasic Cu chloride (TBCC, IntelliBond®C, Selko, USA), and monovalent Cu (CoRouge®, CR, Animine, France). In vitro: *S. suis* (DSM 9682) in Brain-Heart-Infusion medium was tested with each Cu source saturated at 10g/L at a concentration of 32 µg Cu/mL. Growth was monitored over 24 h, and minimum inhibitory concentrations (MICs) were determined. In vivo: 1189 piglets (PIC 800 × Fast 276) were fed from 42 to 63 days of age. Diets included 10 ppm Cu from premix (CuSO₄), with treatments: negative control (NC), positive control (PC, 150 ppm TBCC), and CR at 50, 75, and 100 ppm. Data were analyzed using one-factorial ANOVA with Bonferroni post-hoc tests to identify significant differences among Cu sources. Results showed that CR had the strongest inhibition of *S. suis*, with delayed growth onset (9 h vs. 3 h for divalent Cu) and lower MIC (64 µM vs. 256 µM). In vivo, all treatments improved growth vs. NC (P=0.014) in the first week. CR100 showed the highest ADFI (P=0.002). Overall, CR75 and CR100 had higher ADG than NC (P=0.004), and CR100 had higher ADFI than NC, TBCC, and CR50 (P=0.002). Morbidity and mortality did not differ between treatments. These findings align with prior studies highlighting the superior antibacterial properties of monovalent vs. divalent copper. In conclusion, the in vitro trial demonstrated that the monovalent copper had the highest inhibitory potential against *S. suis*. Furthermore, CR at 75 and 100 ppm provided similar or improved performance to TBCC at 150 ppm while reducing supplemental copper and environmental excretion.

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Theatre 12

Design animal experiments with statistical power: “pwr4exp” – An R-based user-friendly tool supporting 3R principle

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Robust experimental design in animal nutrition research requires rigorous power analysis to ensure valid and reproducible findings. However, a review of 4,376 animal nutrition publications (1995–2023) revealed that only 4.73% reported power analysis, with 59% lacking methodological details and most relying on non-open-source tools like SAS. While SAS-based approaches (e.g., Stroup, 1999, 2002) are versatile, they lack automated degrees-of-freedom (DF) approximation, a limitation for models requiring covariance adjustments, making them challenging to implement effectively. To address these gaps, we introduce pwr4exp (<https://cran.r-project.org/web/packages/pwr4exp/index.html>), an open-source R package enabling model-oriented power analysis. Built on linear mixed model theory, pwr4exp automates DF approximation and enables power calculations for standard designs (e.g., randomized block, split-plot, and Latin Square) while supporting custom experimental designs tailored to diverse study objectives. This package provides actionable sample size estimates, guiding decisions on experimental logistics – e.g., prioritizing primary objectives in large commercial herds or detailed multi-variable sampling in smaller trials. Validation against SAS confirmed accuracy, with pwr4exp resolving DF challenges and offering an intuitive interface. By enabling precise power approximation, pwr4exp advances the 3R principles by reducing animal use (Reduction) while maintaining statistical rigour. Integration into research workflows enhances statistical robustness, minimizes Type II errors, and improves translational relevance, aligning ethics with scientific impact. In the EAAP presentation, we will introduce pwr4exp with validated examples and discuss its implementation as an integral tool in experimental design before conducting animal trials. Keywords: Power analysis, Experimental design, R package, pwr4exp, 3R principle

Predicting rumen pH based on exhalomics features in dairy cows: A pilot study combining machine learning and generalized additive model framework

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Rumen pH is vital for microbial fermentation, volatile fatty acid production, and nutrient digestion in dairy cows. Previous models using diet composition, feeding behavior, ruminal metabolites, and gas emissions offer moderate predictive power but rely on indirect proxies. This study aimed to predict the rumen pH of dairy cows using non-invasive exhalomics features, leveraging statistical and machine learning methods. A switchback design with 4 rumen-cannulated Swiss Brown cows fed low/high-starch (6.31 vs. 16.2% DM) diets across three 9-day periods was used. Exhalome samples (GreenFeed) were collected 8 times/period (every 3 h) and analyzed via secondary electrospray ionization-mass spectrometry. Simultaneously, rumen fluid was collected via cannula, and pH was measured immediately (range 6.31–6.62). Random Forest (RF) and Gradient Boosting Machine (GBM) were first used to prescreen the top 10 exhalome features based on importance scores. The union of RF + GBM-selected features was retained and controlled multicollinearity (variance inflation factor < 5). Features were putatively identified by exact mass-to-charge ratios cross-referenced with the Livestock and Human Metabolome Databases (± 5 ppm tolerance). Data were fitted using generalized additive mixed model (GAMM), including random effect of cow and smoothed exhalomics features using thin plate regression splines. Leave-one-out cross-validation (data from each individual cow were used for validation each time) was conducted for model evaluation. The developed model demonstrated moderate accuracy, with performance metrics of $R^2 = 0.216$, root mean square prediction error (RMSPE = 0.355, RMSPE as % of average = 5.52%), and concordance correlation coefficient (CCC = 0.362). Feature importance via Shapley values (SHAP) identified lactate, butyrate, butyronitrile, and acetyl-acetate as key metabolites (mean SHAP > 0.075). Acetyl-acetate, butyronitrile, and lactate showed non-linear trends, while butyrate had a near-linear positive relationship. In summary, the model predicted rumen pH with moderate accuracy, likely due to the narrow pH range. Further, incorporating a broader pH range could enhance prediction accuracy and interpretability.

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Poster 14

Mammary epithelium cell, mammary organoid and the possibility to the develop the artificial mammary gland

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Mammary gland is the specific mammalian organ that could synthesize body fluid required for young offspring. During lactation, three major milk components are actively synthesized from this gland via mammary epithelial cell (MEC). In addition to the physiological function of mammary gland, breast cancer becomes the most cancer incidence. The role of MEC as an in vitro model will be summarized here to understand the mechanisms of milk synthesis and the biological mechanisms of breast cancer. We reveal the information of MEC from different 2 sources. While the MECs from mammary tissue contain stem cell that can be reconstituted the mammary gland and produced the mammary organoid, the major MECs population from goat fresh milk is the epithelial progenitor. Based on our investigation, we provide the information that MEC culture could be enriched for artificial mammary gland as bioreactor to produce artificial milk or milk components. We extend the protein producing capacity of MEC culture to the pharmaceutical target protein. This hypothesis bases on the idea that MEC is the mammalian cell contained the intact post-translation processes. Finally, we also introduce the idea to develop MEC to mammary cell line. This is based on the fact that the studies of breast cancer biology and the development of target cancer therapy require the appropriate mammary cell line. Taken together, the roles of MEC culture are underscored for the future research to enhance the livestock well-being, to provide an alternative choice for recombinant proteins and to refine the understanding of breast cancer.

Release of polyphenols from raspberry pomace into rumen fluid during in vitro incubation

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Raspberry pomace, which is a leftover from juice production, is a product rich not only in nutrients such as fiber, protein and fats, but also contains bioactive components in the form of polyphenols, which have proven antioxidant activity. Based on previous knowledge, we assume that raspberry pomace can be a good antioxidant addition to ruminant diets due to its high polyphenol content. The aim of this study was to investigate the release of polyphenols from raspberry pomace into the rumen fluid. Raspberry pomace crushed with a cryogenic mill was used for the study. Polyphenol release was analyzed in vitro using an Ankom DaisyII incubator. The bagged pomace samples were incubated in rumen fluid collected from sheep, respectively: 0, 2, 4, 8, 24 and 48 hours. Rumen fluid samples were taken at the designated hours. The polyphenol content of the pomace and fluid were determined by HPLC. The analyses showed that raspberry pomace is a good source of polyphenols – 3.19% DM. Analysis of rumen fluid as early as 0 h incubation showed the presence of phenolic acids: ellagic acid, p-coumaric acid, syringic acid, 4-hydroxybenzoic acid, protocatechuic acid, caffeic acid, ferulic acid, sinapic acid, as well as flavonoids: quercetin, apigenin, kaempferol, luteolin, isorhamnetin, epicatechin. Initially, the content of these compounds was fairly constant; however, a decrease in the content of all metabolites was noted at 4 h of incubation. 24 h of incubation brought an increase in content, which persisted until the end of incubation. The exceptions were p-coumaric and syringic acids, the content of which increased at 4 h of incubation and decreased with each subsequent hour. In conclusion, the research showed that raspberry pomace is a good source of polyphenols, which are released into the rumen fluid during fermentation. This may indicate the possibility of using raspberry pomace as an antioxidant additive, however, it requires investigating the absorption of polyphenols from rumen fluid and utilization by animals.

Session 38

Theatre 1

From the framework to the field: Simulating genotype by environment interaction in farmed animals

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There is increasing interest in modelling and leveraging genotype by environment interaction (GEI) in animal breeding programs, particularly with the emergence of more variable and extreme environments amid climate change. Stochastic simulations offer the potential to explore the implications of GEI in breeding programs, such as the optimal design of genomic reference populations, selection strategies and models for estimation. However, current approaches for simulating GEI in animals typically use overly simplistic assumptions, such as the presence of a single underlying environmental covariate driving GEI, or the presence of only a few environments. There is a need for more realistic and reproducible simulations of GEI, which can be scaled to real-world animal breeding settings. Our objective is to demonstrate a flexible framework for simulating GEI in animals with controllable levels of complexity and structure. The framework is an extension of Falconer's original treatment of GEI (Falconer, 1952), where performance in different environments is considered as separate but potentially correlated traits. There are several key advantages of the new simulation framework for GEI: Flexibility: The simulated GEI pattern can range from simple and noisy, through to complex and highly structured. Scalability: A very large number of environments (i.e., >1000) can be generated from the underlying distribution. This enables the user to reproduce the environmental sampling process involved when testing animals on-farm. Efficient: A multi-trait model for GEI with p environments requires simulating p traits (e.g., 1000), whereas the reduced rank parameterisation underpinning the framework requires only a small set of k traits (i.e., ~10). Informative: The framework enables genetic gain to be tracked for the target population of farming environments, in addition to those sampled during testing. Here, we demonstrate the utility of the new framework to investigate response to selection with different selection strategies and complexity of GEI in a livestock population.

Optimizing management of non-additive genetic variation in selective breeding programmes

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Intense selection, particularly in nucleus breeding programmes, has been hugely successful in achieving genetic gain and improving commercially important production and health traits. Alongside genetic drift, selection leads to changes in allele frequencies over time, and loss of genetic variance and heterozygosity, which give rise to inbreeding depression. Breeding programmes in livestock are typically based solely on additive genetic architecture, while non-additive genetic effects (e.g., dominance) are overlooked. The aim of this study is to explore methods to estimate quantitative genetic parameters and assess the impact of dominance variance on the breeding programme over time, in the presence of both additive and dominance genetic effects. We have employed stochastic simulations within the AlphaSimR package, to examine scenarios for a complex trait influenced by only additive or by both additive and dominance genetic effects, in populations of varying numbers of breeding animals, and assuming random mating and equal contributions. An initial burn-in of 10 generations was followed by 20 generations of truncation selection based on phenotypes or on genomic estimated breeding values (GEBV). We estimated GEBV using either an additive or an additive and dominance model via ridge-regression best linear unbiased prediction (RR-BLUP / SNP-BLUP). The temporal trends and long-term performance of the simulated breeding programmes were assessed in terms of genetic gain, changes in genetic variance, inbreeding depression, and prediction accuracy. We expect that accurately estimating and directly accounting for and exploiting dominance effects can enhance the long-term performance of the breeding programme and better inform the management of genetic variance and inbreeding depression. Our findings will support further work to optimize the trade-off between genetic gain and loss of genetic diversity, and to devise breeding strategies for how to best estimate and exploit non-additive variation in practice in commercial breeding programmes.

Session 38

Theatre 3

Inbreeding depression for milk yield under heat stress in Italian Holstein cows

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This study aimed at assessing inbreeding depression for milk yield under heat stress in Italian Holstein cows. A total of 414,514 milk yield records were available for 24,395 cows, raised on 703 herds, which were visited on 25,001 herd-test-days. For each herd-test-date, the average temperature, humidity, and temperature-humidity index (THI) for the week before the visit was available. Milk yield averaged 37.9 kg/d (± 8.92 kg/d). Genomic inbreeding estimates were assessed via three methods: Fgrm, derived from the diagonal of the genomic relationship matrix (GRM); Froh_genome, calculated as the ratio of the sum of runs of homozygosity (ROH) segments to the entire genome; and Froh_class, which categorizes ROH segments into six classes based on their sizes. A first mixed model was implemented to assess the impact of environmental stress on milk yield. Heat stress related covariates (temperature, relative humidity or THI) were fitted in the model as cross-classified effects with 5 levels, with relative humidity resulting with the strongest impact. A second model was implemented to assess the impact of inbreeding depending on environmental conditions. This model included the fixed effects of lactation stage by lactation number (30 classes), relative humidity (5 classes), and the inbreeding covariate nested within relative humidity class. Additionally, herd-test-date, cow permanent environment and additive genetic random effects were fitted. Results show that cows had higher milk yield under low, medium-low and medium humidity classes, while yield decreased with humidity increasing to medium-high or high. Inbreeding depression was strong and statistically significant at the high relative humidity class as well as at the low relative humidity class. Depression was not statistically significant for the medium class, and weak for the medium-low and medium-high classes.

The impact of using individual-specific genetic correlations on the ranking and genetic covariance parameters of selection candidates

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In breeding programs, genetic correlations between traits are treated as parameters that are equal to all individuals featuring in a genetic evaluation. This use of genetic correlations as parameters defining the multivariate distribution of breeding values (BV), and considered in the construction of the unified selection index (SI), has been very successful in ensuring genetic progress for many traits, while managing their trade-offs, when existent. However, since genetic correlations are strongly dependent of the involved traits' architecture and on the genotype presented by each individual, different individuals may present different potential for genetic correlations. When very few phenotypic records are available per individual, statistical methods prevent us from adequately estimating individual-specific genetic correlations (iSGC). However, in populations where certain individuals have a large number of offspring such as dairy cattle, iSGC can be obtained for sires as a correlation between the BV of their daughters – these corrected for the maternal BV. Two traits were simulated with heritabilities 0.3 and 0.01, and -0.2 genetic correlation, and iSGC were calculated from several simulated gametes per individual. Two SI with economical weights 0.75 and 0.25 for traits 1 and 2 respectively were built: SI_par with genetic correlation -0.2 for all individuals, and SI_iSGC with the iSGC of each individual, resulting in moderate different rankings among the top 20% sires. Moreover, the top 20% sires selected with SI_iSGC presented greater genetic variance than that of the top 20% sires selected with SI_par, as well as a less negative genetic correlation between the two traits. These results suggest that selection based on SI_iSGC may achieve equivalent genetic progress for the traits of interest, while maintaining a greater genetic diversity, and better control of the trade-off between antagonistic traits.

Estimating heritability in small populations in the genomics era: a case study on Istrian sheep

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Accurate and reliable estimation of variance components is crucial in animal breeding programmes, as it guides selection and conservation strategies. Estimation can be particularly complicated in small-scale programmes at the start of or transition from traditional to genomic selection. In this study, we evaluate heritability estimates for milk, fat and protein yield in a local Croatian sheep breed using four approaches: pedigree- and genomic-based residual maximum likelihood (REML), single-step REML (ssREML), and REML using imputed data. We also explore how data availability and genetic relationships impact variance estimation for flocks of varying sizes and pedigree depth. Our dataset consisted of ~9500 lactation records (milk, fat and protein yield) for ~4500 Istrian sheep across ~40 flocks (ranging from 5 (6) to 430 (1129) animals (records)), ~63K in pedigree, ~1300 genotyped with 50K SNP array and 15 key animals sequenced with 30X coverage. The results demonstrate that while pedigree-based estimation is reliable for well-recorded flocks with many individuals, it becomes unreliable for smaller and more fragmented flocks. In such cases, genomic-based estimation, which captures additional genetic relationships, provides more reliable heritability estimates. Our study highlights the importance of reliable heritability estimation in small-scale breeding programmes and contributes to optimising cost-effective phenotyping and genotyping strategies where large-scale actions may not be feasible due to economic constraints.

Variance components estimation with Monte-Carlo single-step genomic REML

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Variance components estimation with restricted maximum likelihood (REML) requires the inverse of the mixed model equations (MME) to calculate the trace term of the first derivatives of the restricted likelihood. Inverting the MME is computationally unfeasible when the number of traits and genotyped animals is too large. Monte Carlo REML (MC-REML) uses a stochastic estimator of the trace to overcome inverting the MME. This process involves simulating the data and solving the MME for each sample. The current MC-REML does not consider genomic information for the data simulation process, making it unsuitable for single-step genomic BLUP (ssGBLUP) models. In this study, we aimed to derive Monte Carlo REML for ssGBLUP models, which we called Monte Carlo single-step genomic REML (MC-ssGREML). We derived formulas to simulate the ssGBLUP data-generating process. This involved simulating marker effects, residual polygenic effects, J factors, and imputation errors. We simulated correlated random effects and traits simultaneously to enhance computing efficiency. Previous research demonstrated that an additional convergence criterion is needed for MC-REML due to Monte Carlo errors. We proposed a new convergence criterion consisting of the coefficient of variation of the five previous runs of the approximated restricted likelihood. We tested MC-ssGREML for a beef cattle's birth weight (BW) and weaning weight (WW) model. The model included contemporary group as a fixed effect, additive genetic and maternal effects for both traits, and maternal permanent environmental effect for WW. We compared the exact Average Information (AI) REML against AI-MC-ssGREML for a small dataset of 100,000 animals, from which 10,000 were genotyped. The model had 607,796 equations and 13 variance components to estimate. We used 40 samples to estimate the trace terms, whereas only one sample was used for the likelihood approximation. There were no significant differences between the exact AI-REML and MC-ssGREML, whereas the running time for each approach was 70 and 11 hours, respectively. Variance components estimation with MC-ssGREML seems feasible for large datasets with many genotyped animals.

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Theatre 7

Modelling genetics of the multidimensional feed intake complex of dairy cows: fitting many variance components simultaneously using factor analytical models

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The feed intake complex of dairy cows contains many dimensions: traits (milk yield (ECM), dry matter intake (DMI), and liveweight (LW)), different parities, and lactation stages within parity. Energy partitioning and mobilisation of tissues make the associations between the traits dynamic. However, the genetic (co-)variance among 3 traits x 3 parities x 6 lactation stages is a matrix of 54 columns and rows. Estimating this matrix using a multitrait model gave convergence issues, as the full matrix hits the boundaries of the parameter space every iteration. Initially random regression models offered a solution for reduced rank modelling, but the order was still limiting. In this study we tested factor analytical models (XFA) in ASReml to reduce the rank of the full piecewise-constant model. After careful selection, the dataset included 6,019 Holstein cows, 19,783 lactations, and 611,580 weekly averages for ECM, DMI, and LW. The model included fixed effects for breed, age at calving and year-herd of calving, and random effects for the additive genetic and permanent environmental covariances. The experimental treatment/recording periods per herd were fitted as random effects, within trait and parity, and across lactation stage with a covariance fitted between the traits and parity. The 54 trait model was too ambitious given computing time, but three analyses combining 3 traits, 2 parities and 6 stages (36 traits) was performed. The XFA models of order 5, 6 and 7 had 108, 126, and 145 free parameters respectively, and gave already a reasonable representation of the full model with 666 distinct genetic (co-)variances to be estimated, but higher orders are still needed based on likelihood improvement. These estimates appear more realistic than those of random regressions, in particular at the beginning and at the end of the lactation, where they do not suffer from strong inflation., and might reflect the genetics and dynamics of the feed utilisation complex in dairy cows better.

Approximation of the logarithm of the determinant of large matrices applied to animal breeding and genetics computations

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Evaluating the (restricted) log-likelihood of a statistical model is widely used when estimating variance components and doing hypothesis testing in animal breeding and genetics. Due to memory usage and extremely long computational times, performing likelihood evaluations is impossible for large datasets. A major computational bottleneck in evaluating the likelihood is calculating the logarithm of the determinant (log-determinant) of high-dimensional matrices. This study aimed to present a method to approximate the log-determinant of positive semi-definite matrices, making likelihood evaluations feasible for very large datasets. Our method uses repeated matrix-vector products, notions of complex calculus, and contour integrals. We tested the approximation on beef and dairy cattle, chicken, and pig datasets, including single and multiple-trait models. The average relative difference between approximated and exact values was around 10-3. The approximation was 2 to 500 times faster than the exact calculation. Differences in computing time between the exact and approximated log-determinant increased with dataset size. For the largest dataset (dairy cattle), with 9.4M animals, 34.5K genotyped individuals, and a coefficient matrix rank of 19.2M from the mixed model equations, the exact calculation took 19 days, while the approximation took just one hour. As an application example, we calculated the restricted likelihood and estimated variance components with expectation-maximization (EM), average information (AI) REML, and two derivative-free (DF) methods using the approximation of the log-determinant for a single-trait model. The exact and approximated log-likelihood showed maximums at the same heritability value among the REML methods. Our proposed approach lifts the computing time and memory limitations for likelihood evaluations in large datasets. The method allows approximating the log-determinant of positive semi-definite matrices, opening the possibility of performing likelihood evaluations for large datasets in animal breeding.

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Theatre 9

Computation with a branch GRM for millions of individuals

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Genetic relatedness is a central concept in genetics and typically stored as a genetic relatedness matrix (GRM). This relatedness has been defined in various contexts based on pedigree, genotype, phylogeny, coalescent times, and, recently, ancestral recombination graph (ARG). ARG-based GRMs have been found to better capture the structure of a population and improve association studies relative to the genotype GRM. However, calculating GRMs and further operations with them is fundamentally challenging due to inherent quadratic time and space complexity. We show how different definitions of relatedness can be unified by making use of the additive model of a quantitative trait and provide a definition of branch relatedness and the corresponding branch GRM. We explore the relationship between branch relatedness and pedigree relatedness through a case study of French-Canadian individuals that have a known pedigree. Through the tree sequence encoding of an ARG, we then derive an efficient algorithm for computing products between the branch GRM and a general vector, without explicitly forming the branch GRM. This algorithm leverages the sparse encoding of genomes with the tree sequence and hence enables large-scale computations with the branch GRM. We demonstrate the power of this algorithm by developing a randomized principal components algorithm for tree sequences that easily scales to millions of genomes. All algorithms are implemented in the open source tskit Python package. Taken together, this work consolidates the different notions of relatedness as branch relatedness and by leveraging the tree sequence encoding of an ARG it provides efficient algorithms that enable computations with the branch GRM that scale to mega-scale genomic datasets.

Piecewise Constant Model Outperforms Repeatability Model for Genetic Evaluation of Longitudinal Feed Intake in Dairy Cattle

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Accurate genetic evaluations of feed intake are needed for breeding programs which aim at improving production efficiency and sustainability in the dairy industry. Often, repeatability genetic evaluation models are applied to longitudinal feed intake data, which assume constant genetic effects throughout the lactations. We developed an alternative approach that allows breeding values to vary across lactation stages, enabling better utilization of sparse data patterns and more flexible incorporation of the trait within selection indices. Our analysis used 389,967 records of weekly dry matter intake collected across 22,144 lactations and across 10 farms, from 11,230 Dutch and Belgian Holstein cows. We implemented various modeling strategies including repeatability, random regression, and piecewise constant models. Cross-validation methodology was employed to evaluate prediction accuracy, taking into consideration the longitudinal structure of the data. Results showed that the piecewise constant model significantly outperformed both the conventional repeatability model and alternative random regression specifications, throughout all lactation stages, in terms of prediction accuracy. The piecewise approach successfully captured stage-specific genetic effects, providing more nuanced genetic evaluations by estimating breeding values specific to different lactation periods. This allows for more precise selection decisions based on feed intake at various physiological stages. We developed genomic estimations and reliabilities for both period-specific and whole-lactation breeding values. Our findings suggest that replacing a repeatability model with a piecewise constant approach could substantially increase the accuracy of genetic evaluations for feed intake, could support more targeted and effective breeding decisions, and ultimately contribute to more cost-efficient and environmentally sustainable dairy production systems.

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Theatre 11

Multi-trait genomic models with trait and marker specific weights can substantially increase the accuracy of genomic breeding values

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The GWABLUP (Genome-Wide Association based Best Linear Unbiased Prediction) approach used GWA analysis results to differentially weigh the SNPs in genomic prediction, and was found to improve the reliabilities of genomic predictions. However, the proposed multi-trait GWABLUP method assumed that the SNP weights were the same across the traits. Here we extended and validated the multi-trait GWABLUP method towards using trait-specific SNP weights. In a 3-trait dairy data set, multi-trait GWAS estimates of SNP effects and their standard errors were translated into trait-specific likelihood ratios for the SNPs having trait effects and posterior probabilities using the GWABLUP approach. This produced trait-specific prior (co)variance matrices for each SNP, which were applied in a SNP-BLUP model for genomic predictions, implemented in the APEX linear model suite. In a validation population, the trait-specific SNP weights resulted in more reliable predictions for all three traits. Especially, for somatic cell count, which was hardly related to the other traits, the use of the same weights across all traits was harming genomic predictions. The use of trait-specific SNP weights overcame this problem. In multi-trait GWABLUP analyses of ~30,000 reference population cows, trait-specific SNP weights resulted in up to 13% more reliable genomic predictions than unweighted SNP-BLUP, and improved genomic predictions for all three studied traits.

Genomic partition: Partitioning allele frequency trends and identifying locus contributions to genetic mean
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Understanding sources of genetic change is crucial for the effective implementation of breeding programmes. However, these programmes are characterised by numerous interdependent decisions, making it challenging to identify which actions contribute most to genetic change. We have previously developed an R package AlphaPart, that implements a method for partitioning breeding values and genetic trends into contribution of selection paths defined by groups of animals. Here, we extend the package to study the partition of locus-wise allele frequencies and downstream trends, which we call “genomic partition”. This extension provides deeper insights into the dynamics and drivers of genetic change in mean and variance along the genome. However, the limitation of this method is its dependency on the number of genotyped generations, since the contributions in the base generation of genotyped animals are assigned to the category of base animals. This limitation could be overcome by imputing genotypes of the ancestors, conditional on the pedigree information. We evaluated the performance of this approach using multi-population simulated data, where a target population performed selection on a single trait and received imports from populations selected on a genetically correlated trait. We evaluated the accuracy of the genomic partitioning when imputing ancestral genotypes with different cut-offs for the genotyped base population. Based on the simulated results, we applied the best pipeline to a real dataset of ~7000 genotyped Brown-Swiss animals. We demonstrate the value of the method for understanding how selection decisions change allele frequency trajectories and genomic variation, and how the contributions change along the genome. This can aid in identifying the most influential selection decisions or origins, which will guide future breeding decisions.

Session 38

Poster 13

Benefits of machine learning for genomic prediction across heritabilities, QTL numbers, and reference population sizes

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Applying machine learning (ML) models in genomic prediction is of growing interest nowadays. Some studies using actual datasets reported that ML models outperform GBLUP in terms of accuracy, while other studies did not find such an advantage. The benefit of ML models seems to depend on the studied trait, the ML model used, and the reference population size. In addition, evidence has shown that some ML models tend to perform poorly for traits influenced by a large number of QTL. Together, the results of these studies did not uncover a clear pattern that shows under what conditions ML models outperform GBLUP. The objective of this study is to investigate under what conditions (i.e. heritability, QTL number, and reference population size) ML models have a benefit over GBLUP in terms of prediction accuracy. We simulated datasets of animal populations under selection on an additive trait with heritabilities of 0.1 or 0.5, and QTL numbers of 50, 100 or 5000. The reference population size was varied between 500 to 50,000 animals. GBLUP and three representative ML models (namely multilayer perceptron, random forest and support vector regression), will be used to predict the genetic value of young animals. Models will be compared on prediction accuracy, bias and computation time. The results of this study will determine whether there are conditions in which ML models outperform GBLUP, and identify which ML model performs best under different conditions. We expect to present the results of this study at the conference. This study systematically explores the interaction between multiple key factors on the performance of ML models in genomic prediction. By using simulated data, we can assess these effects under controlled conditions, providing insights that may be difficult to obtain from real datasets. The findings will enhance our understanding about the potential benefit of applying machine learning models to genomic prediction.

Genomic Data Augmentation with Variation Autoencoders (VAE): Effect on prediction accuracy and non-additive genetic variance

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In genomic studies, the challenge of limited sample size persists, which affects the accuracy of genetic evaluations and estimation of genetic variance. VAE is a commonly used deep learning (DL) method to generate synthetic data, and it can be used to moderately increase the sample size. The VAE architecture comprises an encoder that encodes the essential features of the input data into a lower-dimensional latent space represented by a probability distribution and a decoder that samples from this latent space, thereby synthesizing new samples. This study evaluates the predictive performance of feed efficiency and the proportion of non-additive genetic variance captured using DL methods, specifically Multilayer Perceptron (MLP) and Convolutional Neural Networks (CNN) models, in real and augmented pig datasets. The boar datasets provided by Topigs Norsvin were augmented with 1,000 synthetic samples per breed, in addition to the 13620 Duroc and 11969 Landrace records. The predictive ability of the DL method was evaluated using a novel approach to average predictions along with conventional split methods, whose predictive abilities were averaged. In addition, we introduced a novel method to decouple the additive and non-additive predictions captured by DL models. MLP outperformed CNN in the real Duroc dataset, achieving higher predictive accuracies in a test population using the averaged prediction method (0.381 for MLP and 0.377 for CNN) and when compared to the augmented dataset (0.378 for MLP and 0.372 for CNN). MLP achieved an accuracy of 0.364 for the real Landrace dataset, whereas CNN achieved 0.353, which slightly increased to 0.358 with augmentation. The proportion of non-additive genetic variance captured by MLP was lower in the real dataset (0.018 and 0.017 for Duroc and Landrace, respectively) than in the augmented dataset (0.030 in Duroc and 0.035 in Landrace). Data augmentation did not improve the predictive ability of feed efficiency using DL methods but significantly increased the capacity to capture non-additive genetic variance.

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Poster 15

Genomic prediction including dominance effect for important traits in Huaxi cattle

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The dominance effects are an important non-additive genetic effect, the inclusion of dominance effects in prediction models can increase the accuracy of genomic estimated breeding value in animal breeding. Previous studies have been conducted genomic prediction including dominance effects in various species and achieved higher accuracies compared with additive model. In this study, a two-component GREML was applied to obtain the additive genetic variance and dominance variance for carcass weight (CW), dressing percentage (DP), meat percentage (MP), fattening period daily gain (DG) and chunk roll (CR) in 1233 Huaxi genotyped with Bovine HD Beadchip. The prediction model of GBLUP-D and BayesAD included additive and dominance effect. The BayesAD assumes that the marker additive effects follow Student's t-distribution, that is, the normal distribution of variance following the scaled inverse chi-square distribution. The absolute value of the additive effects following a folded t-distribution has a half-normal distribution, since it is conditional on an inverse chi-square distributed parameter. We compared the prediction accuracy between GBLUP and Bayesian method including dominance effects (GBLUP-D and BayesAD). We found the estimated dominance variances were accounted for 16.6%, 24.5%, 15.1%, 5% and 15.1% of the total phenotypic variance for CW, DP, MP, DG and CR, respectively. Compared with GBLUP and BayesA, we observed 0.5%~1.1% increase in predictive accuracies of BayesAD and 0.5%~0.9% increase accuracies of GBLUP-D, respectively. Our results suggested that dominance effects yield considerable contribution to the total genetic variance of studied traits. BayesAD and GBLUP-D is convenient for the improvement of genomic prediction in cattle.

Performance of a heteroscedastic model involving both direct and maternal genetic effect on simulated data
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Selection to reduce the variability of traits is gaining interest in animal production because of its impact on animal performance, robustness and welfare. The model used to solve the heteroscedastic model is highly complex and usually only accounts for the genetic effects of the animal affecting the trait and its environmental variability. GSEVM software was developed to solve the model, allowing a Bayesian resolution. Subsequently, the double hierarchical model was developed under a frequentist methodology to solve the model under ASREML, allowing greater versatility. One of the demanded improvements was the possibility of including two genetic effects affecting the trait, as it is usually considered in traits measured at early ages, such as birth or weaning weights. However, it has not still been tested whether this model is capable of estimating all the parameters in such a complex model in which three correlated genetic effects are fitted. The objective of this work was to study by simulation whether the double hierarchical model methodology would adequately estimate two different genetic components affecting the trait and another one affecting the variability, and the correlations among them. A total of 1,000 founder individuals having 2,000 offspring in generation one, and 3,000 in a generation two were simulated so that only 50 females were mothers of the next generation to ensure a strong data structure (around fifty records per female), only the last 5,000 registering phenotype. Two sets of parameters reported in the literature for birth weight in pigs were simulated, so that the data included or not the direct genetic effect in addition to the maternal genetic effect. They were solved with the double hierarchical model assuming the existence and non-existence of the direct genetic effect. The results showed that the model was able to adequately estimate the simulated structure, although it showed the need for a strong structure to perform satisfactorily. It is still necessary to verify the performance of the model in real populations.

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Poster 17

Genetic parameters for heat tolerance in growing Chianina Bulls

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The objective of this study was to provide a framework for the estimation of genetic parameters and breeding values for tolerance to heat stress in Chianina beef cattle. Performance test data was used, which included genomic and phenotypic information for growing bulls' information. A total of 1173 bulls, genotyped with the Illumina 33k beadchip, were included in the analysis. Phenotypic information included body weight measures taken during performance test. Bulls were allocated into contemporary groups at on-testing (CG.pt), which occurred 10 times per year, on average, from 1988 to 2023. Weight dates (wei.d) were also available as information. As performance testing each bull took about 6 months, multiple wei.d were to be found for each CG.pt, and bulls from multiple CG.pt were weighted on each wei.d. Climatic information was downloaded from the NASA-POWER resource portal. Relative humidity, temperature and temperature-humidity index were used in this study. A random-regression animal model was implemented using the MCMCglmm package in R. The model had ADG as the dependent variable. The fixed effects included were the intercept, the polynomial regression on the age at weighting (fourth order), and the effect of relative humidity as regression. The random effects were the CG.pt, wei.d, the additive genetic animal effect of the intercept and regression on relative humidity and the permanent environmental animal effect of the intercept and regression on relative humidity. Residual variance was considered heterogeneous, with 9 classes defined by the stage of the performance test. A genomic relationship matrix was used for the additive genetic animal effect. Results show different values of heritability depending on relative humidity. Heritability values were higher at lower values of humidity, being up to 30% with a 40% humidity, decreasing to 20% or less starting from humidity values of 50% or more. Genetic correlations were close to '0' between extreme values of relative humidity. Results suggest that genetic evaluations for heat tolerance can be implemented.

Modelling non-genetic correlations between litter traits and production traits affects genetic covariances

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In standard multi-trait models, non-genetic covariances between production traits and litter traits are often ignored. This study investigated the hypothesis that including non-genetic covariances between production and litter traits in a multi-trait model changes the estimates of genetic variances and covariances. The non-genetic covariances covered covariances between residuals for litter traits and common birth litter effects for production traits, as well as covariances between residuals for production traits and permanent sow effects for litter traits. Using large multi-trait models for DanBred Landrace pigs, we estimated variances and covariances both with and without accounting for non-genetic covariances. The model included the production traits: piglet and weaner growth, finisher growth, lean meat percentage, feed conversion ratio, and conformation score, as well as the litter traits: litter weight gain, litter size, and piglet survival. The results showed that almost all estimated non-genetic covariances between production and litter traits were significantly different from zero. Furthermore, incorporating the non-genetic covariances influenced several of the genetic covariances. Particularly the covariances among traits measured around farrowing, i.e. piglet and weaner growth, litter size, and litter weight gain. The most significant change in genetic covariance was between litter size and piglet and weaner growth, which changed from negative to positive. Additionally, some genetic variances were notably influenced by the different models. These findings suggest that multi-trait models combining litter and production traits should include non-genetic litter covariances and non-genetic animal covariances to accurately separate genetic and non-genetic (co)variances, leading to more precise estimates.

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Poster 19

Assessing Genotype by Environment interactions in Dutch dairy farms: Impacts of Farming System, Feeding Regime, and Nitrogen Use Efficiency on Milk Production Traits

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As dairy farming transitions towards more sustainable practices, including reduced feed-food competition and improved nitrogen use efficiency (NUE), future farm managements may differ from currently prevailing practices, potentially leading to genotype-by-environment interactions (G×E). This study analysed G×E between organic (OF) and conventional (CF) dairy farming systems using a bivariate model, and across a range of feeding and NUE environments using reaction norm models. Data was available on 974 Dutch dairy farms (2016-2021), including data on farming system (OF or CF) and herd-level average values of dietary concentrate proportion (% of total dry matter (DM)), net energy for lactation (NEL) content of the diet, and NUE (% of N intake retained in milk). Records on 305-d milk yield, fat- and protein content from first lactation Holstein Friesian cows were included, resulting in 6,126 records on OF and 96,015 on CF. Heritability for milk yield was 0.36 ± 0.04 in OF and 0.44 ± 0.01 in CF. Genetic correlations between OF and CF were high for all three traits (0.94 ± 0.03 to 0.99 ± 0.03). Heritability for milk yield increased from 0.17 ± 0.02 at a dietary concentrate level of 13% to 0.54 ± 0.01 at a concentrate level of 43%. Similarly, heritability for milk yield increased from 0.10 ± 0.02 at 6.14 MJ NEL/kg DM to 0.59 ± 0.02 at 7.21 MJ NEL/kg DM, and from 0.11 ± 0.02 at a NUE of 20% to 0.53 ± 0.01 at a NUE of 30%. These changes in heritability were primarily due to changes in additive genetic variance. In contrast, smaller differences in heritability were observed across the same ranges of environments for fat content (0.66 ± 0.01 to 0.74 ± 0.01) and protein content (0.71 ± 0.01 to 0.74 ± 0.01). Moreover, high genetic correlations (> 0.84) were found across environments for all three traits. To conclude, the change in genetic variance across environments indicates a scaling effect. However, this study provides little evidence of genotype re-ranking across environments, suggesting no need for separate breeding programs.

What is a Working Horse in the 21st Century –some implications for Welfare and Veterinary Science

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It becoming increasingly clear that we need to consider re-evaluating the value and impact of recreational, therapeutic and leisure activities as actual work done by horses. If this is so, we need to rethink horse welfare beyond the traditional “oh, they like to work” to see the finer details of what that work means and what effects it might have on them. And, just as in human Occupational Medicine, where a new set of challenges arose as a result of the dominance of service sector work, so too we need to study for the similar impacts on our equine co-workers.

Improving climate parameters in horse husbandry by using a tube ventilation system

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High dust levels and sub-optimal climate parameters often cause respiratory diseases in horse herds. So a series of measurements took place in a closed building for horses with individual stalls for testing a tube ventilation system for optimizing air quality and targeted supply of fresh air. A comparison compartment was ventilated by windows and doors (natural ventilation). The study focused on comparing climate parameters such as temperature, relative humidity, airflow velocity and dust at several points inside the barn. In each unit temperature and humidity were measured with a testo-175 H1 from 04/07/2024 to 15/01/2025 at intervals of 10 minutes continuously. Dust concentrations were measured with a Grimm Aerosol Spectrometer from 04/07/2024 to 23/07/2024 in the ventilated barn and from 27/11/2024 to 15/01/2025 with a 1-minute measuring interval under cold conditions in both barns. A mikromec-multisens logger with a Kumho velocity sensor recorded airflow inside the tube as well as inside the barn in the animal area. The data were statistically analyzed using SPSS 29.0. The results show a maximum temperature of 29.1°C and a median of 22.9°C (SD 2.475) in the tube-ventilated barn, compared to a maximum of 30.7°C and a median of 23.5°C (SD 3.035) in the naturally ventilated barn from July to August 2024 (n=8,496). The velocity inside the tube was a median of 2.04 m/sec. and around the horses a median of 0.25 m/sec. Between December and January tube ventilation optimized relative humidity to a median of 69.10%, compared to 74.60% in the naturally ventilated barn (n=6,625). Dust concentrations were lower with controlled ventilation, showing a median of 245,306 count particles of dust per liter of air, compared to 258,369 particles/liter air in the naturally ventilated barn during a 10-day example period in December (n=14,400). User-friendly and reliable functionality was achieved through the automated control of the tube ventilation system. The goal was to achieve year-round ventilation using tube systems with a focus on reducing air temperature in summer and ensuring moderate air changes as well as low-dust in winter. In this respect, the study showed positive results with reliable functionality of the system.

Body temperature monitoring in equines raised in inner areas using GPS tracking

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Accurate monitoring of both physiological and environmental parameters is essential to understand animal adaptation potential and behavior. Global Positioning System (GPS) and Geographic Information System (GIS) collars were used to monitor location and external body temperature (sensor at neck level) of grazing equines raised for 27 days in winter in semi-extensive conditions in inner areas of Italian Apennines. The study was conducted at once in two pastures areas: area 1 in Capracotta (41°50'N 14°16'E, 1150-1500 m altitude), and area 2 in Capestrano (42°16'N 13°46'E, 350-400 m altitude). A total of 12 female equines, n= 10 pregnant mares in area 1 and n= 2 lactating jennies in area 2, were fitted with GPS collars (Digitanimal®, Spain). During the study period, precipitations and air temperatures were recorded. Jennies had an average (2416 observations) external body temperature of 26.8 ($\pm$ 0.11 SEM) °C, ranging from 14.6 to 37.2 °C. Mares had an average (7881 observations) external body temperature of 24.9 ($\pm$ 0.16) °C, ranging from 14.7 to 35.5 °C. In area 1, total precipitation included 21.9 cm of snow and 4 mm of rain, with 2.3 ($\pm$ 0.39) °C average mean temperature, min-max from -3.3 to 8.5 °C. In area 2, total rainfall was 33.7 mm, with an average mean temperature of 6.5 ($\pm$ 0.38) °C, with min and max from -4.3 to 17.7 °C. As expected, observed external body temperature was generally lower than the average internal body temperature reported in literature. During the study, variations in body temperature did not match variations of air temperatures and precipitations. These results suggest a further potential application of GPS-based monitoring systems to be included in the extensive equine production of inner areas. More studies are needed to boost data collection methodologies and enhance the accuracy of body temperature monitoring systems for equine applications. The survey was approved by the Bioethics Committee of the University of Molise. The research is supported by project PRIN 20224L4WSR, funded by Next Generation EU (CUP: H53D23005120).

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Theatre 4

Wearable continuous fitness tracker device coupled with omics to evaluate horse resilience and adaptation to heat and exercise stresses

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The homogeneous genetic background and physical features make the horse a key model to understand complex traits such as resilience to heat and exercise stresses. In horses, heat and physical stresses activate the hypothalamic-pituitary-adrenal axis while the hypothalamic-pituitary-thyroid axis is suppressed to regulate body temperature. Prolonged exposure stabilizes this response through genome plasticity, adapting and keeping the "footprints" of this new homeostasis, but combined with strenuous exercise, it can lead to anhidrosis or heat stroke. This research aimed to examine molecular resilience to heat and exercise stress in horses using continuous monitoring with a wearable fitness tracker (Equimetre™) and Illumina sequencing of circulating small RNAs. Horses trained for endurance or gallop races in Italy and Dubai were enrolled. ANOVA and Student's T test evaluated differences in blood parameters pre and post-exercise and wearable device measures. Blood analysis showed mild dehydration post-exercise, with significant increases in hematocrit, red blood cells, and hemoglobin ($p < 0.001$). Based on a critical rectal temperature (Tre) of 39.7°C for metabolic imbalance, two groups were identified. No differences were detected in sensor parameters related to exercise intensity (average heart rate at gallop=161.9 bpm, SD=13.7; max heart rate=178.4 bpm, SD=16.8; final heart rate=85.6 bpm, SD=7.3; average heart rate at speed >18 km/h=178.4 bpm, SD=16.8), indicating similar training and fitness. Interestingly, a tendency ($p < 0.07$) toward better recovery capacity was observed in horses with lower Tre. Illumina sequencing is ongoing on circulating small RNAs from horses trained in hot and thermoneutral conditions. Comparing disciplines helps evaluate molecular mechanisms of exercise stress and adaptation, while different climates reveal thermal stress responses. The obtained results enhance the understanding of resilience and aid in identifying stress-resistant animals, improving welfare.

Prevalence and risk of orthopedic diagnoses in insured Swedish Warmblood horses

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Sweden has a strong tradition of insuring horses for veterinary costs, and orthopedic diagnoses are the most common for insurance claims. The Swedish Warmblood (SWB) horse population has become more specialized towards either show jumping (J) or dressage (D) and the aim of this study was to investigate differences in prevalence, risk and type of orthopedic diagnoses between these two categories of horses. SWB horses born between 2010 and 2020 were categorized as J or D horses according to their pedigree. Data from young horse tests and competitions were used, as well as insurance data from the insurance company Agria. In total, 15,619 SWB horses were found in the insurance data, corresponding to 56% of horses born in the time period. A total of 915 diagnoses connected to the locomotor (LOC) system were categorized into eight subgroups. The prevalence of these diagnoses was investigated using LS means from logistic regression analysis, including effects of sex, discipline category (J or D), young horse test status (1/0), and competition status (1/0). The time from birth to the first LOC insurance claim was investigated by LS means from general linear models, and survival analysis for each group of horses and for three birth year cohorts (2010, 2015, 2020) was performed. The results showed that D horses were more predisposed to LOC diagnoses than J horses (50.9% vs 43.9%), and males (geldings and stallions) were more predisposed to LOC diagnoses than mares (49.7% vs 45.0%). Tested horses had a higher prevalence of LOC diagnoses than non-tested horses (49.9% vs 44.9%), whereas no difference was seen between competed and non-competed horses (48.2% vs 46.5%). However, competed horses had different types of LOC diagnoses than non-competed horses. Survival analysis revealed that later cohorts were more likely to have insurance claims for LOC diagnoses at a younger age than earlier cohorts. In conclusion, insurance data can be useful to understand underlying factors of diagnoses to improve the health status of the SWB population.

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Theatre 6

Relationship between gastric content ecosystem, mucosal microbiota and integrity of glandular mucosa in horses

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Equine Glandular Gastric Disease (EGGD) can be critical to the health and performance of horses. Prevention is difficult due to the unclear pathogenesis. Previous works suggested that EGGD may result from the composition of the mucosal microbiota or from the activity of the luminal microbiota in the stomach. The aim of this study was to investigate the relationship between gastric ecosystem parameters and the integrity of the glandular mucosa in horses. Ten French Trotters (6±2yo; 546±37kg BW) previously in training were enrolled in a cross-sectional study. During the 35-d follow-up, horses were not exercised and fed a high-fiber (59.5±0.6% DMI), low-starch (1.1±0.6% DMI) diet to meet their requirements. On d30, gastric contents were collected individually 2h after the meal by nasogastric intubation. Bacterial diversity and composition were determined using 16S rRNA sequencing, concentrations of fermentation end-products (VFA and lactate) were quantified by gas chromatography and enzymatic methods, and pH was measured. A gastroscopy was performed on d35 after 12h fast. The integrity of the glandular mucosa was scored macroscopically (0-4 scale). Biopsies of the glandular mucosa were taken from non-ulcerated area to study the mucosal microbiota (16S sequencing) and to evaluate histologically the inflammation of the mucosa (0-4 scale). Correlations were performed to investigate the relationship between ecosystem parameters and glandular scores (PROC CORR, SAS). Ulcers with infectious foci were macroscopically observed in 2/10 horses, and 8/10 horses had histologically congestive mucosa. Glandular scores assigned during gastroscopy or after histological analysis were correlated ($r=0.67$; $P=0.049$). These scores were not correlated with the diversity nor the relative abundance of the bacterial phyla identified in the mucosal and luminal microbiotas. In contrast, correlations between scores and microbial activity were noticeable. A positive correlation was observed between histological score and total VFA concentration ($r=0.71$; $P=0.032$), notably propionate ($r=0.67$; $P=0.047$). This suggests that some fermentation end-products in the contents have a greater impact on EGGD than gastric microbiota composition, at least for gastritis.

The effect of composting on anthelmintic residues in horse manure

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The standard treatment for helminth infections, which are a constant threat to the health of horses of all ages, is the administration of anthelmintics. This study was initiated to investigate the potential of composting on the degradation of anthelmintics. Fecal samples were spiked with Fenbendazole and placed at in different treated manure heaps. After composting for six weeks, the samples were dried, homogenized and transferred to a centrifuge tube. Fenbendazole-d₃, water, acetonitrile and ammonium formate solution were added. The mixture was vortexed, ultrasonicated in a water bath and centrifuged. The resulting supernatant was filtered, aliquoted into appropriate vials and immediately frozen at -80°C. The quantification of the anthelmintic residues was performed using high performance liquid chromatography-electrospray ion trap tandem mass spectrometry. The results show that the degradation of Fenbendazole varies in the different muck heaps and trial runs. No significant differences in the degradation behavior of Fenbendazole were found between the different treated muck heaps. In addition, the analysis revealed significant differences between the amount of Fenbendazole residues in the muck heaps and the control samples. Compared to the degradation of Fenbendazole by the composting process, Fenbendazole was less degraded in the control samples. Both the outside temperature and precipitation had an influence on the amount of Fenbendazole residues detected. In conclusion, the method allows the detection of anthelmintic residues in horse manure. This provides valuable insights into the degradation behavior of active substances. Furthermore, the study suggests that the composting process can have a positive influence on the degradation of Fenbendazole in horse manure.

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Theatre 8

Economic comparison of different methods of processing horse manure into a valuable fertilizer and the associated ecosystem services

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The large amount of horse manure produced in Germany poses environmental and economic challenges. Rising disposal costs and high bedding percentages reduce its value as a fertilizer. The study examines five methods for processing horse manure into nitrogen-rich fertilizer, focusing on processing costs and environmental performance. The following methods were analysed: Storage and application (M1), utilization in biogas plants and application of the fermentation residues (M2), single manure chopping (M3), manure chopping and application (M4), double manure chopping (M5). The analysis is based on a systematic literature review and operational data from a large horse stud farm. Processing, storage, application, and labor costs were evaluated along with an ecosystem service analysis using pre-defined ecological criteria. The results indicate that anaerobic digestion in biogas plants (M2) is the most cost-effective and ecologically beneficial approach for the utilization of horse manure. This method enable the production of a high nitrogen fertilizer (8.12 kg N/t, 30% dry matter) with low processing costs (8.68 €/t fresh mass) while generating renewable energy. M2 also ranks first in the analysis of ecosystem services, as the fermentation residues improve soil fertility and contribute to pest control. Chopped and composted manure (M4) also improves soil quality and nitrogen availability, but is associated with higher processing costs. Both the M2 and M4 utilization methods promote humus formation and soil health. In contrast, M3 and M5 are less effective due to low nitrogen fixation and high machinery costs. Although M1 is the most economical option, it provides limited nutrient availability. In conclusion, the fermentation of horse manure in biogas plants is a sustainable and beneficial processing method that provides long-term environmental and economic benefits.

Equine milk production in France: challenges and solutions

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Equine milk production has been developing in France since the end of the 1990s. Today, more than a hundred producers (around 120) use jenny and mare's milk, mostly in cosmetics and occasionally in the food market, because of the numerous qualities of the product. This niche sector, poorly structured and federated, includes farms with small herds that rely on an alternative agricultural model. It is often associated with the maintenance of rare breeds and specific local areas, illustrating its impact in the conservation of biological and cultural diversity. In this context, the objective of this research is to better understand the characteristics and organizations of equine milk producers in France, the problems they encounter and the possible solutions to structure this sector. To this end, a qualitative approach was used with around fifty equine milk producers, combining participant observations on farms (for around ten of them) and face-to-face or remote interviews (for all). The comparison in discourses between desired and effective practices highlights coherences and paradoxes that can influence producers' organization. The description of equine milk producers highlights differences in management methods, production practices and ethical choices. Difficulties come from questions about the refusal of slaughtering, animal wellbeing, characteristics (purebred or not), and sale, product certification, marketing, and economic viability of structures. They are notably caused by a lack of knowledge, available training, and references on the production of equine milk. Finally, by identifying the recurring problems encountered by producers, the research will lay the foundations for improving the organization of this production and strengthening collaborations between the different actors of the sector. Moreover, this study paves the way to identify avenues of scientific research that could support the development of this sector under construction.

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Theatre 10

Equine assisted services in France: current status and challenges for the future

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Equine assisted services consist of connecting a horse and a beneficiary into a therapeutic, educational or social setting. This practice has grown significantly in France over the last ten years, leading to a considerable heterogeneity in practices, methods used, economic models, trainings offered, and the structures involved. This exploratory study seeks to identify the different profiles of professionals working in this sector and to analyse their practices, organisations, needs and challenges for the future. To this end, qualitative interviews were first conducted with twelve key professional stakeholders in this sector (practitioners and actors within training, funding or structuring organisations in the sector). In a second step, the analysis of this data made it possible to design a quantitative questionnaire to which 188 equine assisted services practitioners responded. The first results highlight that 93% of respondents are women, the majority work part-time, 63% have a second professional activity (not always related to equines). The main issue encountered is related to financial constraints, which impacts different aspects of the activity, such as covering the cost of horse care, renewing horses, but also purchasing customized equipment. Nevertheless, respondents show a certain optimism about the pursuit of their activity. Among the respondents, 91% express a need for additional training on varied subjects such as equine ethology, human psychology, or specific knowledge about the beneficiaries they work with. The research topics considered as both useful and a priority by the respondents are the benefits of equine assisted services (87%), the human-animal relationship (59%), and equine welfare (55%). This study will help support the development of this emerging sector and identify avenues for scientific research that would further support these activities.

Horse-Assisted Priming: An Uncharted Area? The Impact of Horses on Neurobiological Reactions, Interaction, Attachment and their Potential Role in our Current Crisis-Ridden Society

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Throughout history, horses have played a crucial role in human development, shaping societies through transport, agriculture, and warfare. While no longer essential for transportation, they remain vital in sports, breeding, and therapy. An unexplored aspect is their impact on humans as social beings, influencing emotional regulation, attachment, and behavior. This study examines horse-assisted priming and its effects on stress regulation and behavior in children with different attachment backgrounds. A qualitative approach compared traumatized children unable to live with their families, whose stress axis was highly activated and who had documented disorganized attachment patterns, with securely attached children. Eight children (N=8) participated in 27 priming sessions, with video-based behavioral analysis and cortisol measurements before, during, and after interventions. Cortisol samples assessed Morning Awakening Response (MAR) and stress axis (HPA-axis) regulation using enzyme immunoassay (EIA). Behavioral data were coded via Solomoncoder and statistically analyzed. Findings show a significant improvement in stress regulation and behavior, a positive shift in interactions, and a reduction in avoidant, controlling, and defiant behaviors, while exploration and social openness increased. Horses had a calming effect on the stress axis, acting as “door openers” for communication, interaction, learning, and attachment. This study highlights the broader societal relevance of horse-assisted interventions. Given rising mental health challenges, integrating horses into prevention and resilience-building efforts presents promising opportunities. Horse-assisted priming could serve as a valuable tool in enhancing emotional well-being and fostering social engagement. Horses function as powerful, non-verbal facilitators of emotional regulation and attachment, offering immense potential in child development and mental health care. Further research and interdisciplinary collaboration will be key to harnessing their transformative role in fostering resilience and well-being. The role of horses in society has changed, but their importance remains.

Session 39

Poster 12

Effect of fenbendazole administration on the fecal microbiota and secretory IgA in young foals

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Fenbendazole is commonly administered to 2-month-old foals to control parasitism. In adult horses, anthelmintic treatment can alter the structure of the hindgut microbiota, reducing the diversity and the concentration of fibrolytic bacteria. This can lead to microbiota-related diseases, including large intestine inflammation. This study aimed to evaluate the effect of fenbendazole in 2-month-old foals, whose hindgut microbiota and immunity are still developing. Fourteen French Trotter foals (62±3 d, 135±16 kg) were treated with fenbendazole (1500 mg PO). Feces were collected before treatment (d0), 2 days (d2) and 7 days (d7) after. Bacterial community structure and diversity were determined by 16S rRNA sequencing and secretory immunoglobulin A (SIgA) concentration was quantified by a colorimetric assay. The day effect was assessed using a GLM model including “day” as repeated measure and “horse” as random effect. Bray-Curtis distances were generated to evaluate inter-individual diversity and the day effect was assessed using PERMANOVA. Fenbendazole had no effect on inter (p=1.000) and intra-individual diversity (Chao1, p=0.111, InvSimpson, p=0.464). However, when considering individual responses, a large variability was highlighted (e.g. intra-individual diversity decreased at d2 for 7/14 foals). Relative abundance of some taxa changed with day, mostly at d2, returning to baseline at d7: at phylum level, Bacteroidota decreased (p=0.035); at family level, Rikenellaceae (p=0.028) and Ruminococcaceae (p=0.024) increased; at genus level, Agathobacter (p=0.023), Lachnospiraceae UCG-002 (p<0.001), V9D2013 group (p=0.019) decreased, while Ruminococcus (p=0.028) increased. Few genera were modified only at d7. Fecal SIgA concentrations tended to decrease at d2, and to return to initial levels at d7 (p=0.099). These results suggest that both microbiota and immunity are transiently disrupted by fenbendazole in foals. Variability in response to fenbendazole may be due to the high plasticity of foal’s microbiota, or to different coprophagic practices among individuals. Some potential fibrolytic taxa appeared altered. Additional data on fibrolytic activity would clarify the implications for foal nutrition and health.

Cobalamin in stallion sperm extender reduces sperm lipid peroxidation and improves kinetic factors after thawing

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Abstract Numerous studies have documented the impact of cobalamin (vitamin B12) on sperm physiology and semen quality. A deficiency in cobalamin may result in spermatogenesis disruption, atrophic changes, diminished sperm motility, and lowered fertility. This study aimed to investigate the effects of cobalamin on stallion sperm during the freeze-thaw process. Cobalamin was incorporated into the homemade freezing media for stallions at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 mg/ml. Semen was collected from three adult Arabian stallions using a Missouri artificial vagina, with collections occurring five times per stallion over a five-week period. Following initial assessment and treatment, the sperm was frozen in 0.5 ml straws. For the freezing process, semen was first cooled to 4 °C for 75 minutes. The straws were then exposed to nitrogen vapor for ten minutes before being transferred to liquid nitrogen. Post freeze-thaw evaluations included kinetic parameters, lipid peroxidation (measured as MDA), membrane integrity (using HOST), and sperm viability. Data analysis was conducted using SAS 9.2 software and the MIXED method, comparing means with the Tukey method. The findings demonstrated significant enhancements in sperm total and progressive motility and viability at cobalamin concentrations of 1.5 and 2 mg/ml in the freezing extender. MDA levels were notably lower with cobalamin at 1.5 and 2 mg/ml compared to the control and other groups. Additionally, the use of 2.5 mg of cobalamin in extender caused a significant decrease in total and progressive motility and viability, and increase in MDA level. It is thought that high doses of cobalamin have toxic effects on stallion spermatozoa. **Keywords:** Stallion, Sperm, Cobalamin (vitamin B12), Freezing, Evaluation

Session 39

Poster 14

First insights into effects of an early start of training on performance and health of German Thoroughbred horses

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The early training and racing of young racehorses is discussed controversially. The present study investigated data of one cohort of German Thoroughbred racehorses to identify potential effects of an early start of training on horses' health and thus animal welfare. All German Thoroughbred racehorses born in 2020 and trained by listed German trainers were considered for the present study. Data were collected from the archiving program of the German Racehorse Association as well as via an interview conducted in 2023 with the trainers (n=41). Finally, information given by trainers on horses' training and racing performance as well as on disease and injuries from 311 horses were gathered (diseases classified in infection, respiratory system, gastrointestinal tract, skin, muscles, bones, cartilage/joints; injuries classified in skin, muscles, bones/joints, tendons/ligaments). For further analyses, horses were assigned to two groups according to their age at first training (AFT1 ≤20 months, n=167; AFT2 >20 months, n=138). For statistical comparison, a Chi-squared test was used. In the assessed three-year period, 10% (32/311) of the analysed horses showed a disease (most common: respiratory system 38% (12/32)) and 24% an injury (76/311) (most common: bones/joints 68% (52/76)). Comparing the two groups of different AFT (AFT1 vs. AFT2), statistical differences were neither found in the number of successful races (success: place 1-4, p=0.77) nor in the total number of races completed (p=0.07). Moreover, the number of documented injuries with different training starts did not differ (p=0.11). In these first results, AFT showed no effect on performance and health of these German Thoroughbred racehorses. Further statistical evaluations regarding additional influencing factors are urgently required to generate further knowledge.

Effect of feed supplement on locomotion score and joint health biomarkers in sport horses

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Sport horses need an adequate diet to support career longevity. This study evaluated the effect of a supplement (HAPPI MOBILITY, Terrya) given to 9 active jumping horses (6–16 y.o., 1–2 competitions/month). All were fed 3.46 kg of complete feed before the trial, of which 1.26 kg was replaced during the 45-day study by a supplement containing *Saccharomyces cerevisiae* CNCM I-1077, selenium-yeast, and melon juice concentrate (Lallemand). Seventeen blood variables were measured, 15 locomotion parameters assessed using a non-invasive sensor (Equisym, Arioneo), and a questionnaire covered 37 individual criteria (age, training, competition, health history). Paired t-tests compared values between day 0 (baseline) and day 45 (end). Locomotion scores were based on sensor data, and percentage of horses improving or declining was analyzed. Non-parametric tests evaluated questionnaire factors. Osteocalcin and glutathione peroxidase (GPx) levels increased significantly after 45 days (+55% and +59%, $p < 0.01$). Phosphorus rose significantly ($p < 0.05$), while calcium, zinc, and vitamin D remained stable. Locomotion during circular movements on hard ground improved ($p < 0.01$), showing greater flexibility. At baseline, osteocalcin tended to be lower in horses > 10 y.o., those with bone history, no recovery protocol post-competition, or asymmetry during training ($p < 0.10$). No subgroup differences were seen at d45. This trial highlights the benefits of supplementing jumping horses for joint health, likely due to primary antioxidants (superoxide dismutase, catalase) and organic selenium, a GPx cofactor essential for bone integrity.

Session 39

Poster 16

The impact of yeast supplementation on horses' coat condition, hoof health and feces quality

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The aim of this study was to evaluate the impact of yeast supplementation on coat condition, hoof health and feces quality in a population of horses. The study involved 23 horses (control group $n=11$, experimental group $n=12$), aged 3–23 years, of various breeds, used for recreational riding. The experimental group received a daily inactivated brewer's yeast product (postbiotic) at 25 g per 100 kg of body weight for 90 days. Assessments were conducted at four time points: day 0 (before the start of supplementation) and days 30, 60 and 90. On day 30, a transition from winter to summer feed occurred due to external factors. The condition of the coat (0–2, poor to good), hooves (0–4, based on number of defects) and feces (0–4, firm to watery) was visually assessed by the same person. Statistical analyses included the Shapiro-Wilk and Lilliefors tests, followed by the Mann-Whitney U test and Spearman's rank correlation. No statistically significant differences ($p > 0.05$) between the control and experimental groups were found for coat condition, although significant correlations with supplementation duration were observed. Seasonal effects may also have influenced outcomes. Hoof condition showed no significant group differences ($p > 0.05$), but a temporal trend in the experimental group suggested faster resolution of hoof damage. Fecal consistency remained stable throughout the study and watery feces were not observed in previously affected horses, though differences between groups were not statistically confirmed. Although no statistically significant effects were demonstrated, the observed trends suggest potential benefits of yeast supplementation for coat and hoof recovery and possible stabilization of fecal quality. Visual assessments indicated a more rapid improvement in coat and hoof condition in the experimental group over the study period. Funded by the Minister of Science under the "Regional Initiative of Excellence Program".

Occurrence and prevalence of endophyte toxins in meadows and pastures for horses across different European countries

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Temperate pasture grasses form the basis for grazing and production of roughage for horses. Their fungal symbionts are endophytes that produce bioactive compounds protecting the plants from stress conditions. Some endophyte compounds are toxic to animals, e.g., ergovaline is an ergot alkaloid and lolitrem B is a potent tremorgen responsible for perennial ryegrass staggers. There is limited knowledge about the presence of endophyte toxins in European grasslands. However, this information is important for predicting the exposure and health risk to grazing animals. From 2021 – 2023 we monitored the presence of ergovaline, lolitrem B, and paxilline (a possible precursor of lolitrem B) in selected meadows and pastures providing roughage for horses. A total of 375 samples of *Festuca*, *Festulolium* and *Lolium* spp. were taken from Austria (AT, n=42), France (FR, n=127), Hungary (HU, n=14) and Germany (DE, n=192, 4 locations). Concentrations of target metabolites were determined using LC-MS/MS. There were 63 positive samples with the rate of 0-50% of samples per location and year were positive for at least one of the metabolites. Ergovaline concentrations reached up to 3.4 mg/kg on a dry matter basis. Locations may differ in the occurrence of endophyte toxins due to variation in botanical composition and geo-climatic factors. Location differences were observed for the occurrence of ergovaline and paxilline ($P < 0.01$) with two DE locations having low concentrations of ergovaline ($< 10 \mu\text{g/kg}$), whereas the HU location showed significantly higher ergovaline concentration ($265 \mu\text{g/kg}$) ($P < 0.05$). Using frequency data, the incident rate of ergovaline was not different across locations. Temperatures before sampling (1-3 months) were more positively associated with the concentration of target metabolites than that at sampling week. In summary, unexpectedly high concentrations of endophyte toxins can be present in individual plants but their presence in meadows and pastures are not ubiquitous. Warm temperature could contribute to increased concentrations of endophyte toxins in grasses, warranting a surveillance of their prevalence.

Session 40

Theatre 1

The future of animal breeding in Europe

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The European animal breeding sector is at a crossroads, facing the challenge of attracting young talent while maintaining competitiveness and addressing complex societal expectations. This FABRE TP Challenge session at EAAP 2025 will explore the multifaceted landscape of animal breeding through an interactive “challenge” format designed to foster constructive dialogue with the audience and speakers. Animal breeding and genetics and the dissemination of genetic improvement play a crucial role in sustainability, better health and welfare of animals, and food security. Also, animal production is under pressure, particularly among new generations. There is a need for attractiveness among young geneticists and other scientists and for interdisciplinary training and innovative collaboration between academic institutions and breeding companies. Diverse perspectives, from academia to private sector and early career researchers, will explore critical issues such as talent attraction, skill development, funding mechanisms, and the changing role of animal breeding in sustainable food systems. The discussion will highlight the importance of animal breeding beyond the traditional genetic perspectives, emphasising the role in technological innovation, sustainable supply, agricultural development and food security. This challenge session will feature a dynamic and interactive approach; speakers from different European regions and career stages will provide short, focused presentation followed by a structured roundtable discussion. This format aims to encourage direct engagement between speakers and the audience, allowing for real-time exchanges to address the challenges and potential solutions. In conclusion, this challenge session aims to provide the audience with a comprehensive understanding of current barriers to the future of animal breeding in the EU, possible approaches for attracting and supporting young talent, collaborative solutions for bridging the gap between academia and the private sector, and a roadmap to reimagine the future of animal breeding in Europe.

Link Between Academia and Industry in EU Research: can we finally close the Gap?

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Research & innovation is acknowledged as a “catalysts of change” by the recently launched EU vision for Agriculture and Food. However, animal farming and research is not an easy area to work on. Yes we need R&I to provide for answers to the current and future challenges, and for that we need students and researchers and we need resources and motivation. How can (should) we motivate young students and researchers to animal science? How to ensure that R&I outputs are being of use? A crucial challenge is integrating basic and applied research—fundamental discoveries must translate into societal impact while application-driven inquiries can refine theoretical frameworks. Synergies between academia, private sector, farmers and policymaking have the potential to shape a research continuum, ensuring knowledge transfer and technological advancements. Academic institutions focus on fundamental research, exploring theories and principles that may not have immediate commercial applications. On the other hand, the private sector are driven by market needs, prioritizing research with tangible products, services, or solutions. A bidirectional flow of knowledge and people is encouraged: academia provides with new scientific insights, innovative technologies, and skilled talent; the private sector offers real-world issues and knowledge, funding and infrastructure, and that shape academic inquiry.

Session 40

Theatre 3

The Future of the Animals Breeding Sector in the EU: The Role of Academia and the Private Sector

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Sustenance of livestock production into the future relies on genetic improvement of animals to increase resilience and efficiency, reduce waste, facilitate adaptability to changing environments and varying production systems. This is more so with climate change and its impact on the availability and supply of farming inputs. In this regard, the role of structured breeding programmes and the human capacity to bring about genetic improvement cannot be over-emphasised. Because of biological differences between livestock species and available technologies, breeding programs vary in structure. A common factor, however, is the reliance on highly skilled personnel needed to make genetic improvement of animals possible in practice. These include skilled personnel in commercial breeding programmes researchers in academic institutions, as well as stockmen with skills required in all aspects of practical livestock breeding. Naturally, therefore, the sustainability of this process into the future requires continuous training, recruitment and retention of young people from generation to generation. This is true in Europe and other Western countries, where most improved seed stock for the livestock industry is currently developed and in evolving economies with a preponderant role in livestock production. Demand for skilled manpower in developing countries is increasing because of the need for a sustainable increase in production to meet growing demand and ensure food security. Attaining the full potential of high genetic merit animals in the field requires strong knowledge of the local production systems and the skill to care for the animals. It is therefore imperative that young professionals be trained and retained in all sectors of the livestock breeding and production chain.

How to attract top talent and shape the future of animal breeding?

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Livestock production is one of the defining sources of foods for human consumption. Yet, the attractiveness of the sector is not aligned with its crucial importance in the general society. The direct reflection of these tendencies is the dwindling number of students in animal sciences, in particular in animal breeding, across universities throughout Europe. Our shared goal in this sector should be to reverse the negative trend, attract top talent, and prepare for future challenges. The question remains: how to do it? One crucial factor is establishing attractive, interdisciplinary study programs—like the international European Master in Animal Biodiversity and Genomics (EMABG)—as well as other national programs offered by universities across Europe. Such programs (in particular with availability of scholarships) attract excellent students from outside Europe. Interestingly, even the incentive of a full scholarship does not generate a similar response among European students. The solution for this problem is not clear. One important measure could be to improve employability among graduates. As a natural extension of this process, enhancing connections between academic and industry partners comes to mind. While some universities have a closely interlinked teaching and research process involving academic and industry partners, others are less well developed in this regard. Internships, guest lectures, research partnerships and aligned research goals could increase the exposure of students towards real applications and show a clear career path. Such interventions, combined with strong communication to the general public, could reverse negative trends and help attract top talent.

Session 40

Theatre 5

You can't pursue a career in animal science if you don't know it's an option – perspectives from a 'geriatric' student

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As the lucky ones who have chosen to pursue a career in animal science, we understand the value of animal agriculture, not just in relation to food production, but also the larger role it plays environmentally, economically and culturally. Unfortunately, there is a growing disconnect between those of us who live and breathe livestock production and the general public. Often times those of us who don't have a direct connection to the industry, are not aware of the amazing variety of career opportunities within animal science. There are many challenges faced by those of us who choose to pursue animal science, however, our personal and professional journeys are shaped by these challenges. We face biases inside and outside the industry while developing an understanding of how we value ourselves, the work that we do, and the perception of our work to the outside world.

Animal breeding innovation; the need of long-term support

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The long timelines and considerable resources required to develop and bring terrestrial and aquatic animal genetics to the market, especially for less mainstreamed species and breeds, create a high dependency on funding. This situation needs a clear identification of the priorities for research. It is therefore essential that the efforts of the public sector are accompanied by collaborations with the private sector and adequate financial support. The results of discovery research and pre-competitive breeding would feed into developing a broad range of traits and breeding objectives and strategies for terrestrial and aquatic animals. Enabling knowledge and technology development and, crucially, innovation transfer between sectors and species are also part of the dedicated support needed by public authorities. Although Horizon 2020, Horizon Europe, and various (inter)national funding schemes have funded/are funding various projects supporting the development of breeding strategies for terrestrial and aquatic animals, these projects tend to be scattered and poorly aligned. As a result, their outcomes are often not effectively utilised beyond the scope of the individual projects. FABRE TP recently conducted a study identifying the public investment in animal breeding and genetics R&I in the EU covering the period between 2007 and 2025 (until end-March). FABRE TP's mission is to promote research and innovation in animal breeding and genetics by identifying the main priorities with its members. Based on those, FABRE TP advocates for sufficient, extensive, and appropriate funding opportunities from public authorities for the sector. In the current policy context, it's more important than ever to change the prevailing narrative that focuses solely on the negative effects of animal farming. We need to highlight the shared research and innovation priorities of both the private and public sectors and bring these to the attention of policymakers. This will help ensure continued long-term investment in animal science. The aim is to enhance the discussion with concrete elements.

Session 40

Theatre 7

Navigating the EU academia landscape from the PhD to a career in academia with EU funding: opportunities and challenges

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EU programs for the support of PhD and Postdocs include the Marie Skłodowska-Curie Actions (MSCA), among others. These programs promote excellence and mobility in EU and abroad. By promoting all domains of research and innovation allow harmonisation. Moreover, the mobility contributes to encourage international cooperation to set-up strategic collaborations. These programs, aim to promote the development of attractive research careers to support excellence in research and innovation across Europe. Since 1996, the MSCA have provided grants to train excellent researchers at all stages of their careers while encouraging transnational, inter-sectoral and interdisciplinary mobility. The Marie Skłodowska-Curie Actions (MSCA) program has supported approximately 39,000 PhD candidates. While focusing on the initial training networks, we can observe that the involvement of countries throughout the EU is quite uneven. In the field of animal production, it seems to clearly overlap with the distribution of established animal production chains that include a link to research&development (R&D) infrastructures from the industry. If we look at animal health for example, countries like Portugal, Latvia and Lithuania do not have centres for production or R&D just logistics. In regards to animal breeding and genetics, R&D is also located in a few countries. This lack of harmony throughout EU presents challenges that we have for the future that also represent opportunities. Animal production from EU is stronger with a country-wide higher level of production. But how can we achieve that? How can this be stimulated? In this talk these subjects will be approached in a data-driven manner and from the perspective of my personal experience as a researcher, with an EU-funded career in Portugal, a target-inclusiveness country.

The Future of the European breeding Sector: Discussion and closing remarks

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European research infrastructures are essential for advancing research and innovation in terrestrial, aquaculture, and insect breeding. As the European Forum of Farm Animal Breeding (EFFAB) and the Farm Animal Breeding & Reproduction Technology Platform (FABRE TP), we represent both public and private sector stakeholders, working to bridge the gap between research and application. Ensuring that infrastructures effectively serve the needs of breeding and animal science requires stronger collaboration with the private sector, addressing key challenges such as data accessibility, intellectual property management, and long-term sector engagement. This session will explore strategies to enhance public-private cooperation, making research infrastructures more accessible, industry-relevant, and sustainable. Through case studies and discussion, we will highlight best practices for fostering meaningful partnerships that drive innovation and ensure research infrastructures remain key enablers of progress in animal breeding and sustainable food production.

Session 41

Theatre 1

Valorization of co-products in the formulation of growth media for cellular foods

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The food industry is facing a debate on sustainability, driving research towards innovative solutions to reduce environmental impact. In this context, cultured meat represents a good alternative. However, its development is still hampered by major challenges, including the formulation of ethical, economically viable and cell-compatible culture media. For this, the aim of the study was to evaluate the use of food co-products as components of C2C12 muscle cell culture medium and to analyse their effect during cell proliferation and differentiation. In particular, protein hydrolysates obtained from hemp flowers (HF), shrimp processing (SP) and a chemical extract (50:50, H₂O/EtOH) from oil samsa (OS) were investigated. Besides presenting a valuable protein content, in particular SP (46.72±1.31%), all matrices showed high antioxidant activity, with highest values for OS (262.82±8.77 mg FeSO₄/100 g) and SP (201.51±16.17 mg FeSO₄/100 g), tested with FRAP assay. Proliferation assay (Alamar Blue test), performed after 24 and 48 h of muscle cell culture with HF (11.7-0.02 mg/mL), SP (12.5-0.01 mg/mL) e OS (33.3-0.015 mg/mL), showed that concentrations between 1.46-0.37 mg/mL, 1.56-0.20 mg/mL and 1.04-0.26 mg/mL, respectively for HF, SP and OS, supported cell proliferation. Cell differentiation assay, conducted by assessing Citrate Synthase and Creatine Kinase activity, showed promising results, especially at lower concentrations. Future research will focus on combining the BEST concentrations of HF, OS and SP to evaluate a synergistic effect that can improve cell growth and differentiation. The integration of ingredients in cell culture media could open up new perspectives for cultured food production in line with the principles of the circular economy. Financed by the European Union- Next Generation EU, Mission 4 Component 1 CUP_G53D23003940006 and by Mission 4 Component 2 Investment 1.3 – Project code PE000003

Effects of Exogenous Protease Levels on Intestinal Morphology, Tight Junction Proteins, and Inflammatory Response in Pigs Fed Reduced-Protein Diets

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Exogenous protease may improve gut health in pigs by enhancing glycinin degradation, reducing inflammation, and improving barrier function. In this context, the present study evaluated the impacts of including exogenous proteases in a diet with reduced crude protein and amino acid content on intestinal morphology, tight junction proteins, and inflammation in growing-finishing pigs. Thus, 135 pigs were housed for 104 days in pens under a randomized block design with five treatments and nine replications per treatment. The dietary treatments were: positive control (PC) – basal diet with a 5% reduction in protein and amino acid requirements; negative control (NC) – basal diet with a 7.5% reduction in protein and amino acid requirements; NC100 – NC + 100 g/T of aspartic protease; NC150 – NC + 150 g/T of aspartic protease; and NC200 – NC + 200 g/T of neutral serine protease. On day 105, 8 pigs per treatment were slaughtered for jejunum sample collection to analyze inflammatory markers (IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-12p40, IFN- α , IFN- γ , TNF- α), intestinal morphology, and tight junction proteins (claudin, occludin, and zonula occludens). Procedures were approved by the ethics committee. For all parameters, ANOVA was used followed by Tukey's test. A statistical difference was observed between the PC and NC treatments for IL-1 β , with PC showing higher IL-1 β levels. For TNF- α , a higher concentration in the NC200 group compared to NC and NC100 was observed. No statistical differences were found among treatments for the other inflammatory markers, intestinal morphology, or tight junction proteins. The results suggest that the inflammatory response varied among treatments, with PC and the highest protease level increasing inflammation; however, these treatments did not affect intestinal morphology or tight junction proteins.

Session 41

Theatre 3

Effect of early-life supplementation with rumen fluid inoculum and feed additives on growth performance in naturally and artificially reared goats

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The rearing system (artificial vs. natural) can influence rumen development in early life. While some studies suggest that maternal rearing may promote rumen maturation, there is limited data on its impact on overall productivity until weaning, as well as its long-term effects on adult performance. Additionally, oral administration of rumen fluid from donors has been shown to accelerate rumen development in young ruminants. Specific feed additives, such as probiotic live yeasts and butyric acid, have also been reported to promote the structural development of the rumen epithelium. In this experiment, thirty-two individual pens were set up across two separate animal houses. Following birth, kids were assigned to two groups: the ArtiG (artificial rearing) and the NatuG (natural rearing), each consisting of 16 newborn kids. Both groups were initially fed colostrum for the first two days in individual pens, after which ArtiG kids were fed milk replacer and solid feed until weaning, while NatuG kids remained with their dams. From the 10th to the 30th day of age, 8 kids from each group received 5 ml of fresh rumen fluid inoculum. After day 30, the inoculated kids were supplemented with 1% wt/wt tri-butyrin and 500 mg/head/day of *Saccharomyces cerevisiae* live yeast (Actisaf, Phileo). Four kids from each subgroup were slaughtered at 70 days of age to collect gastrointestinal tract epithelium samples. The study assessed the effects of natural vs. artificial rearing, rumen fluid inoculation, and rumen-promoting feed additives on growth rate and immune system response at weaning. Additionally, the relative abundance of ruminal protozoa, fungi, and fibrolytic bacteria, such as *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Ruminococcus albus*, were evaluated. Finally, histological analysis of the rumen mucosa examined the appearance and distribution of extracellular matrix components (collagen and reticular fibers), as well as prekeratin and keratin contents, and the overall structure of the epithelium.

Acid-neutralising capacity of different commercial dietary buffers

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Dietary buffers (DB) mitigate rumen acidity, enhancing ruminal fermentations when high-concentrate diets are fed to cattle. The acid-neutralizing capacity (ANC) assessment is a reliable measure of the buffering agents' effectiveness. The trial aimed to compare the ANC of 6 commercial DB (DB1-6) and NaHCO₃ (SB), or their different combinations (cDB) within an acidogenic diet for lactating dairy cattle. The DB differed in calcite content, which was <5 wt% in DB5; 5-15 wt% in DB1, DB4 and DB6; 15-25 wt% in DB3; and >75% in DB2. Only DB4 and DB5 contained more than 15 wt% of aragonite. The ANC was tested in triplicate by titrating 200 mg of DB or SB in an aqueous solution with 0.1 M HCl (strong acid – SA) or 0.5 N acetic acid (weak acid – WA) to reach the target pH 6, 5 and 4. Additionally, based on the commercial inclusion levels, the equivalent of 160 g of SB (0.65 % DM) in an acidogenic ration (27% starch, 30% NDF) for high-producing dairy cows (assumed DMI: 24.6 kg) were replaced by 80 g of each DB (1-6) or a mixture (1:1 ratio) of SB-DB5, or 60 g of DB5. The 9 cDB were incubated in duplicate in an in vitro batch fermentation system with RF for 0, 1, 4, 12 and 24 h. The pH and the residual ANC in SA were evaluated. The volumes of WA and SA required to reach each target pH were compared among DB or cDB. Differences were assessed by ANOVA using SPSS software v.29 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY). The LSD post-hoc test was applied for pairwise comparisons. At pH 6, the highest ANC was achieved by SB (20.7 mL of SA; $p < 0.001$), and DB6 (4.2 mL of WA; $p < 0.001$) challenged with SA and WA respectively. At pH 5, the strongest ANC was obtained by SB (23.5; $p < 0.001$), and DB3 (8.5 mL of WA; $p < 0.001$) when tested with SA with WA respectively. At pH 4 DB1, DB2 and DB3 showed the highest ANC both with SA and WA ($p < 0.001$). The main differences among the cDB were observed after 24 h of incubation ($p < 0.001$), with DB5 (60 g) having higher ANC with SA at pH 6 compared to DB1 and DB6. At the other target pH and incubation intervals the cDB showed similar ANC.

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Theatre 5

Effects of β -mannanase supplementation on growth performance, nutrient digestibility, intestinal morphology and immunity in weanling pigs fed with low energy dietP. Neves Silvestre¹, S. H. Ha¹, S. D. Choi¹, S. S. Lee¹, J. Y. Mun¹, J. S. Kim¹¹ Kangwon National University, Department of Animal Science, 24341 Chuncheon -Si, South Korea

In this study, a total of two hundred and forty weaned piglets (Landrace $\times$ Yorkshire $\times$ Duroc) were assigned to three dietary treatments: positive control (PC), negative control (NC: -90 kcal/kg energy), and DGM2 (-90 kcal/kg energy with 0.02% β -mannanase), divided in phase 1 and 2. BW was higher ($P < 0.05$) in the PC and DGM2 compared to NC. In phase 1 and 2 ADG and G:F were greater ($P < 0.05$) in the PC and DGM2, and overall, ADG and G:F increased in PC and DGM2 treatments. Nutrient digestibility of DM increased ($P < 0.05$) in DGM2 and PC, and non-starch polysaccharides and mannan increased ($P < 0.05$) in all treatments in phase 1. During phase 2 there was a tendency ($p = 0.095$) for higher DM in DMG2. Non-starch polysaccharides digestibility was enhanced ($P < 0.05$) in DGM2, PC and NC. Furthermore, mannan levels were greater in DGM2 and PC. There were no significant differences between amino acid and monosaccharides and mannan oligosaccharides digestibility. Intestinal viscosity was lower in DGM2 compared to PC and NC. Duodenal VH was higher ($P < 0.05$) in DGM2, and NC, while the VH/CD ratio tended to be greater ($P = 0.084$) in these groups. In jejunum, VH was higher ($P < 0.05$) in DGM2, and VH/CD ratio was higher ($P < 0.05$) in the DGM2 and NC. In ileum, VH was greater ($P < 0.05$) in DGM2, and VH/CD ratio was higher ($P < 0.05$) in DGM2 and NC. No significant differences were observed in the colony-forming unit in the ileum, cecum, and feces. However, *Escherichia coli* in the ileum and cecum decreased ($P < 0.05$) in the DGM2 compared to the PC. No significant differences were found in IgG, IgM, IL-1 β , IL-12, and IL-18 across treatments in both phases; however, insulin and glucose concentrations were higher ($P < 0.05$) in DGM2 and NC. In conclusion, the supplementation of β -mannanase for weanling pigs fed a low-energy diet, decreased intestinal viscosity, improved growth performance, nutrient digestibility, and enhanced intestinal morphology. Key words: energy, enzyme, mannan, piglets, villus, viscosity

Selenium and Zinc Biofortification in Black Soldier Fly Larvae

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Insects are natural bio-accumulators of different micro nutrients and minerals. Minerals such as selenium (Se) and zinc (Zn) are essential for both animals and humans. Therefore, the aim of this study was to examine the Zn and/or Se bioaccumulation potential of black soldier fly larvae (BSFL) reared on fortified substrates. To study this, BSFL were reared on five distinct substrates: 1) control substrate (CTR) of plant-ingredients (Gainesville diet); 2) Okara and potato waste-based substrate (OP); 3) OP fortified with 150 mg/kg of Zn (Zn); 4) OP-Se fortified with 0.3 mg/kg of Se (Se); and 5) OP fortified with 150 mg/kg of Zn + 0.3 mg/kg of Se (SeZn). Each treatment had five replicates. Se was fortified in the inorganic form of sodium selenate, while Zn was administered in the form of zinc sulfate monohydrate. All experiments were conducted under conditions of darkness (26°C, 60% relative humidity), and the growth performance of the larvae was observed. The analysis found that the mean larval weight was unaffected by the treatments. However, the CTR group exhibited significantly higher total weight at the end of the trial ($P < 0.05$). The survival rate was not affected by the treatment. The nutritional composition of the larva was affected by the type of substrate and not the fortification of Se and Zn. Larvae reared on OP substrates had elevated levels of lipids ($P < 0.05$) and reduced ash content ($P < 0.05$) compared with the other groups. Furthermore, the fortification of Se and/or Zn affected their bioaccumulation in the harvested larvae. The results confirm that OP, when supplemented with Zn and Se, is a suitable substrate for BSFL, resulting in “fortified” larvae characterised by a favourable nutrient profile. However, it is imperative to consider the content of lipids, Zn and Se when utilising BSFL for feed production. Nevertheless, further research is required to ascertain the unintended effects of these substrates on BSFL metabolism.

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Theatre 7

Effects of dietary supplementation with microalgae *Schizochytrium* spp and *Nannochloropsis gaditana* on rumen microbial community, enzymatic activity and fermentation of dairy ewesA. Mavrommatis¹, D. Skiros², P. Kyriakaki¹, R. Andreaki¹, F. F. Satolias¹, E. Godevenou¹, N. V. Karakonstanti¹, E. Flietakis², E. Tsiplakou¹¹ Agricultural University of Athens, Department of Animal Science, Iera Odos 75, GR-11855 Athens, Greece, ² Agricultural University of Athens, Department of Biotechnology, Iera Odos 75, GR-11855 Athens, Greece

Supplementing dairy ruminant diets with polyunsaturated fatty acids (PUFAs) can enhance the nutritional quality of milk. However, long-chain PUFAs may alter rumen microbial communities and their biochemical processes. This study investigates the effects of microalgae blend supplementation on the rumen ecosystem in ewes, analyzing bacteriome and archaeome composition using 16S metagenomic analysis and microbiome profiling via Whole Genome Sequencing (WGS; shotgun). Rumen biochemistry was assessed through volatile and free fatty acid profiling and digestive enzyme activity. Rumen fluid was collected on days 30, 60, and 90 of dietary supplementation. Thirty-two early-lactation dairy ewes were divided into four groups: control (CON), 30 g *Schizochytrium* spp./ewe/day (SC30), 30 g microalgae blend (70% *Schizochytrium* spp. and 30% *Nannochloropsis gaditana*)/ewe/day (MB30), and 40 g microalgae blend/ewe/day (MB40). *Methanobrevibacter ruminantium* increased significantly in all microalgae-supplemented groups compared to the CON group, while *Methanobrevibacter millerae* decreased in the MB30 group. Fibrolytic genera, such as *Ruminococcus*, *Fibrobacter*, and *Treponema*, were unaffected by supplementation, while *Butyrivibrio* was more abundant in the microalgae-supplemented groups. The abundance of *Prevotella* tended to decrease in these groups. WGS analysis identified numerous viral genomes, with 75-80% of them being phages. The molar proportion of volatile fatty acids (VFAs) was largely unchanged, except for a decrease in propionate in the MB30 group. Total VFAs increased in the MB40 group, with acetate significantly rising. Enzymatic activities of amylase, protease, xylanase, and cellulase were not significantly altered. These results suggest that microalgae supplementation modulates rumen microbiota, while its biochemistry and enzymatic activity rebalance.

The effect of a microbial cell protein on weaned piglets' growth performance

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Raising protein demand calls for novel protein-ingredients without compromising piglet health and performance along with sustainable sourcing. Microbial cell proteins (MCP) may substitute feedstuffs as soybean meal without adverse effects and benefitting pig growth. This trial evaluated MCP's (Uniprotein®, Unibio A/S, Denmark) effect at 4 and 8% inclusion on post-weaning (PW) piglet performance, hypothesizing MCP could substitute, fully or partially, skimmed milk powder (SMP) and soy protein concentrate (SPC) without performance drawbacks. Weaned piglets (8.1 kg; 29.4 days) were housed in groups of six. Six dietary treatments (T) were tested over 2 PW periods (day (d) 0-14 and 14-35) with 12 replicates per T. In period 1 (0-14 d PW) diets consisted of: negative control (NC, T1), NC+4% MCP1 partly replacing SMP (T2) or SPC (T3), NC+8% MCP1 fully replacing SMP (T4) or SPC1 (T5) and NC+4% MCP2 partly replacing SPC (T6). In period 2, a different NC T was fed; T 2-3 a NC+2% MCP partly replacing SPC; T 4-5 a NC+4% MCP fully replacing SPC; NC+2% MCP2. Feed was provided ad libitum, with an abrupt transition between period 1 and 2. Piglets were weighed at d 0 and 35 PW; pen weights were taken at d 14 PW. Body weight (BW, kg), ear and tail lesions were measured at d 0, 14, and 35 PW, and ADG, ADFI, FCR, and faecal consistency (FS) between d 0-14, 14-35, and 0-35 PW. All conducted over two weaning rounds. Statistical analysis (ANOVA, GenStat®) evaluated on 1) T effects and 2) CP source×MCP1 dose, with significance set at P<0.05, T means were compared by LSD. Treatment had no effect (P>0.05) on mortality, medical treatments, or FS. In period 1, piglets on T4, T5 and T6 had a higher FCR (P=0.04) than T1, but no FCR differences were measured in period 2. No effects of CP source, MCP1 dose or its interaction were measured for ADG, ADFI, and FS in period 1. In period 2, ADG was affected (P=0.03) by CP source×MCP1 dose. Piglets fed 2% MCP1 had higher ADG than those fed 4% (642 vs. 599 g/d, respectively), but this was only the case for the SPC group in period 1. This suggests that MCP can replace SPC and SMP in weaner diets without overall adverse effects on performance and health.

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Theatre 9

Potential of selected, heat-treated peat meal as a dietary component for alleviating mycotoxin-induced challenges for pigs and poultry

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Mycotoxins in feeds may impair intestinal functions and decrease the performance of farm animals. Commercial mycotoxin deactivators often contain silicates, yeast hydrolysates and specific enzymes, but novel materials for neutralizing the effects of mycotoxins are still needed. The present multi-trial project examined the potential of a carefully selected, humic acid rich, heat-treated Finnish peat in alleviating the challenge of mycotoxins for poultry and pigs. First, the ability of peat meal to adsorb zearalenone (ZEA), ochratoxin A (OTA) and deoxynivalenol (DON) from an aqueous medium was studied in vitro, using hydrated sodium calcium aluminosilicate (HSCAS) as a comparison. At pH 6.5, peat adsorbed more ZEA and DON than HSCAS, while the binding of OTA was similar for both materials. At pH 2.5, HSCAS bound more OTA and ZEA than peat, but both products failed to bind DON. In trial 2, 420 male Ross 308 broiler chickens were given starter diets which were intentionally contaminated with low levels of DON (900 µg/kg) and ZEA (250 µg/kg). Peat meal was included in the 3 diet groups (20 pens of 7 chicks/tr.) at 0% (Control) 0.5% or 1.0%. On day 21, the chicks were sampled for ileal and caecal contents. Dietary peat inclusion improved bird weight gain and feed conversion, and beneficially modulated intestinal fermentation and caecal microbial populations. Finally, the ability of peat to modulate fermentation by authentic microbial community of porcine colon was studied in an elaborate ex vivo simulation system. Compared to control treatment, peat increased butyrate production and decreased the production of branched chain volatile fatty acids, suggesting positive effects of peat to microbial fermentation. In conclusion, peat meal demonstrated promising potential for becoming a functional ingredient in mycotoxin-neutralizing feed products: peat may bind mycotoxins, positively modulate gut microbiota, and alleviate the negative effects of mycotoxins on animal performance. More experiments are needed for dosage optimization and for validating these effects in commercial-scale animal farming.

Enhancing Growth and Nutrient Utilization of Pigs with N-Carbamylglutamate Supplementation

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Abstract This study aimed to evaluate the effects of dietary N-carbamylglutamate (NCG) supplementation on growth performance, nutrient digestibility, and blood profiles in crossbred grower-finisher pigs. A hundred and fifty pigs with an average initial body weight (BW) of 50.72 ± 3.15 kg were assigned to five feeding strategies. The treatments consisted of a control diet (NP), and low protein diets (LP) with NCG supplementation (0, 0.04, 0.07, and 0.1%) over three phases (phase 1, d 1-21; phase 2, d 22-42; phase 3, d 43-63). The BW at phases 2 and 3 was linearly increased with supplementation of NCG. Moreover, the NP group showed a greater BW in phases 2 and 3 compared to LP treatment. The NCG supplementation linearly improved gain to feed ratio in phases 1 and 2, and overall. The NP group showed a greater gain to feed ratio in phases 1 and 3 and a greater overall average daily gain compared to LP treatment. The digestibility of crude protein was linearly increased with the supplementation of NCG. The digestibility of Arg, His, Leu, Lys, Phe, Thr, Val, Ala, Glu, Gly, Ser Pro, Cys, Cit, and Ornithine was linearly improved with NCG supplementation. Moreover, the digestibility of Arg, His, Ile, Val, Glu, Pro, and Cit was higher in the NC treatment compared with the LP. The gene expression of solute carrier family (SLC)6A19, SLC7A5, SLC7A7, SLC7A9, SLC38A2, and mTOR in the jejunum was linearly increased, and the gene expression of SLC1A5 and SLC7A1 tended to increase with supplementation of NCG. The marketing age and backfat thickness were linearly reduced with supplementation of NCG, and the slaughter weight, carcass weight, and loin muscle area were linearly increased. Moreover, the NC treatment showed a lower marketing age, and a higher slaughter weight, carcass weight, and dressing percentage compared to the LP. In conclusion, 0.1% dietary NCG supplementation is recommended for pigs due to linear increase in growth performance, feed efficiency, amino acid utilization, and carcass quality.

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Theatre 11

Hybrid-rye as alternative cereal for feeding of dairy calves: effects on performance and rumen fermentation

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Different cereals are used in starter feeds for dairy calves to stimulate rumen development, although rye has rarely been used. The use of hybrid-rye in livestock feed is becoming increasingly attractive due to its higher resistance to ergot and drought compared to other cereals. Moreover, due to its specific hemicellulose content compared to wheat, rye may stimulate rumen butyrate production and hence rumen development. The present study aimed to investigate whether hybrid-rye could replace wheat in concentrate for dairy calves by evaluating its effect on performance and development of rumen fermentation. A total of 32 Simmental calves (half female, half male) were assigned to one of two dietary groups (wheat- or rye-based concentrate) after birth. Calves were monitored until weaning (12 weeks). Calves had free access to hay and water and were fed milk replacer and concentrate semi-ad libitum via automatic feeders. Concentrates included 46% hybrid-rye or wheat. Rumen fluid was collected via an esophageal tube for pH, volatile fatty acid (VFA) and microbiome (16S rRNA sequencing, V3-V4 region) analysis. An ANOVA was performed using the PROC MIXED and repeated measures model in SAS. Daily concentrate intake was low in the first 4 weeks and then steadily increased to about 2.2 kg at 12 weeks. Total intake of milk replacer and concentrate was similar between dietary groups. Likewise, growth performance was similar between dietary groups. Rumen pH was above 6 throughout the experiment and developed similarly between dietary groups. Concentrations of VFA (i.e., acetate, propionate and butyrate) were higher in rye-fed calves at 5 weeks of age ($p < 0.05$) and butyrate tended ($p < 0.1$) to remain higher at 6 weeks compared to wheat-fed calves. Differences in bacterial composition in rumen fluid of rye- and wheat-fed calves were small, indicating that differences were mainly at functional level. Overall, the present data demonstrate that hybrid-rye can safely replace wheat in concentrate for dairy calves. Promoting butyrate fermentation through hybrid-rye likely provided an additional benefit for rumen development.

Effects of astaxanthin supplementation on diarrhea and rumen fermentation in dairy calves

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Calves are often exposed to oxidative stress, and the efficacy of antioxidants is discussed. The objective of this study was to evaluate effects of astaxanthin supplementation, a carotenoid with high antioxidant capacity, on diarrhea and rumen fermentation in Holstein calves. Sixteen Holstein male calves were divided into 2 dietary groups: a control diet (CON; n = 8) or the same diet with Panaferd®, which contains 2.3% astaxanthin derived from the carotenoid-rich microorganism *Paracoccus carotinifaciens* (AX; n = 8); added to milk replacer (MR) at 1.2g/d from 7 d, 1.6 g/d from 14 d, 2.4 g/d from 21 d, and to calf starter at 2.4 g/d after weaning. All calves were fed MR (30% crude protein, 21% fat), gradually increased from 600 to 1200 g/d (powder basis) in 7 to 20 d, then maintained for 2 weeks, and gradually reduced from 1200 to 600 g/d in 42 to 55 d, and weaned at 56 d. All calves were fed calf starter and chopped hay ad libitum. Blood samples and body measurements were collected weekly, and rumen fluid was collected at 56 and 91 d. Data were analyzed by PROC MIXED of JMP® 18 pro (SAS Institute Inc., Cary, NC). AX group had higher plasma astaxanthin concentration and total antioxidant capacity throughout the experiment ($P < 0.05$). Dry matter intake and average daily gain were not affected by treatment before weaning, however AX group had lower incidence of diarrhea from 7 to 20 d ($P < 0.15$) and plasma γ -glutamyltransferase concentration at 7 and 14 d ($P < 0.05$). After weaning, hay intake was lower in AX group from 57 to 70 d ($P < 0.05$), and then rumen endotoxin activity was lower at 91 d ($P < 0.05$). These results suggested that astaxanthin supplementation to calves improved antioxidant capacity and decreased diarrhea in neonatal period and affected rumen fermentation in post weaning.

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Poster 13

Dietary yeast hydrolysate improved production performance and reduced milk somatic cell count of dairy cows under an organic production system

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Direct-fed microbials, such as yeast and lactobacilli, can be used to modulate rumen fermentation and thus improve feed digestibility, production performance, and wellbeing of dairy cows. The present experiment studied the effects of an acid-hydrolyzed *Saccharomyces* yeast product (YHP) on dairy cows. The commercially available product has been approved for organic dairy production. The study was conducted at Mustiala Teaching and Research Farm, a facility specializing in organic milk production. The 75 cows were mainly of Ayrshire and Holstein breeds, with mean ECM of 10,426 kg/cow/year. Their diet consisted of grass silage with ~39% concentrate proportion. The study compared production and feed intake data from two 35-day periods: before and after the introduction of YHP to the diet. The product was dispensed through a milking robot as a part of concentrate at the level of 6-8 g/cow/day, depending on milk production and the stage of lactation. Comparison of milking robot data between diet periods was carried out at both herd and individual cow levels. At the herd level, dietary YHP amendment increased feed intake and milk yield: the dry matter intake was 21.3 kg/cow/day and 22.1 kg/cow/day, for control and YHP period, respectively, while milk yield was 30.2 kg/cow/day in the control period and 33.9 kg/cow/day in the YHP period. The energy-corrected milk yield was 13% higher in the YHP than control period (34.9 vs. 31.0 kg/cow/day). In the YHP period, the fat content of milk was decreased by 1%, while the protein content was increased by 4%, in comparison to the control period. Feed conversion efficiency was improved by 9% during the YHP period. The average SCC in milk was 106,000 cells/ml in control period and 78,000 cells/ml in YHP period. In summary, the results demonstrated increased dry matter intake, higher daily milk yield, improved feed conversion efficiency, and a reduction in SCC during the YHP period. These findings indicate that YHP can have positive effects on production performance, rumen function, and udder condition of dairy cows under organic farming systems.

Fresh and ensiled *Rosa rugosa* Thunb.: Impact on in vitro ruminal parameters in dairy cows

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Rugosa rose (*Rosa rugosa* Thunb.) and its by-products, known for their high content of biologically active components such as polyphenols, tannins, and unsaturated fatty acids, offer promising benefits in ruminant nutrition, including dairy cattle. This study aimed to investigate the potential of fresh (RFPF) and ensiled (RFPS) Rugosa rose fruit pomace as a feed supplement, focusing on evaluating their effects on in vitro ruminal fermentation, methane production, and biohydrogenation processes. The evaluation was conducted through a short-term incubation system – batch culture. Treatment groups consisted of 200 mg samples of grass silage as a control (n=15), RFPF (n=15), and RFPS (n=15). The addition of rose pomace led to notable alterations in several ruminal fermentation characteristics in ruminal fluid such as, ruminal pH, ammonia concentrations, and volatile fatty acids (VFAs) profiles. Both RFPF and RFPS increased ruminal pH ($p<0.05$) from 6.02 to 6.47 and 6.28, respectively, while ammonia concentration decreased ($p<0.05$) by 31.2% and 26.8% respectively. The inclusion of both RFPF and RFPS resulted in a reduction in in vitro methane production ($p<0.05$) by 23.4% and 18.3%, respectively. The study concluded that incorporating Rugosa rose fruit pomace into dairy cows' diets has the potential to enhance the nutritive value of ruminant feed, help mitigate environmental pollution, and thereby promote sustainable livestock production practices

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Poster 15

Maize can be partially replaced by sugar beet in the diet of goat kids in order to reduce the carbon footprint of caprine meat production systems

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The MEDGOAT project (PRIMA 2022) addresses the need to mitigate the contribution of livestock to global warming in the Mediterranean countries by (1) enhancing the use of goats, and (2) integrating local alternative feed resources into feeding calendars. In southern Europe, it is imperative to find low-cost and drought-tolerant alternative feed resources based on high-yielding crops that increase CO₂ sequestration using less irrigation water than maize. The cultivation of sugar beet as a feed for goats might be an economically viable option in the Mediterranean region, which is exposed to severe drought conditions that may hamper maize production. Therefore, our hypothesis was that sugar beet might be used to replace, at least partially, maize in the diet of fattening kid goats, without affecting feed efficiency or metabolism. Therefore, 24 goat kids being 5 months-old, divided into three experimental groups (n=8 animals each) received three complete pelleted diets (CPD) formulated either with 18 % maize (control group), 9% (T09 group) or 18% (T18 group) of sugar beet in replacement of the same amounts of maize in the CPD. According to the results observed, maize can be partially replaced by sugar beet in the diet of fattening goat kids with no significant effects on residual feed intake (feed efficiency). However, a significant decrease in insulin level was observed in the groups receiving sugar beet, likely due to the presence of betaine, a natural compound commonly found in this feed. Betaine is known to enhance insulin sensitivity of cells and support lipid metabolism; therefore, further studies are needed to determine whether this effect may have an impact on meat quality traits.

Effects of Exogenous Protease Levels on Microbiome Composition and Short-Chain Fatty Acid Production in Pigs Fed Reduced-Protein and Amino Acid Diets

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Exogenous protease is a feed additive used in monogastric diets to enhance protein digestion into amino acids. Amino acids may modulate intestinal microbiome by influencing short-chain fatty acids (SCFA) producing bacteria. This study investigated the effects of reduced crude protein and amino acids, along with varying protease doses and types, on microbiome composition and SCFA production. Thus, 135 pigs were allocated to pens in a randomized block design for 104 days, with five treatments and nine replicates per treatment. The diet treatments were: positive control (PC) – basal diet with a 5% reduction in protein and amino acid requirements; negative control (NC) – basal diet with a 7.5% reduction in protein and amino acid requirements; NC100 – NC + 100 g/T of aspartic protease; NC150 – NC + 150 g/T of aspartic protease; and NC200 – NC + 200 g/T of neutral serine protease. On day 105, 8 animals per treatment were slaughtered for cecal content collection to quantify acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. DNA extraction was performed on six samples per treatment for microbiome analysis. For the SCFA, ANOVA was employed, followed by Tukey's test. Alpha diversity was analyzed using the Wilcoxon test, and beta diversity was assessed by PERMANOVA. There was no statistical difference between treatments for the concentration of SCFA. The NC150 treatment showed significantly lower alpha diversity, as reflected in the Shannon and Simpson index, compared to the PC and NC treatments. NC150 also exhibited a lower abundance of Erysipelotrichaceae than NC 100. The results suggest that pigs receiving NC150 had reduced species diversity. Nevertheless, they exhibited a decreased abundance of a family associated with metabolic disorders in the pigs, while keeping SCFA concentrations stable.

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Poster 17

Rumen protected calcium gluconate improves lactational performance on 12 farms in Italy

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Several trials under research conditions and on commercial farms have shown that feeding rumen protected calcium gluconate increases milk production of dairy cows. RPCG was tested on 12 farms in Italy. The effect of feeding RPCG was tested in a total of 2,339 cows in 12 commercial herds in the Po valley in Italy. Farms had between 90 and 400 lactating cows. RPCG was fed at a dose of 16g/day/cow for a period between 30 and 90 days. Production parameters were recorded before, during and after the period RPCG was fed. Production of Energy Corrected Milk, g milk fat/day and g milk protein/day before RPCG treatment ranged from 29.6 to 45.5 kg/day, 1.192 and 1.644 g and 945 to 1.453 g respectively. Production of Energy Corrected Milk went up in 10 out of the 12 farms and was reduced in 1 farm. On the farm that saw a reduction of milk production, 0.2 kg/head of bypass fat was removed from the ration and replaced with RPCG at the beginning of the test period. Average increase of ECM production during the period RPCG was fed was 1.63 kg/day. Average milk fat production went up by 63 g/day, average milk protein production went up by 32 g/day. Average daily production of ECM, milk fat and milk protein increased in 2,339 dairy cows in 12 different commercial farms as a result of feeding RPCG. This result is in line with the results of trials in individual commercial farms.

Global environmental impacts of feed production were reduced and growing pig performance was maintained by adding phytase and reducing phosphorus, calcium and amino acids

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The objective of this study was to evaluate the increasing dietary phytase and reduced phosphorus, calcium and SID amino acids on the growth performance, blood parameters and global environmental impacts of feed production. Forty crossbred barrows (30.32±0.88 kg) were allotted in a randomized block design, with four treatments and ten replicates. Treatments consisted of diets with four phytase levels (0, 250, 500 and 1000 FTU) and the respective reductions in the concentrations of phosphorus (0.357, 0.297, 0.237, and 0.117%), calcium (0.722, 0.662, 0.602 and 0.428%), SID lysine (0.007%/250 FTU), SID methionine (0.004/250 FTU), SID threonine (0.004/250 FTU), SID valine (0.002% / 250 FTU) and SID tryptophan (0.214, 0.213, 0.212 and 0.208%). Pigs and diets were weighed at the beginning and end (50,28±1,21 kg) to determine the daily weight gain, daily feed intake and feed:gain. At the end, blood samples were collected to determine serum calcium and phosphorus concentrations. The ingredients inventories, based on life cycle assessment, were applied in the feed formulations to determine global environmental impacts (land occupation, soil acidification, water eutrophication, cumulated energy demand and terrestrial ecotoxicity). Analysis of variance was performed and, in case of significance, Dunnett's test and polynomial regression were applied. No differences were observed for growth performance and serum phosphorus concentrations. Serum calcium concentrations decreased linearly ($P=0.0647$) as dietary phytase concentrations increased. Regarding the environmental impacts, diets with higher phytase concentrations provided lower global environmental impacts of feed production. It is concluded that increasing dietary phytase concentrations, with the respective reduction in the concentrations of phosphorus, calcium and amino acids for growing barrows (30 to 50 kg), despite reducing the serum calcium concentration, maintained the performance and reduced the global environmental impacts of feed production.

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Poster 19

Effect of Rumiviv' phytochemicals on protein degradation using in-sacco method

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Some phytochemicals are known to improve by-pass protein level in the diet by binding protein and then limiting ruminal degradation, which may improve milk yield and milk protein synthesis. The objective of this study was to evaluate the effects of dietary phytochemicals [30g/day of RUMIVIV ; CCPA, Janze, France] as rumen modifiers on protein degradation. 3 different fistulated dairy cows in lactation were used for in-sacco analysis. The diet was based on corn silage. The control was collected at the beginning of the study, then cows were fed during 21 days with RUMIVIV before the 2nd rumen juice sample (Rumiviv group). Dry matter and protein degradation were determined with soybean meal 43,2% CP and rapeseed meal 33,7% CP. Samples were incubated in bags for 0, 2, 7, 24, and 48 h. Dry matter (48 h at 60°C) and nitrogen content of the residues (Kjeldahl) were analyzed for both raw materials. The diet of dairy cow and the group influenced dry matter degradability and protein degradation for soybean meal and rapeseed meal. Indeed, Rumiviv significantly decreased the protein degradability for soybean meal at 2h and 4h (respectively -4% and -10,4%). The group effect was not affected by the diet (no interaction for soybean meal and for rapeseed meal). These results suggest that using Rumiviv can significantly affect rumen fermentation, and these effects were reflected in decreased protein degradation. It's a solution to enhance nitrogen efficiency and reduce waste in environment.

Impact of rumen protected calcium gluconate on milk production on a farm in Croatia

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Calcium gluconate has a prebiotic effect in the hindgut, feeding rumen protected calcium gluconate increases volatile fatty acid production, which improves hindgut health and reduces the risk of leaky gut. Earlier trials have shown that RPCG increases milk production of dairy cows. Purpose of the current trial was to test RPCG on a farm in Croatia. A total of 276 cows were divided into two groups of 188 cows each. Average lactation number of the cows fed RPCG was 1.8, for the controls 1.85. Average DIM was 107 for the group fed RPCG and 111 for the control group. The control group was fed the same diet but without RPCG. RPCG was fed for 30 days at a dose of 16 g/cow/day. Milk production and milk fat percentage were compared between the 2 groups. Cows in the RPCG group produced 34.40 kg of milk per day compared to 33.25 kg for the controls. Milk fat percentage in the RPCG group was 4.58 compared to 4.43 for the controls. It was concluded that feeding RPCG increased milk production by 1.15 kg in combination with an increase of milk fat percentage of 0.15%. This result is in line with the results of other trials in commercial farms in various countries around the world.

Endo-1,4-beta-xylanase improves the performance, nutrient digestibility, and intestinal health of weanling pigs fed low energy diet

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In this experiment, 240 piglets (Landrace × Yorkshire × Duroc) with an initial body weight (BW) of 5.90 ± 0.6 kg were randomly assigned to 3 treatments with 10 replicates per treatment, and 8 heads per replicate pen. All pigs with mixed sex selected for this project were clinically and physically healthy. The dietary treatments include (1) corn-wheat-soybean meal (SBM) basal diet without xylanase (XYL) enzyme as control (CON), (2) corn-wheat-SBM basal diet deprived -90kcal/kg metabolizable energy without XYL enzyme (NC), and (3) corn-wheat-SBM basal deprived -90kcal/kg metabolizable energy + 0.02 XYL (DG2). The diets were administered in mash form for 42 days in 2 phases (phase 1, days 0-21; phase 2, days 22-42). The results show XYL supplementation increased final BW ($p < 0.01$) and overall average daily gain compared to the NC. Moreover, the feed to gain ratio in phases 1, 2, and overall was higher in the CON compared with the NC. Pigs fed NC diet showed a higher feed intake compared with the CON. In phase 1, digestibility of gross energy was higher in the CON compared with the NC, and the DG2 treatment showed the highest digestibility of xylan and non-starch polysaccharides (NSP). In phase 2, digestibility of gross energy was higher in the CON and DG2 compared with the NC, and the CON treatment showed a greater digestibility of ether extract compared with the NC. Moreover, the DG2 treatment showed the highest digestibility of xylan and NSP. The ileal digestibility of amino acids, villus height, and crypt depth were not affected. The digesta viscosity was the lowest in the DG2 treatment. The content of monosaccharide (MOS) and xylooligosaccharide (XOS) in the ileum was the highest in the DG2. The content of MOS in the cecum was higher in the NC compared with the CON, however, the content of XOS was the highest in the DG2. The population of *Lactobacillus* in the feces tended to increase and the colonization of *Escherichia coli* in the cecum tended to decrease in the CON and DG2 groups. Overall, XYL supplementation effectively enhanced performance and intestinal health in weanling pigs.

Assessing the impact of plant additives on fermentation kinetics during simulated in-vitro rumen hyperthermia

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Several commercial feed additives are available and marketed as effective solutions to mitigate heat stress in dairy animals. However, adding such products to the diet changes the fermentation kinetics. In this ongoing study, we aim to evaluate the effects of different commercially available plant additives on the rumen fermentation kinetics and fermentation products under simulated hyperthermia in in-vitro environment. We tested 2 different commercially available plant additives (Product A and B) and a total mix ration (TMR) in four different temperature settings (39.5, 40.5, 41.5, and 42.5 °C) for 48 h and measured pH, total gas production, methane yield, and volatile fatty acid. Product A has a crude fat content of 52.71% of DM (dry matter) and an ash content of 7.67%. Product B has a crude fat content of 4.36% of DM, and an ash content of 10.87%. The TMR had a crude fat content of 4.89% of DM and an ash content of 7.23%. Orthogonal polynomials of degree 3 were generated to analyze the effects of temperature on fermentation kinetics and products. With increasing incubation temperature, the fermentation of TMR leads to a quadratic trend ($p < 0.05$) methane yield/g DM and a linear trend ($p < 0.05$) for butyric acid production (39.5°C: 6.38, 40.5°C: 6.06, 41.5°C: 5.75 and 42.5°C: 53.8 mmol/g organic matter). For product A, there was a significant quadratic trend of temperature on acetic acid production during the fermentation ($p < 0.05$). For product B, pH follows a cubical trend ($p < 0.05$). However, the increase in temperature did not have any significant (linear, quadratic, or cubic) effect on the rest of the fermentation products. This study evaluated the effects of different plant additives on the variability of fermentation kinetics under a simulated in-vitro rumen hyperthermia environment. These insights help to understand how plant additives modulate fermentation kinetics during rumen hyperthermia, providing valuable information for enhancing dairy cattle nutrition under heat stress conditions.

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Poster 23

Effects of microalgae on in vitro rumen degradation, fermentation characteristics, and methane yield

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Microalgae are rich in bioactive compounds and are therefore evaluated for their potential to enhance animal health and productivity while also serving as methane mitigators. This study investigated the effects of four microalgae, namely *Dunaliella salina* (DUNA), *Spirulina platensis* (SP), *Nannochloropsis gaditana* (NANNO), and *Odontella aurita* (ODO) on rumen degradation, fermentation characteristics, and methane yield compared to a diet without microalgae (CON) using a 72-hour in vitro gas production system and three runs. The CON diet consisted of dried, ground grass silage, wheat grain, soyabean meal, and brewers' spent grains. Microalgae partially replaced soyabean meal, except in SP and the combination of DUNA+SP (DUSP), where soyabean meal was completely replaced. Wheat was partially replaced in all treatments except DUSP. Diets were isonitrogenous. Rumen fluid samples collected from four donor animals were strained and incubated with a fermentation medium at a 10:90 ratio ($n=4$ replicates/treatment). A linear mixed model was used, with treatment as a fixed factor and cow ID and run as random factors. Fisher's Least Significant Difference test was used for pairwise comparisons. In vitro dry matter degradability (IVDMD), ammonia concentration, and pH did not differ ($P > 0.05$). Acetate, propionate, and valerate concentrations (mM) were reduced ($P < 0.05$) in ODO and DUSP compared with CON, while propionate concentration was also lower ($P=0.014$) in NANNO than in CON. The propionate-to-acetate ratio and methane yield (g/kg substrate and g/kg IVDMD) did not differ ($P > 0.05$). These findings suggest that microalgae inclusion does not significantly alter rumen degradation or methane production under in vitro conditions. The reduction in specific volatile fatty acids in ODO and DUSP needs further investigation to determine implications for rumen metabolism. In vivo trials should assess the long-term effects on efficiency, animal performance, and methane emissions under practical feeding conditions.

Immune stimulated gilts fed diets supplemented with inosine-5'-monophosphate and reduced metabolizable energy on the immune system response

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To evaluate the effects of dietary inosine-5'-monophosphate (5'-IMP), and respective metabolizable energy (ME) reduction, fed to immuno stimulated (IS) gilts on the immune system response, 40 gilts with initial and final weight of 75.58±0.94 and 101.13±1.99 kg, were randomly assigned in a 2x2x3 factorial design, with 10 replicates/treatment. The studied factors consisted of a conventional (3,330 kcal ME/kg) or a 5'-IMP (0.100% 5'-IMP, 3,200 kcal ME/kg) diets. Gilts were subjected to a placebo or immune stimulation protocol, consisting in inoculating 1 mg bovine serum albumin with 0.5 mL of phosphate-buffered saline in association with 0.5 ml of Freund's complete adjuvant (day 1). The same procedure was adopted on day 12, but in association with Freund's incomplete adjuvant. The last factor consisted of blood collection period (days 0, 12 and 24). Blood proteinogram was determined by electrophoresis. Data was subjected to ANOVA and polynomial regression. Diets x periods interactions were observed for transferrin (P<0.001) and heavy chain IgG (P=0.026), increasing during the 24 days in gilts fed the 5'-IMP diet. The same interaction was observed for IgG (P<0.001), but increasing only in pigs fed the conventional diet. Immune stimulation x periods interactions were observed for IgA (P=0.010), ceruloplasmin (P<0.001), haptoglobin (P=0.029) and IgG (P=0.008). The IgA concentration of IS gilts decreased over the period. The IS gilts showed an increased ceruloplasmin as the period progressed, as the 5'-IMP diet led to increased transferrin. Albumin increased in placebo treated gilts, whereas IS gilts displayed a reduction over the period. Gilts treated with placebo showed a reduction, while IS gilts exhibited an increase in haptoglobin as, the period progressed. The 5'-IMP diet provided an increase in heavy chain IgG throughout the period. It was concluded that immune system shows different responses according to dietary 5'-IMP supplementation and immune stimulation of finishing gilts during a 24 days period.

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Poster 25

Pleurotus ostreatus and Mucor sp. as alternative cell culture medium ingredients for cellular food production E. Petrosillo¹, D. Bulgari², D. Lanzoni¹, G. Ghilardi³, R. Cavaliere³, T. S. Sundaram¹, C. Giromini^{1,4}, A. Baldi¹, F. Cheli¹

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Fungal extracts in cell culture media offer a sustainable solution to protein limitations in cellular agriculture. With their rich nutritional composition, they provide bioactive compounds supporting cell adhesion, differentiation and matrix formation. In this study, the protein content of *Pleurotus ostreatus* (PL) and *Mucor* sp. (M) mycelia was quantified, and their antioxidant (ABTS, FRAP) and ACE inhibitory capacities were assessed. Subsequently, the effect of the aqueous extracts from mycelia was evaluated on proliferation and differentiation of C2C12 muscle cells. C2C12 cells were cultured for 24 and 48 hours, during which fungi extracts were administered at different concentrations (from 12.50 to 0.01 mg/ml). Subsequently, cell differentiation was evaluated using the two most effective PL mycelium aqueous extract concentrations (PL1, PL2). After 48 hours of proliferation, different treatments were administered for six days as follows: fetal bovine serum (FBS) + horse serum (HS); PL1 + HS; PL2 + HS. Cell proliferation was assessed using the Alamar Blue assay. PCR was performed to analyze genes involved in proliferation (Cyclin B1 and D1, CDK4) and differentiation (Myh2, MyoG, MRF4, Desmin). Protein content of the two mycelia was 21.50% for PL and 19.80% for M. PL mycelium exhibited antioxidant capacity (243.97±18.03mg Trolox Equivalent/100g and 61.94±1.96mg Acid Ascorbic Equivalent/100g for ABTS and FRAP respectively). PL ACE inhibitory capacity was 38.90±4.57%. PL extract enhanced C2C12 proliferation at concentrations from 0.8 to 0.1mg/ml. These findings suggest that fungi extracts, rich in bioactive compounds, could enhance C2C12 growth. Funding: PRIN – Bando 2022. Prot. 20229S4T77, Cellular agriculture for sustainable and innovative food production “CELLtoFOOD” (Milan, Italy) – NRPP Mission 4 Component 1 code PE000000003

Co-expression network analysis evidenced relationship between lncRNA and milk leukocyte populations in cows with subclinical mastitis

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Long noncoding RNAs (lncRNAs) are a class of endogenous noncoding RNA molecules that constitute a significant portion of the transcriptome and may influence inflammatory and immune processes, such as those involved in bovine mastitis. This study aimed to identify regulatory networks of co-expressed genes and lncRNA associated with milk immune cells subpopulations in dairy cows having subclinical intramammary infection (sIMI). Two experimental groups were defined after a first bacteriological screening (n=188) as follows: cows with no history of mastitis and negative at the bacteriological examination (NEG; n=9) and, (ii) positive animals for two different pathogens (*S. agalactiae* and *Prototheca* spp.; sIMI; n=22). RNA sequencing (MGISEq platform) was conducted with a paired-end approach and lncRNA were predicted using FEELnc. Weighted gene co-expression network analysis (WGCNA) was conducted to identify differentially co-expressed modules of genes and lncRNA in NEG and sIMI animals and their associations with leukocyte subpopulations (i.e. total leucocytes, PMN, macrophages, T-helper cells, T-killer cells and B cells) measured with flow cytometry. Results showed 7 modules significantly correlated with total leucocytes, 7 with B cells, 6 with PMN, 8 with macrophages, 16 with T helper cells and 19 with T killer cells. Notably, the blue module ($P < 0.01$) had a strong positive correlation with T helper cells ($r = 0.8$) and a moderate negative one with PMNs ($r = -0.57$). This module contained 26 and 21 significant genes for T helper cells and PMNs, respectively, along with two lncRNAs. Interestingly, TCONS_00158275 targeted SRSF4, a gene linked to mitochondrial functions and immune energy metabolism. Functional analyses of the differentially co-expressed modules will further clarify sIMI regulatory mechanisms in dairy cattle. Acknowledgments: This work was part of the Agritech National Research Center funded by the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPO-NENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022) and of 2024DAFNAEIDOR-00172 funded by University of Padova.

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Theatre 2

Calves and their consideration in genetic evaluation in Fleckvieh

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Calves and youngstock have long been more or less neglected in cattle breeding. Mostly, only calving ease – directly affecting neonatal survival – and stillbirth have found their way into breeding goals. More recently, calf and youngstock survival and health have also gained importance. In Austria, genetic evaluations for calving ease (for first and higher lactations) and stillbirth (born dead or death within 48h), both direct and maternal, were introduced for all dairy and dual purpose breeds in 1995 and 1998, respectively. In Fleckvieh, heritabilities (h^2) are between 0.04 and 0.08 for calving ease and are 0.01 for stillbirth. Genetic correlations within direct and maternal calving ease traits are 0.75 and 0.90, respectively, and between direct and maternal traits they range from -0.03 to -0.35 (calving ease) and are -0.01 for stillbirth. In 2016, the calf vitality index, including stillbirth and rearing losses, i.e. deaths up to 30 days (both sexes) and from 31 days to 10 months (males) or 15 months (females), was implemented in the then joint Austrian-German-Czech genetic evaluation. Heritabilities for rearing losses range from 0.01 to 0.02, genetic correlations between these traits are around 0.50. In 2021, a single-step genomic evaluation was established for these traits. In Fleckvieh, the number of difficult calvings has been markedly reduced, especially in first calving cows. This is not only the result of considering calving ease in the breeding goal but also of avoiding bulls with lower calving ease direct breeding value when mating with heifers. Slightly positive and stable phenotypic trends have been observed for stillbirth and rearing losses, respectively. The genetic trends are clearly positive for both calving ease and the vitality index. In the future, calf and youngstock health traits, also using other data sources such as slaughter houses and rendering plants, should be incorporated into routine evaluation in order to improve health and further reduce early age losses.

Genetic relationship between environmental variability at birth and pre-weaning weights in a selected population of Iberian pigs

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Selection for uniformity has benefits in terms of productivity and animal welfare. Since 2022, a selection experiment has been carried out in a Sánchez Romero Carvajal Iberian pig population. Two generations of selection have been performed until now to reduce the environmental variability of the birth weight (BW). The experiment design includes two replicates (154 sows and 14 boars in each one in the design) per discrete generation. The mating is optimised to maximise the genetic response while maintaining the global relationship in the offspring in each replicate. The objective of this study was to analyse the expected correlated response in the environmental variability of pre-weaning weights (W21) in this Iberian pig population selected for birth weight uniformity. The data included 3569 piglets from 637 primiparous sows having both weights (BW and W21). The pedigree records contained 6075 individuals. The model included age of the sow (345-481 days), sex (2 levels), the batch (7 levels), litter size at birth (10 levels), and the age (for the later weight) as fixed effects. The trait was assigned to the mother and same effects were fitted affecting the trait and its variability. The bivariate analysis was done using ASReml 4.4, solving with the double hierarchical generalized linear model. The genetic variance for the environmental BW variability was 0.51 ± 0.06 and 0.20 ± 0.04 for W21. The genetic correlation between BW and W21 mean traits was high and positive (0.62 ± 0.04). Regarding the genetic correlation between mean of both traits and its variation was negative for BW (-0.20 ± 0.07) and not different from zero for W21 (-0.03 ± 0.09). The genetic correlation between both environmental variabilities was 0.91 ± 0.06 indicating that selection for BW uniformity would also imply homogeneity in later pre-weaning weights. This study was funded by a grant from the Spanish Ministry of Science, Innovation, and Universities: PID2023-149012OB-I00 (RoPig).

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Theatre 4

Leveraging Genomic and RNA-seq Data to Decode Dynamic Genetic Basis Underlying Cattle Feed Intake across Lactation

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This study aims to integrate genome-wide association (GWA) with RNA-seq analyses to identify temporal genomic variants and transcriptomic patterns across lactation associated with cattle feed intake (FI). An experimental herd of UK Holstein cows has been recorded for FI since 1970s, accumulating >700K daily FI records for 7,500 lactations of 2,300 cows with genotype information. Total RNA-seq from blood were obtained from 121 individuals, with 90 individuals sampled at two different lactation stages. To study the genetic basis underlying FI across time, data were split into primiparous and multiparous populations, and into early lactation stage (weeks 1-8) and later lactation stage (weeks 9 onwards). GWA was used to identify genomic variants associated with FI in primiparous/multiparous cows and at different lactation stages, identifying candidate genes associated with FI across time. Using RNA-seq data, we investigated temporal gene expression patterns of candidate genes in regions associated with FI from GWA by comparing expression levels between RNA-seq samples obtained during and outside specific lactation stages, and identified significant differences in expression levels using t-tests. We also performed differential expression gene analyses for all genes in the cattle genome to identify genes with significant temporal changes in expression. These genes underwent gene ontology enrichment and pathway analyses. Our multi-omic analyses provide molecular evidence of the change in the genetic basis of cattle FI across lactation. Temporal genomic variants associated with FI were identified with their transcriptomic patterns investigated, decoding the molecular mechanisms underlying FI. Our findings also highlighted the general change of gene expression in the cattle genome linked to lactation. To conclude, this study identified biomarkers associated with FI and provided evidence of gene expression changes linked to lactation. We acknowledge funding from the Biotechnology and Biological Sciences Research Council (BBSRC) BB/X009505/1.

Discovery of deleterious genetic variants in candidate genes influencing bovine paratuberculosis resistance or susceptibility using transcriptomics

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Bovine paratuberculosis (PTB) is a chronic granulomatous enteritis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), and leads to significant losses in the dairy industry worldwide. Genetics plays a crucial role in the response to MAP infection, making genomic selection a viable strategy for controlling PTB. Although several studies have identified single nucleotide polymorphisms (SNPs) associated with PTB susceptibility and resistance, many of these SNPs are in non-coding regions, making their functional significance difficult to interpret. RNA-Sequencing (RNA-Seq) is a powerful tool for identifying SNPs in coding regions (cSNPs), which can alter protein sequence and potentially play a crucial role in disease susceptibility or resistance. In this study, RNA-Seq data from blood and ileocecal valve tissue samples collected from Holstein cattle with focal and diffuse PTB-associated lesions, as well as cattle without gut tissue lesions, was analyzed to identify deleterious cSNPs unique to each group of animals. SNP calling was performed using BCFtools, applying a stringent SNP filtering pipeline to ensure high-quality variant detection. To assess the functional impact of the identified variants, Variant Effect Predictor (VEP) and ClusterProfiler were employed for variant effect prediction and functional analysis of candidate genes affected by deleterious variants, respectively. In total, 856, 625, and 603 cSNPs were identified in control cows, and in cows with focal and diffuse lesions, respectively. Among them, 31, 15, and 31 were predicted to have deleterious effects. Pathway enrichment analysis revealed significant enrichment in genes involved in the regulation of apoptosis and lipid biosynthesis in cows with focal lesions. Genes involved in antigen recognition and presentation were enriched in cows with diffuse lesions and without lesions. Targeting these deleterious cSNPs could facilitate the development of rapid multiplex cSNP-profiling assays, enhancing genetic selection for resistance to PTB.

Session 42

Theatre 6

Potential for genomic selection against bovine spastic syndrome (Crampy)

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Bovine spastic syndrome (Crampy) is a genetic neuromuscular disorder where affected cattle (≥ 2 years of age) experience uncontrollable muscle spasms in their hindlimb(s) when attempting movement. This study aimed to evaluate the potential of genomic selection against Crampy through genetic parameter estimation, identifying associated genomic regions, and determining the relationship with current evaluated traits by estimating breeding value correlations. Data from 678 dairy herds with at least one recorded case of Crampy (totalling 1,952 cases) were analyzed. The observed scale heritability was estimated to be 0.068 ± 0.01 using a linear model. This estimate was slightly lower than the threshold model observed scale estimate of 0.085, based on a prevalence of 3.6%. In a genome-wide association study, 41 SNPs were significantly associated with Crampy, revealing the polygenic nature of the disorder and indicating marker-assisted selection is unfeasible. Low favourable breeding value correlations suggest that current selection strategies may reduce the probability of Crampy indirectly and evaluated traits will not be negatively affected if selection against Crampy was implemented. Overall, this study highlighted that selection against Crampy could be achievable and other breeding goals may not be greatly impacted.

Reality check: how well do estimated breeding values predict litter uniformity and phenotypic traits

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Increasing litter sizes in pigs are associated with lower birth weights and increased within-litter variation, which pose challenges to pig farming. The objectives of this study were to 1) evaluate the stability of within-litter body weight uniformity over time and 2) research the relationships between estimated breeding values (EBVs) for uniformity and survival of Piétrain boars and the observed within-litter uniformity and mortality rates of their crossbred progeny. We analysed data of 1292 liveborn piglets from 86 hybrid sows mated to six selected Piétrain boars with contrasting EBVs for uniformity and survival (3 high versus 3 low). The coefficient of variation (CV) decreased from birth to the finishing phase, with considerable reranking of litters around weaning. Low correlations coefficients were observed between the CV at birth and the CVs at the other timepoints between weaning and the start of the finishing phase ($r = 0.049$ to 0.13). On the other hand, high correlation coefficients were found among CVs from weaning to the start of the finishing phase ($r = 0.63$ to 0.87), indicating stability in post-weaning within-litter uniformity. These findings suggest that pre- and post-weaning within-litter uniformity should be considered as two distinct traits. Weak relationships were observed between within-litter uniformity at birth and EBVs, this might imply that EBVs for uniformity and survival of terminal sires have a low predictive value and accuracy for within-litter uniformity at birth, slowing down genetic progress. Nonetheless, significant associations were found between EBVs and specific survival- and uniformity-related traits (e.g., pre-weaning survival and uniformity of growth), supporting their potential for selection.

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Theatre 8

Genetics of iris pigmentation in Swiss pig breeds

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Eye color in pigs is known to be variable with a complex genetic underpinning, but its genetic basis remains largely unexplored. This study investigates the genetic architecture of eye color in Swiss Landrace and Swiss Large White pigs by estimating its prevalence, heritability, genetic correlations and by identifying associated genomic regions. Eye color was phenotyped in 851 Swiss Landrace and 312 Swiss Large White pigs with known ancestry using a scoring scale for each individual eye (dark brown, medium brown, light brown, pale). Moreover, heterochromia iridium (different colored eyes) and heterochromia iridis (different colors within one eye) were recorded separately. Genotype data were available for 704 pigs (77,056 SNPs). After quality control, 675 pigs and 32,175 SNPs remained for analysis. Genetic parameters were estimated using a Bayesian threshold model (gibbsf90+) using both pedigree and genomic data. The SNP genotypes were imputed to the whole-genome sequence level and a genome-wide association study (GWAS) was conducted using GCTA. The Prevalence of brown eyes was 65.7% in Swiss Landrace and 78.2% in Swiss Large White, while heterochromia iridium was notably higher in Swiss Landrace (19.0%) compared to Swiss Large White (6.4%), similar to previous studies in other pig breeds. Pale eyes (7.8% and 5.4%) and heterochromia iridis (7.5% and 10.0%) had a low prevalence. Heritability estimates ranged from 60-85% (Swiss Landrace) and 42-92% (Swiss Large White), indicating a strong but complex genetic basis. Dark brown eyes were negatively correlated with pale eyes ($rg = -0.65$) and heterochromia patterns ($rg = -0.81, -0.63$). A preliminary GWAS identified a significant SNP on SSC4 (86.5 Mb) associated with pale versus dark brown eyes. This study provides the first comprehensive genetic analysis of iris pigmentation in Swiss pig breeds. The high heritability and strong genetic correlations suggest a common but complex genetic basis of iris pigmentation patterns. The high prevalence of heterochromia iridium in Swiss Landrace is an intriguing finding warranting further investigation. Future work will prioritize candidate genes and assess potential selection implications.

Using Encrypted Genotypes and Phenotypes for Reproducible and Collaborative Genomic Analyses to Maintain Data Confidentiality

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With decreasing genotyping costs, genome-to-phenome analyses have gained in popularity. For collaborative studies and reproducible research, data owners may prefer or are required to keep their data private due to privacy concerns, commercial interest, or data sharing policies. Different methods have been proposed to protect genetic information, such as deidentification and encryption. However, most of these have their shortcomings, as information about individuals may still be inferred from unencrypted genotypes, and encryption schemes often require genomic data to be decrypted before analysis. Homomorphic encryption allows specific analyses to be performed on encrypted data without affecting results, decrypted if necessary. Here, we extend homomorphic encryption for genotypes and phenotypes (HEGP), which applies homomorphic encryption to genomic and phenomic data, to wider applications. We show that HEGP can be applied to many popular mixed models, beyond single-marker regression, including Bayesian variable selection methods, that are routinely employed in animal and crop improvement, for quantitative genetic analyses, including genetic parameter estimation, genomic prediction, and GWAS. By testing HEGP on both simulated and real data from pigs, we evaluate the correlation between estimates of marker effects and breeding values based on the encrypted and unencrypted data. We also compare GWAS results from encrypted and unencrypted data, using Bayesian variable selection methods. We demonstrate that HEGP successfully obscures information about individuals for data privacy, without affecting estimates of marker effects using the encrypted and unencrypted data, without the need for decryption. Funding from AG2PI Seed Grant 2020-70412-32615 and 2021-70412-35233, and USDA-NIFA AG2PI grant 2023-70412-41054.

Session 42

Theatre 10

Identifying influential observations in variance component estimation: A Gamma regression equivalent to REML

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Variance components are fundamental parameters in animal breeding that quantify genetic and environmental sources of variation; they provide the basis for heritability estimates and estimations of breeding values in genetic evaluations. While Restricted Maximum Likelihood (REML) is the gold standard for variance component estimation, the resulting estimates can be difficult to interpret in terms of which observations are influential. Contrast this with standard linear regression, where tools like leverage and influence diagnostics readily identify observations driving parameter estimates: equivalent diagnostics for variance components in mixed models are mostly lacking. In this study, we exploit a little-known equivalence between REML and a Gamma generalized linear regression on phenotypic contrasts, which holds for mixed effect models with two variance components (e.g., genetic and residual). This equivalence provides a framework to identify the groups of observations that push estimates upward or downward. We demonstrate the approach using practical examples, showing how outlier identification can improve the robustness of genetic parameter estimates. The computational feasibility of this method and potential extensions to more complex covariance structures are also briefly discussed. This alternative formulation for REML offers practical tools for animal breeders and other animal scientists to better understand the influences behind their variance component estimates.

Exploring Lactation Number-Dependent Energy Balance Regulation in Dairy Cows: RNA-Seq Profiling Reveals Novel Differentially Expressed Genes as Potential Biomarkers

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Introduction: Negative energy balance (NEB) in early-lactation dairy cows risks health and productivity, but its genomic mechanisms are unclear. This study used RNA sequencing to identify genetic markers and pathways linked to energy homeostasis in Holstein-Friesian cows across lactation stages (1–5). **Material and Methods:** Seventy-four cows were allocated into four groups based on lactation number, pre-trial milk yield, body condition score (BCS), and dietary crude protein levels. Daily measurements of weight, milk composition, feed intake, and energy balance were recorded. Blood sampled 4–5 weeks postpartum underwent RNA extraction (Qiagen RNeasy Plus Universal Mini Kit) and sequencing (Illumina TruSeq Stranded mRNA kit). Reads were quality-checked (FastQC), trimmed (Trimmomatic), and aligned to ARS-UCD1.2 (STAR-2.7.11b), with differential expression analyzed via DESeq2. **Results and Discussions:** High-quality RNA (80.23% alignment rate) yielded 20,958 genes post-normalization. Twenty-six genes were differentially expressed (DEGs; adjusted $p < 0.05$), showing lactation-dependent trends: 14 upregulated and 12 downregulated in multiparous (lactation ≥ 3) versus primiparous cows. Parity-specific DEGs included COL1A1 (young: lactation 1–2); SLIT3, TGM3, LOC524810, ACVR1B (medium: lactation 3); and NOX3 (old: lactation 4–5). Functional annotation linked DEGs to lipid/glucose metabolism (PCK1, ACSL1), ECM-receptor interaction (COL1A1, COL18A1; $p = 9.1 \times 10^{-3}$), and PI3K-Akt signaling (PTPRS; $p = 0.11$). Pathway enrichment implicated glycogenolysis, insulin, MAPK, Wnt, and TGF- β cascades, highlighting lactation-stage-specific adaptations to NEB. **Conclusion:** These findings propose novel biomarkers for energy balance regulation, though tissue-specific validation (e.g., liver, adipose) is warranted to address limitations of blood transcriptomics. This work advances genomic strategies for improving feed efficiency and sustainable dairy production.

Session 42

Poster 12

Genetic analysis of the service sire effect on litter size and its relationship with semen quality

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This study aimed to estimate the genetic parameters for service sire effect on the number born alive (NBA) and its correlation with semen quality traits. Data of 7K, 23K, and 49K litter size records collected between 2020 and 2024 from three purebred terminal sire lines were analyzed. The number of sows was 3K, 12K, and 24K, with 235, 554, and 891 service sires for the three respective lines. There were 1M, 4M, and 2M animals in the pedigree of which 72K, 250K, and 350K were genotyped and imputed up to 50K SNPs. The service sire and sow effects were modeled as additive genetic effects, considering a covariance structure among them, given by the relationships matrix. The model also included fixed effects of contemporary group and parity and random permanent environmental effect. Genetic parameters were estimated using the AIREML option in the BLUPF90+ software, and GEBVs were generated by ssGBLUP with the algorithm for proven and young (APY). Heritability for the service sire effect ranged from 0.01 to 0.03, and the heritability for the sow effect ranged from 0.09 to 0.15, with genetic correlations ranging from -0.20 to 0.34. A single-step genome-wide association study (ssGWAS) was also performed, and no strong signals were detected for service sire effects on NBA. Sperm motility and abnormal morphology GEBVs from the three lines were estimated based on about 57K to 630K ejaculates recorded using the computer-assisted semen analysis (CASA) system from 1.8K to 30K boars. Heritabilities ranged from 0.11 to 0.13 for motility and from 0.15 to 0.26 for morphology. The correlations between service sire GEBV for NBA and semen quality were low but in the favorable direction. Results suggest that although paternal genetic contributions to litter size were small compared to maternal genetic contributions, selecting on service sire effects on litter size in addition to semen quality traits will improve overall reproductive success.

The persistency of estimates of SNP effects can speed up routine single-step genomic evaluations

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Single-step genomic evaluations are widely used in animal breeding programs because these models analyze phenotypic data from both genotyped and ungenotyped animals simultaneously. The accumulation of data over time poses computational challenges for routine single-step genomic evaluations, particularly those with short time windows. However, new data added to a previous evaluation represent only a small fraction of the entire dataset, resulting in minimal changes to most estimates. This study investigates firstly the persistency of estimated SNP effects in routine single-step evaluations using one dairy cattle and two pig datasets, and secondly, the potential of a novel approach, called Direct Genomic Values-Pedigree BLUP (DGV-PBLUP) to accelerate single-step genomic evaluations. The results show that estimates of SNP effects exhibit minor changes between subsequent evaluations, spanning up to 4 months, with high correlations (>0.99) across all traits. The observed allele frequencies also remain stable over time, with correlations >0.999 . Based on these results, we developed the DGV-PBLUP approach, which uses estimates of SNP effects from a previous evaluation to compute DGVs and integrate them into a pedigree-based BLUP. When applied to the datasets, DGV-PBLUP yields similar genomic estimated breeding values to a single-step evaluation using the whole dataset, with correlations close to 1, even for young animals and after 2 years of new data in dairy cattle. Based on these results, we conclude that the DGV-PBLUP is a viable option for reducing computational costs and timings on routine genomic evaluations. Indeed, it will provide the same genomic estimated breeding values as a single-step genomic evaluation using the whole data, but with similar computational loads as a pedigree-based evaluation.

Session 42

Poster 14

Using CADD scores as prior information to improve genomic prediction accuracy in mice

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Although standard genomic prediction (GP) models such as GBLUP assume that genomic markers contribute equally to genetic variation, it does not match the reality. Some genomic markers are more informative than others because they are more closely linked to causal variants. GP models could therefore be finetuned by incorporating biological information about the impact of genomic markers. One approach for this is to use Combined Annotation Dependent Depletion (CADD) scores, which reflect the likelihood of a genetic variant being deleterious. Our objective was to determine the benefit of including CADD scores as prior information in genomic prediction. We analyzed 10 traits in a mouse population, and included CADD scores through either 1) selecting genomic markers based on different thresholds of CADD scores, 2) weighing genomic markers based on CADD scores, or 3) both. We used two different types of CADD scores (CADD-marker and CADD-window) for the above procedures. With CADD-marker, we used the CADD score at the position of a genomic marker, and with CADD-window, we used the average CADD score within a ~5 MB flanking region around a genomic marker, representing a window of strong linkage disequilibrium. We employed five GP models including GBLUP, BayesA, BayesB, BayesC, and BayesR. For weighing genomic markers, we employed a weighted GBLUP model. The results showed that using CADD-marker to select genomic markers did not improve prediction accuracy across all scenarios and GP methods. Compared to using all genomic markers, selecting the top 50% of genomic markers based on CADD-window performed best among all scenarios. In this scenario, all five GP models improved prediction accuracy for at least 7 out of 10 traits, with improvements ranging from 0.031 (BayesC) to 0.037 (BayesB). Weighing (selected) genomic markers based on CADD-window had little impact on accuracy for all traits, with changes in accuracy ranging from -0.005 to 0.009. In conclusion, using CADD-window scores to select genomic markers improved prediction accuracy, but the benefit depended on the trait of interest. Using CADD scores to weigh genomic markers did not improve prediction accuracy.

The development of a single-step genomic evaluation system for dairy cattle in the Walloon Region of Belgium

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For several years, the Walloon Region of Belgium provides dairy cattle breeders with locally estimated breeding values for important traits. These evaluations allow Wallonia to participate in the international MACE evaluations performed by INTERBULL. In current genomic evaluations, genomic and pedigree information is then combined with this external information (international EBV from MACE, "MACE_EBV") in a pseudo-single-step genomic evaluation, resulting in genomically enhanced EBV (GEBV). However, these pseudo-single-step evaluations result in potential biases. Therefore, the aim of this study was to implement a single-step genomic BLUP (ssGBLUP) that combines simultaneously all available national information with MACE information, and to validate it using milk production traits. Briefly, the new strategy first consists in defining "pseudo-traits", reflecting MACE-traits that are 305-day across the first three lactation averages for milk, fat, protein yields. MACE information is therefore converted into adjusted pseudo-phenotypes and deregressed effective contributions, avoiding double-counting of Walloon information contained in MACE_EBV. Second, the variance-covariance matrices of the national test-day random regression model was adapted to include the 3 MACE pseudo-traits as traits correlated to the different Legendre polynomials. Finally, an ssGBLUP is performed to analyze simultaneously test-day records and MACE pseudo-phenotypes. This integration of MACE information followed the strategy developed for integrating international EBV and associated REL in beef Italian Limousin evaluation. Validation of pedigree and ssGBLUP were conducted using data from official Walloon genetic evaluations of April 2022. Pearson correlations among MACE EBV and (G)EBVs with integration were close to 1 (0.95 – 0.97) in all cases, demonstrating the robustness of this new method. Next a linear regression validation approach will be used to perform a cross validation on the calculated values.

Session 42

Poster 16

Maturity is associated with casein alpha S1 region in Saanen and Alpine goats

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Maturity, defined as the ratio of milk yield (MY) in first parity to subsequent parities (e.g., MY in first parity divided by MY in second parity), is a trait linked to functional longevity in French dairy goats. The genetic correlation between maturity estimated breeding values (EBV) and longevity EBV is -0.5, highlighting its potential in selection objectives for Alpine and Saanen breeds. This study aimed to identify genomic regions associated with maturity. The dataset included MY records from 175,572 Saanen and 246,504 Alpine goats collected in France between 1990 and 2023. The goat *as1* casein gene is a complex gene: alleles A, B, C associated with increased levels of *as1* casein were coded as « strong effect (S) », allele E as « intermediate effect (I) », allele F were coded as « reduced effect (R) ». Genomic evaluation was performed using BLUPF90+, and the variance explained by 1 Mb windows was calculated with POSTGSF90 software following ssGBLUP. A 1 Mb region on chromosome 6 demonstrated the strongest association with maturity, explaining 3% of the total variance in Saanen and 1% in Alpine. One of the top SNP (snp59427-scaffold980-309132) at position 85,994,216 is located within the CSN1S1 gene. Genotypic frequencies among Saanen AI bucks (N=467) was 15% AA, 55% AG and 30% GG, while in Alpine (N=613), it was 53% AA, 41% AG, and 6% GG. The alpha S1 casein allele associated with each genotype at the significant SNP were in Saanen: AA to homozygote S (75%), AG to heterozygote S-I (87%), and GG to homozygote I-I (94%). Maturity EBVs were highest for AA genotypes, intermediate for AG, and lowest for GG in both breeds. While longevity EBVs did not vary significantly among genotypes, bucks with AA genotype had higher EBVs for fat and protein contents compared to AG and GG genotypes. Notably, GG bucks exhibited higher MY EBVs in both first and sixth parities, with greater differences observed in later parities. This study underscores the genomic influence on maturity and provides insights for integrating this trait into breeding programs to enhance longevity and production efficiency in dairy goats.

Estimation of Genetic Parameters for Production and Somatic Cell Score traits in Korean Holsteins Using a Random Regression Test-Day Model

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In South Korea, the animal model has been used for the national genetic evaluation of milk yield (MY), fat yield (FY), protein yield (PY), and somatic cell score (SCS) traits in Korean Holsteins since 1995. However, many countries have adopted the random regression model (RRM) for more accurate genetic evaluations. This study aimed to estimate variance components to apply an RRM for the national genetic evaluation of production and SCS traits in Korean Holsteins. Test-day records from first to third parity were used, comprising 847,196 test-day records from 42,963 animals across 1,216 herds, with birth years ranging from 2003 to 2023. Variance components were estimated using two separate Bayesian analyses (gibbsf90+ software): One for MY, FY, and PY traits, and another for the SCS trait. Model comparison based on the ratio of Mendelian sampling variance to the additive genetic variance and other criteria revealed that the model including the Herd-Test-Day factor performed worse than the other models. In contrast, the most optimal model included Parity-Year-Month of calving, Parity-Age-Season of calving, Parity-Herd-Year-Season of calving, additive genetic, permanent environmental, and heterogeneous residual effects. In the best model, the average daily heritability estimates for MY across the first three parities were 0.28 (0.01), 0.29 (0.01), and 0.28 (0.01), respectively. For FY, the estimates were 0.23 (0.01), 0.28 (0.01), and 0.28 (0.01), while for PY, the estimates were 0.26 (0.01), 0.29 (0.01), and 0.29 (0.01). For SCS, the estimates were 0.17 (0.01), 0.22 (0.01), and 0.23 (0.01), respectively. The genetic correlations between MY and FY across parities ranged from 0.38 (0.02) to 0.65 (0.01), while those between MY and PY ranged from 0.63 (0.02) to 0.88 (0.00). The genetic correlations between FY and PY ranged from 0.52 (0.01) to 0.77 (0.01). These results will be considered for application in the national genetic evaluation of production and SCS traits in Korean Holsteins.

Session 42

Poster 18

Genomics meets Precision Livestock Farming: combining omics and sensor-based phenotyping in dairy cattle

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The integration of advanced technologies in livestock farming offers new opportunities to improve animal welfare and productivity. This study, within the NextGenerationEU framework of the "Next Generation Animal Production" project, explores the combination of thermal imaging, video surveillance, pedometers, and genomic data to monitor dairy cattle behavior and health. The objective is to develop an integrated system using multiple data sources for precision phenotyping, early health detection, and breeding optimization. Thermal cameras and video surveillance capture physiological and locomotion data, while activity sensors track movement patterns. Phenotyping will include 900 Holstein cattle from four Croatian farms. Genotyping with a 700K SNP array will enable analysis of genomic variability linked to analysed traits. In addition to linear models, machine learning will identify key indicators of disease susceptibility and welfare. Preliminary results show significant correlations between genomic markers and behavioral traits, suggesting a genetic basis for activity levels and stress resilience. Certain allele variants are associated with these traits, emphasizing the potential of genomic selection to improve welfare and productivity, while integrating sensor data facilitates early detection of behavioral anomalies. This study highlights the potential of integrating genomics and digital phenotyping in future breeding programs.

The Țigaie Ruginie sheep breed prolificity and its genetic consolidation in the Transylvanian plateau population

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Prolificity in sheep is influenced by several genes as well as other factors. In the present study, we aimed to perform a phenotypic (calvings on the interval 2021-2024) and a genotypic analysis within the Țigaie Ruginie breed population (96 individuals), located in the Transylvanian plateau. The genomic analysis method used was Axiom™ Ovine Genotyping Array (50K), we performed the analysis of a number of 50k SNPs in relation to economic/health traits. For phenotypic characters, 4 categories of variables were identified, lambing with single, twin lambing, abortion, and infertile mating. Genomically, a number of 693 exomic genes were identified at the level of all 26 autosomal, X mitochondrial chromosomes. Mutations were identified on genes directly involved in prolificacy and/or in relation to prolificacy traits: BMP15/Chr.X; DLG1/Chr.1; GDF9, GDF9/Chr.5; NR5A2/Chr.12. Genotype frequencies were: GDF9-SNP1 was monomorphic; GDF9-SNP2, TC-60%, TT-10%, CC-30%; NR5A2, CC-85.5%, CT-14.5% and DLG1, GG-90%, GA-10%. The association analysis was performed between fertility genotypes and the phenotypic traits. The genotypes with the highest frequency were associated with the highest number of twin lambings g in all analyzed genes. This phenotype/genotype association analysis identified favorable genotypes that can give rise to twin lambings in the Tigaie Ruginie breed. These genotypes could be used in genomic selection applied for the next offspring generations.

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Poster 20

Estimation of genetic parameters for performance traits in Italian beef cattle breeds via a multi-breed multi-trait approach

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Marchigiana (MAR), Chianina (CHI), and Romagnola (ROM) are three Italian autochthonous beef cattle breeds that have been historically selected for meat production. Recent advancements suggest that using genomic data, and multi-breed and multi-trait models to combine information from different populations or phenotypes may help to increase selection accuracy. This work aimed to estimate the genetic components of average daily gain (ADG), weight at one year (WEI), and muscularity (MUS) recorded in performance test for the three breeds. A total of 1092 MAR, 1118 CHI, 1110 ROM phenotyped and genotyped (33K SNPs) bulls, and their pedigrees (11868 animals in total) were available. The three traits were considered either as different or as correlated across the three breeds. Contemporary groups and farm of origin were added as fixed effects for all the traits, while weight at beginning of performance test and age in weeks when muscularity was evaluated were added as covariates for WEI and MUS, respectively. Genetic parameters were estimated through Gibbs sampling with Gibbsf90+ software using a single-step relationship matrix. Results highlighted moderate heritabilities in MAR (ADG 0.35; WEI 0.39; MUS 0.37) and ROM (ADG 0.42; WEI 0.49; MUS 0.44) while CHI showed the lowest heritabilities (ADG 0.16; WEI 0.23; MUS 0.34). Genetic correlations within-breed across-traits were moderate to high in MAR and ROM (0.46-0.87); while CHI had a negative genetic correlation of -0.28 between ADG and MUS. Across-breed, within- and across-traits, genetic correlations were negative between MAR and CHI, but overall positive between CHI and ROM. These findings highlight the potential benefits of exploring new selection models in the three Italian beef cattle breeds that can leverage all available recorded data through a multi-trait multi-breed approach.

Identifying QTL for hoof and leg conformation in Swiss dairy cattle

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Objectives: To evaluate the h^2 of hoof and leg conformation traits and to identify underlying QTL and candidate variants in Swiss Holstein (HO) and Brown Swiss (BS) cattle. **Materials & Methods:** The dataset included 115k HO and 75k BS animals. Microarray-derived genotypes, own performance records and dEBVs for hoof and leg conformation traits were provided by the Association of Swiss Cattle Breeders. For HO, analyzed traits included bone quality (BQ), rear legs rear view (RLRV), rear legs side view (RLSV), heel depth (HDE), hoof angle (HAN), and locomotion (LOC), and for BS, traits examined were RLRV, RLSV, HDE, and HAN. The SNP data were imputed to the sequence level resulting in genotypes for 37M SNVs in HO and 26M SNVs in BS. A subset of 730k biallelic markers was extracted from the imputed data to conduct association tests. Own performance records were corrected for non-genetic factors with the lme4 R package. Analyses used the 730k SNPs, with variance component estimation and MLMA conducted using GCTA. Additive GWAS analyzed both, corrected phenotypes and dEBVs. Non-additive (dominant and recessive) models were applied on corrected phenotypes only. QTL-harboring chromosomes underwent a second MLMA incorporating all imputed SNPs. **Results:** The estimated h^2 using corrected phenotypic data for HO and BS ranged from 0.13 to 0.28 and from 0.10 to 0.26, respectively. In HO, nine additive QTL associated with hoof and leg conformation (LOC, BQ, HDE) were identified on chromosomes 5, 6, 7, 11, 14, and 18. Furthermore, the non-additive GWAS revealed a recessive QTL on chr 22 for BQ. In BS, GWAS identified additive QTL on chr 2, 13, 18, 25, and 26 for HDE and RLRV. **Conclusion:** This study confirmed moderate h^2 for hoof and leg traits in two cattle breeds. Our analyses revealed a genetic complexity underlying hoof and leg conformation traits in HO and BS cattle and identified several significant additive, but also for the first time non-additive QTL associated with these traits.

Session 42

Poster 22

Where to go: breeding for polledness in a Holstein breeders' association in Germany

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In the German Holstein population, breeding for polledness has picked up significantly since about 2015. In the past ten to fifteen years, genetically polled bulls have been used in ever increasing frequencies to raise the allele frequency of the polled allele and ultimately to spare the calves the procedure of dehorning. This means that in Germany, simultaneously with the introduction of genomic selection, a breeding strategy has been implemented with the aim of increasing the proportion of naturally polled animals without sacrificing breeding progress in the standard breeding traits of the breeding goal. Studies at a large German breeders' association (RinderAllianz) show that it is possible to maintain the doubling of genetic gain in the era of genomic selection as predicted by Schaeffer (2006) in relation to conventional breeding, even if a monogenic/qualitative trait is also to be accounted for. Quite naturally, the fact that the polled allele is dominant is an advantage in this process. At the start, the polled allele was present only in a very low allele frequency of rather homeopathic 0.35% in 2015. Since then this has increased to 11.5% for the 2023 birth cohort in the breeding association under consideration, which means that about 20% of a cohort is naturally polled. Regular studies for potential negative correlations seem to be necessary – such as the increased proportion of CDH carriers in polled allele carriers during this process, which led to the exclusion of genetically polled CDH carrier bulls. Although the Friesian variant of the polled allele and thus the phenotype or underlying genotype is almost perfectly determinable (Medugorac et al., 2012), this relationship must be tested regularly to ensure that such the rare variant as described by Brenig (2025), a calf of a PP polled bull was horn-bearing, does not occur more frequently and could lead to a reassessment of the association between presumed and actual status.

Data reduction strategies for large-scale pig genomic evaluations

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Increasing amounts of phenotypic and genomic data are collected for selection purposes in animal populations thanks to reducing genotyping costs and new phenotyping technologies. Over time, breeding organizations have accumulated large datasets that need to be analyzed for routine genetic evaluations, which typically include all or large amounts of historical data. Such large-scale datasets pose computational challenges as (genomic) estimated breeding values (EBVs) must be delivered within specific time limits. This study aims to investigate the impact of several data reduction strategies on the accuracy and computational costs of large-scale, single-step genomic evaluations in pigs. A large multi-trait dataset was available for a three-way crossbred pig population, including ~7.1M pedigree records, ~589K genotypes, and ~10.9M phenotypes across 11 traits (heritability, h^2 , ranging from 0.01 to 0.45). We implemented different scenarios aiming at reducing pedigree, genomic, or phenotypic data, or a combination of these. Scenarios were validated against a full evaluation using all data. Validation animals were selection candidates born in the last two years with their own phenotype and genotype. Results show that removing genotypes of animals without their own or progeny phenotypes had no impact on the EBVs of selection candidates (correlations $\rho \geq 0.99$, and regression coefficients $b1 \geq 0.97$). Progressively removing old phenotypic data showed to impact the EBVs of selection candidates when the ~3 most recent generations of phenotypes were retained, particularly for binary traits with low h^2 ($\rho \geq 0.87$ and $0.86 \leq b1 \leq 1.26$). Traits with higher h^2 showed minimal impact ($\rho \geq 0.99$ and $b1 \geq 0.97$). Retaining 3 generations of pedigree from phenotyped and genotyped animals, the 3 most recent generations of phenotypic data, and genotypes of animals with their own or progeny phenotypes gave consistent EBVs with the full evaluation, whilst reducing time and memory usage up to 10%. Scenarios that reduce further the number of generations and summarize the removed information through informed metafounders are under investigation.

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Poster 24

Genetic analyses of wool fiber quality traits in German Merino sheep

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Wool quality in German Merino sheep is diverse and often low, limiting its use in the textile industry. In the breeding goal, wool quality is included as a score, subjectively graded by an expert. However, objective wool fiber measures as selection traits are expected to improve wool quality. This study aimed to investigate the potential of several wool fiber measures for breeding in the German Merino population. Therefore, 1,360 German Merino ewes of the breeding nucleus were phenotyped for the three wool quality parameters fiber diameter (FDS, FDF, FDR), fiber crimp (CRS, CRF, CRR) and staple length (SLS, SLF, SLR) at three measurement points shoulder, flank, and rump. All animals were genotyped with ovine 50K SNP chips (Illumina, San Diego, CA). Heritabilities for all nine traits were estimated using mixed linear animal models. To estimate genetic correlations between the three measurement points, as well as among the three wool quality parameters, the model was extended to a trivariate approach. Age class (defined by the performance testing protocol) and farm were included as fixed effects based on Wald tests and backward elimination procedures. To infer the genetic background of wool quality, genome-wide association studies (GWAS) were conducted. Heritabilities were found to be significant ($p < 0.001$) and moderate to high (0.60, 0.53, 0.54 for FDS, FDF, FDR; 0.65, 0.58, 0.55 for CRS, CRF, CRR; 0.31, 0.41, 0.39 for SLS, SLF, SLR) with small standard errors (0.06). Genetic correlations between measurement points of a trait were high (≥ 0.9), showing no genetic differences. This implies that a single phenotyping measurement point is appropriate for wool quality traits. Fiber diameter and staple length showed moderate negative correlations with crimp (-0.43 to -0.67) but were not genetically correlated (-0.03 to -0.10) to each other, suggesting that selection based on fiber diameter does not adversely affect crimp or staple length. GWAS results revealed a polygenic trait architecture. These findings suggest potential for breeding towards enhanced wool fiber quality in German Merino sheep.

Paracrine Impact of Fibroblasts on Transcriptomic Changes in Bovine Muscle Satellite Cells in 2D and 3D Cell Culture Systems

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The role of fibroblasts in the proliferation and differentiation of muscle satellite cells (MuSC) – muscle precursors – is well known through cell-cell interactions. However, their paracrine effect on myogenesis still requires more extensive research. This study aims to examine changes in the transcriptome landscape of bovine MuSC co-cultured with fibroblasts during their differentiation in 2D and 3D cell culture systems. Our previous research confirmed the stimulatory paracrine effect of fibroblasts on the proliferation of MuSC. For this purpose, a 0.4 µm insert cell culture system was used to study paracrine signalling between cells in the 2D cell culture, analysed by RNA-Sequencing (RNA-Seq). Then, the three-dimensional (3D) culture was carried out based on a spinner flask culture system and gelatin microcarriers (CultiSpher, Sigma Aldrich). The gene expression of selected crucial genes was assessed by qPCR. RNA-Seq identified 1630 DEGs (differentially expressed genes) indicated as significant (FDR $P < 0.05$, log₂ fold change ≥ 0.5) in the 2D cell culture. Among DEGs, we selected 23 downregulated and 12 upregulated genes involved in myogenesis. ITGA7 showed the highest downregulation (log₂FC = -2.01), while the highest upregulation showed IL-6 (log₂FC = 2.66). Moreover, we noticed upregulation of genes promoting myogenic differentiation like HDAC4, EYA2, SOCS3, EGR1, while genes encoding cytoskeletal and structural proteins like DES, NES, LMOD1, TLN and ELN were downregulated. Gene expression analyses in the 3D culture confirmed the higher gene expression of IL6, EGR1, and genes from the Myogenic Regulator Family (MRF). Notably, ITGA7 expression was higher in the co-culture group under 3D conditions. To summarize, fibroblasts promote myogenic differentiation in conditions of initiating differentiation by medium composition in 2D and 3D cell culture systems. Applying the 3D cell culture technique is crucial, as it is more representative of the in vivo environment.

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Poster 26

Exploring Homozygosity and Genomic Inbreeding Effects on the Some Selected Traits of Dual-Purpose Simmental Cattle in Türkiye

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The objective of the present study was to investigate the patterns of runs of homozygosity (ROH) in Simmental cattle using the model-free approach on “detectRUNS” package in R Environment (MI), and the model-based approach on “bcftools” software (MII). The study focused on 3420 dual-purpose Simmental cows genotyped with 29866 SNPs, of which 2387 were born in Türkiye, and 413, 134, 330, 32, 124 were imported as heifer from 5 different European countries. Inbreeding depression (ID) was assessed by including genomic inbreeding coefficients FROH (FROH) as a covariate in GBLUP model on “BLUPF90+” for lactation milk yield (LMY), age at first calving (AFC), 25 body conformation traits (CONF). The total number of ROH regions detected was 337,390 and 209,289 for MI and MII, respectively. Identified homozygous regions were showed different patterns among countries in terms of distributions of common ROH islands ($>10\%$ of population). On the other hand, BTA6 (between 69.65 and 70.37 Mbp) was consistently found to be highly homozygous ($>34\%$ of population) which includes the KIT gene. ID were found as 0.72 ± 0.407 and 1.14 ± 0.405 days increase for AFC; -12.82 ± 6.541 and -9.75 ± 6.521 liters decrease for LMY using MI and MII, respectively. In addition, strong relationship has been detected between FROH and CONF traits, ID were ranged from -0.51 ± 0.0425 to 0.060 ± 0.0362 .

On the genetic architecture of the inbreeding load in the Rubia Gallega beef cattle population

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Inbreeding results from mating between related individuals and is associated with inbreeding depression. Recently, several models have been proposed to predict individual inbreeding depression loads, aiming to prevent undesirable matings and facilitate artificial purging. These models can be implemented using a single-step GBLUP approach, leveraging the equivalence between GBLUP and SNP-BLUP to estimate SNP effects and identify genomic regions contributing most significantly to the variance of inbreeding loads. In this study, we applied this approach to seven traits—Birth Weight (BW), Weaning Weight (WW), Cold Carcass Weight (CCW), Conformation (CONF), Fatness (FAT), Age at First Parity (AFP), and Calving Interval (CI)—in the Spanish beef cattle population Rubia Gallega. The number of phenotypic records ranged from 72,481 for AFP to 369,226 for BW. The analysis also included a pedigree of 522,885 individuals, with 4,984 of them genotyped using the Axiom_BovM-Dv3 array. The Mendelian decomposition of inbreeding resulted in 6,402,552 partial inbreeding coefficients derived from only 4,086 individuals. The variance of inbreeding load for an inbreeding coefficient of 0.10 ranged from 0.02 for BW to 0.19 for AFP. Correlations between additive genetic effects and inbreeding load effects were consistently negative, ranging from -0.19 for WW to -0.75 for FAT. Following the back-solving of SNP effects, differences were observed in the distribution of additive genetic variance and inbreeding load variance. The most significant genomic regions for additive genetic variance were identified on BTA2 and BTA6, containing the MSTN and LCORL genes. In contrast, the inbreeding load variance was unevenly distributed across the genome, highlighting notable regions on BTA2, BTA16, and BTA26. These regions included potential candidate genes such as MSTN, LDLRAP1, DUSP10, and ADAM12, which may be associated with individual inbreeding loads.

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Poster 28

Genetic analysis and GWAS of hoof pigmentation in Duroc pigs

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Hoof pigmentation (colour) is an important characteristic for the Spanish producers of cured hams and shoulders. A solid black hoof is one of the quality requirements for high-value product ranges. Within this local pork value chain, the Duroc breed has been used as the terminal sire line, and blackness of hooves has been used as a selection criterion for entering Iberian production. In the Hendrix Genetics Swine breeding program, hoof colour has been recorded subjectively along five classes on a percentage scale (0%, 1-25%, 26-50%, 56-75%, 76-100%). This study included 15K male Duroc pigs born between 2019 and 2024. Genetic analyses were performed to calculate heritability for hoof pigmentation, as well as genetic correlations with other production traits. Two transformations, i.e., 'white versus rest' (class 0% recoded to 1, others recoded to 0) and 'black versus rest' (class 76-100% recoded to 1, others recoded to 0), of the original phenotype values were investigated to improve estimates of heritability. The 15K animals also had genotype data available for 12K SNP markers covering the 18 autosomes, and a genome-wide association study (GWAS) was conducted to identify regions associated with hoof pigmentation. A subset (7K) of these animals had imputed whole-genome sequence data available for a high resolution GWAS. The estimated heritability of hoof pigmentation was 0.32, 0.25, and 0.20, for original phenotypes, white versus rest, and black versus rest, respectively. Genetic correlation of hoof pigmentation with other traits were low and varied between -0.07 (back fat) and +0.12 (daily gain). The GWAS identified peaks on chromosomes 1, 5, 7, 14, 15 and 17. The top 10 SNPs explained 13.4% of the phenotypic variance. The GWAS peak detected on chromosome 15 is placed near the OCA2 gene, which has been associated with pigmentation abnormalities in mice. These results suggest that selection for hoof pigmentation in pigs is feasible due to the amount of genetic variance present for that trait in this breeding population. The genetic correlation estimates indicate that selection for hoof pigmentation should not impact other relevant production traits.

Gene Ontology of candidate regions associated with Milk Coagulation Properties and Curd Firmness in Pag Island Sheep

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Milk Coagulation Properties (MCP) and Curd Firmness (CF) are critical for efficient cheese production. Following a previous Genome-Wide Association Study (GWAS) in Pag Island Sheep that identified genomic regions associated with MCP and CF traits, this study aimed to characterize the functional relevance of these regions through Gene Ontology (GO) analysis. Using 291 genotyped individuals and 1,173 milk samples, genes within the significant regions were annotated and functionally classified. A total of 1,311 genes were identified across seven MCP and CF traits. GO analysis revealed 21 parental GO terms, with seven shared between at least two traits, including ATPase activator activity, carboxylic ester hydrolase activity, and metalloproteinase activity. Overall, nine hub GO terms were detected, such as proteolysis, serine-type endopeptidase activity, and insulin-like growth factor binding, while only one hub gene, TGFBR1, was identified. Moreover, APOLD1, CADM2, and CDKN1B, along with PON1, PON2, and PON3, emerged as potential candidate genes influencing multiple traits. These genes and GO terms are involved in protein degradation, lipid metabolism, and vascular function, processes that may play a role in milk coagulation efficiency and curd firmness. The results provide valuable functional insights into the biological pathways underlying MCP and CF in Pag Island Sheep, supporting future genetic selection strategies for improved cheese production efficiency. Funding: European Union – Next Generation EU within “Next Generation Animal Production” (NPOO.C3.2.R3-I1.04.0141) and “Pheno-Geno-IP-2022-10-6914” funded by Croatian Science Foundation.

Session 42

Poster 30

Is indoor feed intake measured on cows with access to pasture genetically the same trait as feed intake of cows fed only indoors?

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In summer, grazing in combination with indoor feeding is common practice in Norwegian dairy production. Geno measure indoor feed intake continuously in some of the herds where the cows have access to pasture. The aim of this study was therefore to genetically compare the indoor feed intake measured during grazing season with the feed intake during the indoor-only season. A question of interest is whether feed intake records during summer months for grazing animals can be useful in genetic evaluation of feed intake. Data on the daily feed intake of 1 224 Norwegian Red cows, including both silage and concentrates, were collected between 2022 and 2024 from 9 herds using feed bins from Biocontrol®. For each herd, the grazing period was defined individually, ranging from May to September. In total, our dataset included 331 360 daily feed intake records, from which the following traits were analyzed; silage intake (SI), dry matter intake from silage (SIDMI), total dry matter intake from silage and concentrate (dDMI), and energy intake from silage and concentrate (EI). All traits were defined as different traits in the grazing vs indoor season and analyzed with bivariate linear mixed repeatability animal models. The results showed that the traits were heritable (0.09 – 0.17) and that genetic correlations ranged from 0.85 to 0.89 with standard errors from 0.06 to 0.11. The highest correlation was between dDMI measured during the grazing season vs the indoor season (0.89). The average dDMI intake when cows were on pasture was 11.69 kg of dry matter per day compared to 13.77 kg of dry matter per day for the indoor season. The strong correlations between the traits suggests that feed intake records during the summer months contributes with valuable genetic information for prediction of breeding values for feed intake in Norwegian Red dairy cows, and further work includes definition of feed efficiency in a sustainable breeding goal for this dual-purpose breed.

Breeding goals and more: the multiple links between livestock farming systems and genetics

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The call for agro ecological transition questions the design of breeding programs. At which conditions breeding programs can contribute to develop agroecology? In other words, how to define relevant breeding goals and relevant breeding programs for livestock farming systems responding to the principles of agroecology? A core question deals with the links between livestock farming systems features and the definition of breeding goals and breeding programs. In this proposal, we elaborated a synthesis of French research works dealing with this issue, at individual and collective levels, merging contributions by researchers from different scientific fields and working on a wide range of livestock species, in a three dimensions framework. The first dimension of the framework concerns how individual farmers choose genetic types in coherence with their livestock farming system. The second dimension concerns the definition of breeding goals, at farm and population level, in link with livestock farming system features. A diversity of reasons explains farmers' choices in terms of breeding goals, and they can combine several levers to get a herd that satisfies them. The third dimension of the framework underlines the need to take into account how farmers add value to products. We invite to an interdisciplinary approach of the links between genetics and farming systems, at several levels of organization. The issue of the relevant breeding goals, and how they are defined, should be an integral part of such a global approach.

Genetic relationships between twinning rate and production traits in Holstein cattle

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Twinning in dairy cattle is associated with reduced dam fertility, increased metabolic disorders, and lower calf survival. Selective breeding is a potential strategy to reduce or at least stabilize twinning rate (TR). To explore the genetic relationships (r_a) between TR and 305-d milk traits, 1.2 million calving records of Italian Holsteins collected between 1992 and 2022 were used. Bivariate animal models accounting for the fixed effect of parity, and the random effects of herd-year-season, permanent environment, and additive genetic animal were used to analyze the calving type (0 = singleton, 1 = twin or triplet) and milk traits. (Co)variance components were estimated through Bayesian methods implemented via Gibbs sampling. Estimated breeding values (EBV) for TR were obtained from a single-trait analysis and used to investigate the genetic trend of TR. The r_a (posterior SD) of TR with milk yield, protein yield, fat yield, protein percentage, and fat percentage were 0.126 (0.020), 0.163 (0.019), 0.075 (0.021), 0.031 (0.016), and -0.049 (0.015), respectively. The genetic trend of TR showed an average annual increase of 0.02%. When sires with ≥ 50 daughters and EBV reliability ≥ 0.50 were ranked for TR, the top 10% had an average EBV of 0.021 and TR of 4.64% among daughters, and the bottom 10% had an average EBV of -0.012 and TR of 1.89% among daughters. Results suggest that decades of genetic selection for production traits may have inadvertently increased TR. At the same time, the weak associations between TR and milk traits suggest that there is no substantial antagonism and thus selecting against twinning occurrence would be possible without impacting production traits.

National genomic evaluation for a novel functional longevity trait in Angus cattle

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Female reproductive efficiency significantly influences the sustainability and profitability of beef cattle. Many trait definitions have been used to select for better reproductive performance including stayability, heifer pregnancy, and longevity. Functional longevity plays an important role in improving reproductive efficiency as it reflects a female's ability to remain in the herd and produce a calf every year. Our objectives were to define functional longevity, evaluate different modeling approaches using random regression models and estimate the genetic parameters. Data was provided by the American Angus Association and contained 8.5M records from 2M cows born between 1990 and 2024. Phenotypes from cows between two and ten years of age were used. Functional longevity was defined as the cumulative number of calves a cow has over a nine-year period. The pedigree included 4.2M animals, of which 1.6M were genotyped for 39K SNP after quality control. To estimate variance components, a data subset with 245K cows was created mimicking the overall data. Different models were compared using Legendre polynomials of order one to three homogeneous and heterogeneous residual variances. Variance components were estimated without genomic information. Once the model was defined, the complete dataset was used to estimate breeding values using single step genomic BLUP. The best model fit was obtained with Legendre polynomials of order three and homogeneous residual variance. Heritabilities ranged from 0 to 0.12 with higher values from age six, but no significant differences between ages 6, 7, 8 and 9. The genetic correlations between ages were positive and high, ranging from 0.86 to 1. The strongest correlations were observed between adjacent years, with correlations of ≥ 0.96 across all ages after age 6. The proposed functional longevity definition provides a feasible selection tool for female reproductive performance. A random regression model with homogeneous residual variance and third-order Legendre polynomials is recommended, with age 6 as the optimal reference point for breeding value prediction and selection.

Session 42

Poster 34

Selection for automated milking system traits

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Automatic milking systems (AMS) provide a wealth of data about production and milkability, which could result in more precise phenotypes for cow production, resilience and functional traits. However, the use of this information may be cumbersome since huge data sets are generated in number of records and traits. This study deals with sampling AMS data for the estimation of genetic parameters of AMS traits with the ultimate goal of producing a reduced set of traits and a combined index that include milking robots and health traits to provide farmers a useful tool to make selection decisions. Data from 157 herds with milking robots serving 30,736 Holstein-Friesian cows in Spain were used. Overall, 17 traits were obtained from the robots. The whole data set contained around 42 million records for each trait. Two sampling schemes involving 1 out of 20 or 1 out of 10 daily records per cow and lactation were repeated 5 times and results compared with the results from the complete data. A high degree of similarity between estimated parameters from the two sampling schemes and the complete was found. Robot traits heritability estimates were highly variable, ranging from low, 0.02 for milking failure, 0.06 for milking interval of 0.10 for number of milkings per day, to moderately high, around 0.5 for fat and protein yields, times (milking, dead), milking speed and teat morphology, and high for distance front to rear udder (0.6) and udder depth (0.8). Genetic correlations were moderate or high between traits in the same group and only relevant for production vs. milking time and vs. morphology, with the exception of udder balance, which did not show relevant correlations with any of the studied robot traits. A reduced set of 11 traits were proposed for genomic evaluations. Finally, joining selected robot traits with claw and udder health traits from the health recording system in this population, a combined index that used economic values for the traits and the estimated correlations was obtained. The research was supported by EU's multiannual financial framework (MFF) for 2021-27 through GO_AMSOS360, EIP-AGRI operational group project, funded with the next generation EU recovery instrument.

Variance components for semen production traits in Swiss pig breeds

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The objective of this study was to estimate variance components (VC) for the semen production traits ejaculate volume, sperm concentration, sperm count and number of semen portions in the Swiss pig breeds Swiss Large White (SLW), Swiss Landrace (SLR), PREMO® (PR), Swiss Piétrain (SP) and Duroc (DU). For this purpose, the semen production traits from 1,298 boars (SLW=203; SLR=147; PR=541; SP=96; DU=311) with 70,066 records (SLW=5,103; SLR=4,718; PR=31,341; SP= 8,725; DU=20,179) were used. The data were collected in the years 2018-2024. Genetic variances and covariances were estimated by REML using the software ASReml 4.1. The univariate model for genetic parameter estimation across all breeds included the fixed effects birthyear of boar x month of collection, age of boar x month of collection, year of collection, collection interval, location of the insemination center, breed and boar handler as well as a random additive genetic component and a permanent environmental effect. The same model without a fixed breed effect (and location for the breeds SLW, SLR and SP) was used to estimate VC separately for each of the breeds SLW, SLR, PR, SP and DU. Estimated heritabilities across all breeds were 0.15, 0.18, 0.20 and 0.16 for ejaculate volume, sperm concentration, sperm count and number of semen portions, respectively. Different heritabilities were estimated for ejaculate volume (0.16; 0.16; 0.05; 0.18; 0.20), sperm concentration (0.17; 0.06; 0.27; 0.32; 0.07), sperm count (0.27; 0.09; 0.30; 0.34; 0.09) and number of semen portions (0.18; 0.30; 0.22; 0.21; 0.15) in SLW, SLR, PR, SP and DU breed, respectively. The results seem plausible and show, that genetic improvement through selection would be possible in semen production traits.

A Bioinformatics Pipeline for Genetic Marker Discovery in Complex Traits with Limited Phenotypic Data: A Case Study on Insect Bite Hypersensitivity in Horses

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Identifying genetic markers for complex traits in species with limited genomic annotations and few affected individuals presents challenges. This study applies a bioinformatics-based approach to prioritize candidate variants, using insect bite hypersensitivity (IBH) in horses as a case study. We first retrieved IBH-associated QTL regions from AnimalQTLdb and identified genes within these regions using Ensembl BioMart. Next, we compared SNP genotypes between an IBH-affected stallion (Arkan) and a healthy mare (Miša) to detect genetic differences. We then applied a 10% frequency threshold, selecting SNPs where Arkan had a genotype frequency of <10% and Miša ≥10% within a population of 432 horses. These were mapped onto IBH-associated QTLs to identify functionally relevant loci. This approach yielded 65 SNPs, including six previously reported IBH-associated markers. Further analysis of two IBH-affected Posavec horses revealed that 12 additional SNPs were shared between Arkan and at least one affected Posavec horse, suggesting novel candidate markers. To assess their functional impact, we performed linkage disequilibrium (LD) analysis using Animal-SNPAtlas, identifying 129 additional SNPs linked to candidate variants. These SNPs were further prioritized based on evolutionary conservation (GERP >2), regulatory potential (CpG islands, transcription factor and miRNA binding sites, polyadenylation sites), and overlap with conserved regulatory elements in humans, pigs, and mice. Functional enrichment (KEGG) and mammalian phenotype associations (MGI) linked candidate genes to biological processes relevant to IBH. This study reconfirmed six known IBH markers and identified 59 novel candidate SNPs, demonstrating how bioinformatics and comparative genomics can aid genetic marker discovery when affected individuals are limited. The findings will be integrated into the Digitization of Livestock Databases (DigŽiv) project, funded by the Recovery and Resilience Plan and the Ministry of Agriculture, Forestry and Food, to support routine selection work for breeders and professional services.

Genetic selection against twin births in Holstein cattle: A promising approach for improved animal welfare
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Twin births in dairy cattle present significant challenges for both animal welfare and economic viability. Compared to singleton pregnancies, twin gestations are associated with increased risk of calving difficulties, stillbirths and postpartum disorders. In this study we calculated relative risk ratios for various diseases linked to twin births. The results indicate an elevated risk for stillbirth (risk ratio 1.6), hypocalcemia (1.7) and retained placenta (5.3), underlining the considerable impact on cow and calf health. Breeding against twin births could therefore reduce economic losses and contribute to animal health and welfare. Additionally, in this study, genetic and genomic background of twin births was investigated and feasibility of selection against this trait was assessed. A single-step SNPBLUP model was applied to estimate breeding values, revealing strong marker effects on chromosome 11. Notably, significant SNP associations were located in the LHCGR and FSHR genes, which also showed prominence in GWAS analyses. To validate the success of potential selection, bulls were categorized based on their estimated breeding values, and the twin birth rates of their daughters were compared. A clear association between breeding values and phenotypic twin birth rate of the daughters was observed, aligning with the estimated genetic variances. These results suggest that selection based on the now-available breeding values represents a promising approach to reducing the incidence of twin births, thereby improving both animal welfare and economic efficiency.

Session 43

Theatre 1

The role of ethics in research innovation

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The role of ethics in research has become increasingly important, particularly in the context of invasive animal research. This type of research, which often involves procedures that may cause suffering or compromise the welfare of animals, is now under scrutiny. The concept of balancing interests is central to this debate: does the potential knowledge gained from invasive procedures justify the ethical costs? One of the key considerations is whether ethical guidelines slow down the progress of knowledge. Critics argue that strict ethical standards can hinder scientific progress by limiting the types of research that can be conducted. However, this perspective overlooks the potential for ethics to drive innovation. If researchers are required to find alternatives to invasive methods, they may be more inclined to develop new techniques and technologies that can achieve the same goals without compromising animal welfare. In addition, ethical considerations can lead to more thoughtful and responsible research practices. By prioritizing animal welfare, researchers may discover more humane and sustainable approaches that could ultimately benefit both science and society. In this way, ethics does not necessarily slow down progress; rather, it can stimulate creativity and innovation, leading to breakthroughs that might not have been possible under less restrictive conditions. In conclusion, ethical scrutiny of invasive animal research presents challenges but also opportunities for innovation. By encouraging researchers to find new ways to balance the interests of animals and the pursuit of knowledge, ethics can play a crucial role in advancing scientific discovery in a responsible and humane manner.

PIGWEB guidelines for enhancing experimental design in pig research

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As part of the PIGWEB project, funded by the European Union's Horizon 2020 research and innovation programme (grant agreement No 101004770), we investigated best practices for designing pig trials. Surveys among PIGWEB partners and pig researchers identified key issues, including misidentification of observational and experimental units and the use of inadequate sample sizes. In response, PIGWEB developed a set of comprehensive guidelines aimed at improving experimental design, particularly in performance, digestibility, and carcass/meat quality trials. These guidelines serve as critical points of attention for researchers when designing new pig experiments. They include defining a clear hypothesis, correctly identifying the experimental unit, conducting power analyses to determine the sample size, applying blocking and randomization, implementing blinding where possible, selecting appropriate statistical methods, and ensuring transparent reporting. An evaluation of 20 recent peer-reviewed pig nutrition publications revealed that most papers formulated clear hypotheses. While the experimental and observational units were generally well defined, several studies used incorrect or ambiguous definitions, which impacted the statistical analysis. A formal power analysis was rarely reported, increasing the risk of underpowered studies or inefficient resource use. Only one study implemented blinding, increasing the risk of subjective bias in the others. Blocking and randomization were commonly used to manage variability, but details were sometimes unclear. Statistical models were generally well described, yet some studies showed issues, such as misalignment with the experimental unit, misinterpretation of main effects when interactions were present, or the use of inappropriate models to test the hypothesis of no difference between treatments. While transparency in reporting was strong, some studies lacked details on, for example, data exclusions or confounding factors. Overall, the selected papers showed strong compliance with the PIGWEB guidelines. Addressing the remaining gaps will further enhance the quality and reproducibility of future pig research.

Session 43

Theatre 3

Ethical Approval and Review in Digital Welfare Research: Navigating the Space Between Research Institutions and Industry

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The rise of Precision Livestock Farming (PLF) technologies since the 2010s has shifted many research-grade tools, such as accelerometers, cameras, audio sensors, and robotics, into the daily operations of commercial farms. These systems, often noninvasive and externally mounted, are deployed to reduce labor demands and enhance monitoring of production traits such as activity, estrus, mobility, and milk yield. However, the development and validation of these technologies depend on large-scale, high-frequency data collected across animal populations. This creates an increasing reliance on collaborations between academic institutions, private companies, and farms, raising new questions around ethical oversight. What constitutes ethical approval when a product is being co-developed or refined outside of traditional research frameworks? How do we navigate farmer-owned data, commercial incentives, and the rigorous needs of behavioral model training and validation, all under ethical constraints originally designed for controlled trials and the peer review process? This talk reflects on these tensions from an industry standpoint, drawing on the author's experience leading a PLF startup that operates alongside universities but outside of institutional research governance. It will also highlight efforts within the EAAP PLF Commission and SENSTARA working group to standardize behavioral validation approaches and sensor ethics. The aim is not to provide fixed answers but to stimulate critical discussion on how ethics frameworks must evolve to reflect the new dynamics of welfare innovation.

Painful husbandry procedures in alternative pig farms: Frequency and farmers' perceptions

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Alternative pig farming systems, which provide animals with outdoor access or deep bedding, are believed to enhance animal welfare compared to confined housing. Although these systems offer improved housing conditions, routine husbandry procedures that cause pain in piglets are used, such as castration, teeth clipping/grinding and tail docking. This study aimed to assess the frequency of these practices in alternative farms and to explore farmers' perceptions of the associated pain. The study involved 92 French alternative farms between 2020 and 2022, each with at least 30 sows. The occurrence of castration, teeth clipping/grinding, and tail docking was recorded, along with any pain management measures. Farmers were also surveyed about their perceptions of animal pain during these procedures. Results showed that castration occurred in 92% of the farms, tail docking in 14% and teeth grinding in 7%. The average ages of intervention were: 6.3 days (range: 1-28 days), 5.2 days (range: 1-28 days) and 1.3 days (range: 1-3 days), respectively. Pain management for castration consisted of: analgesia in 60% of the farms, cold spray in 33% and anaesthesia in 7%. No anaesthesia was administered for teeth grinding or tail docking. Only three amongst the 13 farms doing tail docking used analgesia. Cold spray was used in 2 farms for tail docking. Farmers rated castration as the most painful procedure for male piglets (mean score: 5.9/10) and considered it to be arduous. Among the 85 farmers practising castration, 63 expressed a desire to stop it, with 23% favouring immunocastration. This study highlights that teeth clipping/grinding and tail docking are relatively uncommon in French alternative pig farms, which is in line with the standard of organic farming systems that were predominant in the sample. However, male castration remains widespread, despite being perceived as both painful and arduous, prompting many farmers to seek alternatives for improved One welfare.

Session 44

Theatre 2

Effect of magnesium supplementation in the feed on blood magnesium and cortisol level as well as tail lesions in pigs

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Tail biting is a multifactorial behavioral disorder in production pigs. Managing stress is crucial for reducing tail biting. Magnesium supplementation has been proposed as strategy to reduce stress-induced behaviors in pigs. Data was collected from May to August 2024 in a conventional farrow to finisher farm in Denmark with a history of tail biting in weaners with undocked tails. In total, 851 pigs in 20 pens were followed up throughout the rearing phase in two consecutive batches. Batch 1 (n=466) was supplemented with magnesium oxide (MgO) in five feeding groups (supplementation: 0, 2, 4, 6, 8 mg/kg) and batch 2 (n=385) with magnesium aspartate (MgAsp) in five groups (supplementation: 0, 5, 40, 1000, 2000 mg/kg). Blood samples were taken in the beginning, middle and end of the rearing period and analysed for magnesium and cortisol content. Pigs were monitored twice weekly for tail lesions. Linear regression models were used (applied in R version 4.4.2) to assess the connection between cortisol blood levels, MgO and MgAsp supplementation with the prevalence of tail and ear lesions. Results show a significant drop in magnesium level in both batches in all feeding groups in the middle of the rearing period (p=0.04). Moreover, there was a significant connection between tail lesions and blood magnesium value (p=0.02). Cortisol level was significantly higher at the beginning of the rearing period (p=0.01) but no significant connection between cortisol level and magnesium level or tail lesions was found. The higher cortisol level at the beginning can most probably be explained by stress due to weaning and regrouping. Regarding blood magnesium, results demonstrate a significant drop independent on type and dosage of magnesium supplementation in the feed after three weeks in the rearing stable. This fits to the observation that in undocked pigs, often tail biting outbreaks occur at this time point. Findings of this study support the working hypothesis that a secondary magnesium deficit may be linked to tail biting.

Collaborative research to support the ban of castration in organic pig production

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Keeping and processing intact boars is experiencing bottlenecks in organic pig farming in Belgium, mainly at marketing level. The main two question to address were 1) how to reduce boar taint in organic production and 2) provide information and experience to all pig chain partners. As a first step, bottlenecks and opportunities were discussed with two major retailers for organic pork. Following these consultations, both agreed to collaborate, and a pilot study was launched at three pig farms. On each farm the control diet was compared with a feed reduction measure that fitted the management of the farm manager: an adapted biological variant of a commercially available boar taint reducing feed (BT diet, Farm 1), addition of 1% chestnut tannins (Farm 2), and supplementation of grass silage (Farm 3). Farmers' experience, animal behaviour and boar taint were evaluated. In addition, meat quality was measured (pH, color, drip loss, intramuscular fat, shear force) and evaluated by a trained panel. These assessments were conducted on loin samples from all three farms, as well as on blade loin, bacon, and meat patties from Farm 1, at the request of one of the retailers, who sought more insight into the effects on meat quality. Farmer 2 and 3 had positive experiences with keeping boars. On farm 1, where the boars were given liquid feed and thus fed more limited amounts, there was clearly more aggressive and sexual behavior, so keeping boars intact was also seen as a less good option by the farmer. Overall, with 11 % tainted carcasses based on sensory evaluation, the prevalence of boar taint was remarkably high and also higher than in previous studies. Chemical analysis revealed mainly high androstenone levels (19% > 2000 ppb vs. 4% with skatole >200 ppb). Farm-to-farm differences in androstenone levels were also high (Farm 1: 14%, Farm 2: 6% and Farm 3: 26%). The BT-diet tended to decrease skatole level on farm 1 (35 vs. 50 ppb), while the grass silage fed boars had lower androstenone levels compared to control boars at farm 3 (1228 vs. 2079 ppb). No other significant effects of the feed reducing measures on boar taint were found. Results on meat quality and sensory evaluation of meat products are still ongoing.

Session 44

Theatre 4

Why should farm animals be kept intact, and what does this mean for their welfare?

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Mutilations, such as tail docking, castration, disbudding or beak trimming, are a common part of animal husbandry of most farm animal species. They are carried out for reasons of product quality, human safety and to reduce injury to the animals, but the main underlying motivations are to ease management and lower housing requirements. Despite considerable work on ways to relieve pain during these procedures, it cannot be completely prevented and there is increasing evidence that long-term adverse effects can occur. However, these practices are questioned not only because they cause pain and distress, but also for two further animal welfare reasons: (i) they do not respect the integrity of the animals, (ii) they may mask animal welfare problems caused by inappropriate husbandry conditions. Many welfare issues, such as tail biting, feather pecking or injurious agonistic interactions that are symptomatically mitigated by mutilations, have multifactorial causes and are therefore challenging. Decades of research have not found a sole preventive measure. Instead, there is some evidence that a general optimisation of husbandry conditions can effectively reduce these welfare problems, although this is not easily captured by common scientific research approaches. Refraining from mutilations may lead to increased welfare problems in the short-term and to individual welfare impairments in general, because coping problems with suboptimal husbandry conditions can easier be detected in intact animals. However, practical experience shows that this also facilitates improvements of husbandry practices and a reduction of the welfare problems in the long-term. It becomes then less appropriate to subject all animals to painful procedures in order to prevent sporadic welfare problems. From an animal welfare point of view, the aim should be to keep intact farm animals under husbandry conditions that prevent as far as possible the occurrence of abnormal or injurious behaviour.

Challenge session: Keeping animals intact - why the lack of progress? (Part 1)

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Following the oral presentations the remainder of this session will be devoted to structured workshop like activities during which we will explore the lack of progress in keeping animals intact in our farming systems. Surgical interventions such as tail docking, teeth clipping, castration, beak trimming, and dehorning are commonly applied to animals to adapt them to conventional production systems. However, there is a lack of valid and continuous monitoring systems to assess impact and support improvements. To move beyond these practices, it is essential to explore on-farm strategies related to feeding, housing, and management that allow animals to be kept intact without compromising their welfare. Decades of research explored pain mitigation techniques for painful procedures yet farm-level adoption remains poor. As a result, millions of animals across the EU undergo these interventions without analgesia. Why does the knowledge gained through animal welfare science not translate into meaningful change on the ground? What barriers prevent the widespread adoption of welfare-friendly alternatives in everyday farming practices? There are challenges with keeping animals intact e.g safety concerns with entire males or bulls. While these are difficult to overcome, many other problems like feather pecking and tail biting, are deeply rooted in suboptimal husbandry practices. This highlights the need for broader reflection on how animal production systems can evolve to better support both animal welfare and practical farm management. It is timely to critically examine the persistent routines and assumptions within animal production. Is the system overly reliant on outdated practices, or are there deeper systemic factors that prevent the adoption of more humane alternatives? What innovative approaches could reconcile the practical demands of farming with the need for improved animal welfare? Our aim will be to find meaningful answers through thoughtful, collective conversation about the future of farming practices.

Session 44

Theatre 6

Challenge session: Keeping animals intact - why the lack of progress? (Part 2)

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Disproportionality in high-performing farm animals

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Disproportionality describes any imbalanced anatomical structures and physiological functions of tissues and organs in high-performing farm animals. This imbalance causes physiological limitations; many farm animals appear to be beyond these limitations. Metabolic inflammation, oxidative stress and increased apoptosis/necrosis are symptoms of this condition. Genetical selection for milk, meat and eggs pushed the organism into specific metabolic features which prioritize all structures and functions which are important for performance. However, it is well-known that incidences of metabolic disorders and production diseases are very high in farm animal production systems worldwide. This presentation – based on scientific evidence – aimed to demonstrate that the anatomical and physiological capacities were no longer sufficient enough to maintain health and fertility of many farm animals despite adequate feed intake, growth and performance. Disproportionality is found in fast-growing broilers with relative too small lungs and heart resulting in cardiopulmonary insufficiency and breast muscle myopathies. In laying hens, disproportionality between egg production and maintenance of liver and bones results in high incidences of keel bone fracture and fatty liver hemorrhagic syndrome. In hyperprolific sows, disproportionality between genetically determined numbers of piglets and anatomical features of the uterus results in intrauterine growth restriction of the offspring. In dairy cows, early weaning and low milk supply of calves, early first calving age and high milk performance cause disproportionalities which lead to pre- and postnatal metabolic imprinting of the offspring. To conclude, these disproportionalities are based on structural and functional restrictions which can often not be corrected by improving feeding or management approaches. Farm animal breeding needs a profound reconsideration to avoid disproportionality and improve animal health with optimized performance in producing animal protein for human nutrition. Furthermore, the rearing regimens of farm animal offspring needs to be reassessed to gain the optimal developmental capacity and to diminish epigenetically provoked disproportionalities in the future high-performing farm animals.

Session 45

Theatre 2

Keel bone fractures in egg-laying hens

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Keel bone fractures in laying hens have emerged in commercial layer flocks over the last couple of decades. The fractures are currently considered the most important welfare problem in commercial egg-laying hens, with a prevalence of more than 85% in laying hens at the end of production. In more than 95% of the cases, the fractures are located at the tip of the keel bone, where they have a very uniform appearance. Most of the fractures develop in the period at peak of lay and before the keel bone is fully ossified. The ossification of the keel is not complete until several weeks after the onset of egg-laying. Hens with fractures develop altered moving patterns in particular vertical movements, suggesting a certain impaired welfare and discomfort. Until recently, their origin was hypothesized to be due to external trauma, e.g. collisions with housing equipment. However, morphological characterization of the fractures at the caudal tip of keel suggested that the fractures are more likely to originate from the inside by an unknown mechanism. Radiographic characterisation of the fracture morphology suggests that underlying Ca²⁺ metabolism disorders may contribute to fracture development. It is hypothesized that both the egg-laying and late ossification of the keel bone may play a role in fracture development. Recently, histologic details on the ossification of the keel bone showed that the keel is likely to ossify via endochondral ossification similar to long bone growth.

Options for improving the growth of low-birthweight piglets with optimisation of protein nutrition and functional amino acid supplementation

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The focus on increasing the number of piglets per litter in pig farming systems has pushed sows near the limit of their biological capacity to support the larger number of offspring. This has led to a concomitant rise in the prevalence of negative factors such as increased intrauterine and teat crowding, metabolic stress load on the sow, prolonged labor, a larger number of low birth weight piglets born (L) and increased piglet mortality. Low birth-weight piglets are reported to have higher pre-weaning mortality, impaired organ development, an altered carcass composition and take longer to reach market weight compared to their normal birthweight littermates. In addition to the ethical problems, these impairments represent a significant negative public perception for the industry and an economic loss for the farmer. To address the problems caused by large litters, nutritional intervention is considered as one of the solutions. Two of the most commonly studied interventions to improve the growth of L piglets are optimizing protein nutrition and supplementing with functional amino acids (AA). The timing of these interventions can broadly be divided into three developmental phases: intrauterine, pre-, and post-weaning, each with differing protein needs and potential roles for functional AA. However, despite the large body of protein nutrition and functional AA research which indicate the potential to improve L piglet growth, the results are often conflicting and inconsistent, which can be attributed to differences in genotype, management conditions, feed quality and supplementation regime. Here, we present a critical review of the impact of dietary protein optimisation and functional AA supplementation on improving the growth and vigour of L pigs during the three critical life cycle phases. Funding: German Research Foundation (DFG) – ME 1420/8; ME 1420/10; European Union's Horizon 2020 – MSC Innovative Training Network grant No 955374; *metges@fbn-dummerstorf.de

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Tail tip lesions in dairy cows - a summary of what's new about the old

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Tail tip inflammation is a well-known and widespread problem in fattening bulls. In addition to housing conditions, the main causes are thought to be the influence of feeding in the form of lack of structure or mycotoxin contamination. Recent studies show that dairy cows are also affected by alterations of the tail tips. In a first phase of our studies, different types of alterations in the invertebrate tail end of dairy cows were described. Based on these observations, dairy cows from numerous other farms were examined. Prevalences of up to 99% were found on all farms. Histopathologic examination of the tail ends of affected cows at slaughter revealed coagulation necrosis in deeper layers with undamaged epidermis. Coagulation necrosis occurs as a result of circulatory disturbances and could therefore be a possible cause of the observed changes. These results are in accordance with previous findings on the pathogenesis of hemorrhagic tail lesions, since thermographic examinations of the tail ends have shown that there is a clear correlation between the surface temperature of the tail ends, animal health, and the frequency of alterations at the tail end. Studies in calves have also shown that they were born with intact tail tips. However, weekly scored calves showed a strong accumulation of bloody lesions at the tail tips after dehorning in the third week of life, as well as after other triggers such as weaning from milk or first feeding of a cow TMR. In addition, significant correlations were found between the occurrence of tail tip alterations in dairy cows and the day of lactation, number of parturitions, body condition and milk components. Thus, a purely traumatic origin caused by treads from other animals, manure scrapers or slatted floors seems rather unlikely. In principle, severe lesions such at the tail tip can be expected to be painful and represent, among other things, a potential source of infection and therefore, represent an animal welfare issue. Studies from Denmark or New Zealand also describe similar changes with high prevalences. Severe alterations, including (auto-)amputation of parts of the tail, may therefore be a worldwide phenomenon with an internal cause in high yielding cows.

Clustering of milk lactose dynamics across lactation in dairy cows

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Interest in lactose content (LC) has grown as an indicator of udder health and metabolic disorders. Variability of LC dynamics across lactation has not been described and could help to clarify LC variations due to health or metabolic issues. The aim of this study was to identify distinct patterns of LC dynamics, and assess environmental and genetic contributions. To this end, 1,980,693 test-day records from 183,150 Holstein cows across 2,239 herds in France were analysed. Each cow had at least 2 records in the first 90 d and 4 between 7–300 DIM, averaging 7.3 records/cow. A functional principal component analysis was used to describe LC dynamics across lactation. This approach involves smoothing the LC curves for each cow and then summarising their overall shape using 3 principal components: average LC level across lactation, LC slope and LC level in mid-lactation. LC dynamics were clustered into 6 groups (G). The first 3 groups, representing 86% of the data, shared similar dynamics with minimal LC slopes (<0.01 % per month) and differed in average LC levels (G1: 5.00, G2: 4.79, G3: 4.60%). LC genetic value (G1: 0.59, G2: -0.15, G3: -0.82) and cow parity (G1: 1.8, G2: 2.6, G3: 3.2) explained 68% of the average LC levels. The remaining groups showed LC levels similar to those of G2 until 150 DIM. Thereafter, two showed negative slopes (G4: -0.07% and G5: -0.13% per month), and one a positive slope (G6: +0.05% per month). The variation in LC slope was partly explained by environmental factors (15%). Winter calving were associated with G4 and G5, and summer calving with G6. The proportion of culling during or after the current lactation was higher for G3, G4, and G5 (31-46%) compared to G1, G2, and G6 (16-25%). As a result, clustering lactose revealed distinct LC dynamics with genetic LC values explaining the average LC level. The LC slope variations was partly explained by environmental factors, while the remaining unexplained variability may be caused by health issues, e.g. udder or metabolic disorders, leading to higher rate of culling.

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How harsh environmental conditions affect lifetime efficiency in ewes

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Sheep must be resilient to obtain high lifetime efficiency. However, this compound trait is difficult to measure and highly affected by environmental conditions, suggesting that the animals that fit with current conditions might not be the ones adapted for future conditions. To investigate how environmental conditions affects lifetime efficiency, we extended a mechanistic model from dairy cows to reproductive ewes. The model simulates the female's phenotypic lifetime trajectories, driven by four input parameters describing the individual's resource acquisition- and allocation potential. The obtained resources, resulting from the availability in the environment and the ewe acquisition potential, are split between life functions: growth, gestation, lactation, and body reserves which impact reproductive success and survival probability. The calibration process was based on performance data on more than 2 million Norwegian White Sheep ewes, and previously published research. New elements in the model includes litter size, gestation acquisition and seasonal conception probability. The model is used as a building block in an individual-based simulator to scale-up to flock-level. Individual phenotypic variability emerges from variability in the parameters describing the acquisition and allocation potential and the environmental conditions. We used the simulator to investigate the effect of environmental quality (good and poor) and variability on lifetime efficiency. Results showed that both environmental quality and variability affect ewe performance. In general, ewes in poor environments were smaller, had lower body condition and produced less lambs. Ewes were most affected in early lactation and were not able to recover in the following parity. However, variation in the ability to recover, based on the individual acquisition and allocation potential, suggests that there are optimal resource strategies depending on environmental conditions which can be considered in a breeding goal.

Metabolomic Profiling of Milk from Grazing Beef Cows with Divergent Residual Feed Intake

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This study aimed to evaluate the milk metabolomic profile of grazing, lactating first-calf beef cows with divergent residual feed intake (RFI) during summer, assessing the influence of weather on milk metabolites and their relationship with RFI. Twenty crossbred lactating beef cows (441 ± 33.3 kg body weight; 26 ± 1 mo of age; 79 ± 4 d in milk at the start of the experiment) tested for RFI at 11 ± 1 mo of age and classified as more (-1.4 ± 0.64 kg DM/d, $n = 10$) or less (1.7 ± 0.48 kg DM/d, $n = 10$) feed efficient were used in a completely randomized design. Cows grazed a single pasture for 64 d (June – August). Milk samples were collected at d 0, 13, 28, and 63 following a daily 24-hour peak temperature exposure of 25 to 29°C. Metabolomic analysis was performed using amine/phenol high-performance chemical isotope labeling LC-MS ($^{12}\text{C}/^{13}\text{C}$). A linear model in R software assessed the effects of RFI, day, and its interaction. Human Metabolome Database pathway analysis (MetaboAnalyst 6.0) was conducted for RFI and day, applying a false discovery rate correction ($\text{FDR} \leq 0.1$). Milk contained 2,413 compounds, of which 614 were molecularly identified. Forty-seven compounds were affected by RFI, with 16 contributing to a greater tyrosine pathway activity in LOW-RFI. Across days, 1,400 metabolites varied, with 224 linked to active pathways. Glycine, serine, threonine, and the one-carbon pool by folate were upregulated on d 0 and 28. Valine, leucine, isoleucine, and the arginine-proline pathway increased on d 28, lysine on d 63, and arginine on d 13, 28, and 63. In contrast, alanine, aspartate, glutamate, and lysine were downregulated on d 0, histidine on d 28, tryptophan on d 63, and tyrosine on d 28 and 63. The $\text{RFI} \times \text{day}$ interaction affected several metabolites, though lacking database identification. In conclusion, tyrosine compounds may act as biomarkers for residual feed intake, while extreme weather conditions impact milk metabolism in beef cows. Keywords: beef cows, milk metabolomics, pathway analysis, residual feed intake, weather resilience.

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Metabolomic Profiling Reveals Growth Implant Effects on Key Metabolic Pathways in Beef Calves

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This study evaluated the metabolomic changes of beef calves in two growth implant treatments. Thirty crossbred calves were stratified by birth weight and age and were randomly assigned (10 per treatment) to one of three treatments: Synovex C: 100 mg progesterone and 10 mg estradiol benzoate, Synovex One Grower: 150 mg trenbolone acetate and 21 mg estradiol benzoate, or Control: no implant, at ~75 d of age. Plasma was collected on d 1, 94 (weaning), 108, and 153 of the study and analyzed using a targeted quantitative metabolomics approach. This method integrates direct flow injection mass spectrometry and liquid chromatography tandem mass spectrometry for extensive metabolite profiling. After data cleaning, from 644 metabolites, 401 stayed for statistical analysis. A linear model was fitted using RStudio software to assess the effects of group (calves were implanted at two different time points based on age), time (day of plasma collection), treatment and $\text{time} \times \text{treatment}$ interaction. Statistical significance was set at $P \leq 0.05$ and pathway enrichment analysis was conducted with MetaboAnalyst, using a false discovery rate ($\text{FDR} \leq 0.1$ for significance. Results revealed a significant treatment effect ($P \leq 0.05$) in 43 metabolites. Among these, 42, 7, and 5 displayed significant effects of time, group, and $\text{time} \times \text{treatment}$ interaction, respectively ($P \leq 0.05$). Four metabolic pathways and their significant metabolites were identified as influenced by growth implants: Glycine, serine, and threonine metabolism (L-Serine, Betaine, Guanidinoacetate, N, N-Dimethylglycine, L-Cystathionine and glycine; $\text{FDR} = 1.50\text{E-}5$), one-carbon pool by folate (Glycine, Betaine, N,N-Dimethylglycine, L-Serine and L-Cystathionine; $\text{FDR} = 5.6291\text{E-}5$), sphingolipid metabolism (Sphingomyelin, L-Serine and Lactosylceramide; $\text{FDR} = 0.06$) and Arginine biosynthesis (N-Acetylornithine and L-Ornithine; $\text{FDR} = 0.1$). These pathways are essential for amino acid metabolism, cellular growth, and immune function. Therefore growth implants may improve beef cattle productivity by influencing these metabolic pathways. Keywords: beef calves, FDR, metabolites.

Effects of mechanical milking pulses on bovine mammary cells

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Bovine mammary glands are subjected to large mechanical forces, for example during milking pulses. In other systems it is well known that sustained, repeating forces may cause long term damage to tissues. To investigate potential effects on udders, we set up systems that model the milking forces in culture. We applied milking- like compression and cyclic pulses on bovine mammary epithelial cell lines in 2D and 3D cell cultures. Possible effects on cellular function, as well as on tissue damage and recovery, were examined by employing multidisciplinary approaches. These included cell culture assays, force experiments, microscopy, computational analysis and physical modelling. The udder cells usually maintained normal growth patterns and secretion of milk fat globules in the force experiments. However, the mechanical stresses caused morphological alterations, for example, 3D cell clusters remodeled under compression and were not able to fully recover after the force release. This suggests that also in the animal the tissue undergoes non-reversible modifications during repeating cycles of milking. The in vitro insights can pave the way for optimizing machine milking parameters to minimize long term tissue damage and to maximize the milking performance.

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Can the milk metabolome be used to predict the health of dairy cows in relation to tail tip lesions?

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Current studies are investigating the suitability of examining the tail tip for specific lesions to assess the metabolic health of animals. Lesions on the tail tip of dairy cows may be caused by circulatory disturbances as a result of systemic inflammatory processes. Metabolomic approaches are used to detect and quantify pro-inflammatory metabolite profiles in biological samples to better understand metabolic inflammation in humans and animals. In dairy cows, changes in plasma and milk metabolite profiles have been associated with ketosis, heat stress and mastitis. Systemic inflammatory conditions have been described in dairy cows with increased metabolic load, particularly in the liver, especially during calving and early lactation. To further investigate the potential relationship between these tail tip lesions and metabolic health, milk samples were collected during the early stages of lactation. The tail tip of 51 dairy cows were examined on a weekly basis for the presence of lesions during the entire lactation period. In addition, milk samples were collected on the 14th day of lactation (+/- 1 day) to test the hypothesis that milk metabolites can be used as indicators of systemic inflammation. Milk metabolite profiling was performed using MxP Quant 500 kit (Biocrates, Innsbruck). The results of this study indicated that 56.9 % of the cows exhibited a bleeding lesion at the tail tip during the study period. In 31.4 % of the cows, a loss of a tail part due to auto-amputation was recorded. A comparison was made of cows with bleeding lesions between day 14 a.p. and 80 p.p. with cows without such lesions. Metabolite profiles in milk differ only in the concentration of sarcosine ($p < 0.0108$) with higher values in cows with bleeding lesions. In other studies, an increase in sarcosine levels in the plasma of cows was detected following a LPS challenge. Although single metabolites appear to be associated with tail tip lesions, the milk metabolome determined by MxP Quant 500 kit is not useful to predict metabolic health in association with tail tip lesions.

Optimizing *Hermetia illucens* egg productionC. Coudron¹, D. Deruytter¹, J. Claeys¹¹ Inagro, Insect Research Centre, Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium

Egg production is a key factor in optimizing the efficiency of a closed black soldier fly (BSF) farm without external egg or neonate larvae input. It determines the supply of progeny that will ultimately be harvested as larvae biomass. Ideally, egg harvest should be both predictable and maximized. Reducing the number of females required for reproduction decreases the need for cages and increases the proportion of larvae available for sale. The goal of an optimal BSF reproduction cage is to create an environment where each female can ideally reach her biological maximum for fertilized egg production and deposit them in designated sites. However, due to various (a)biotic factors, achieving the theoretical maximum egg yield per cage remains unrealistic, as there is a tradeoff between maximizing output per fly and per cage. Several parameters influence egg production, including cage design (attractants, oviposition sites, resting areas, watering) as well as fly size, life history traits, and density, all of which affect actual egg yields per cage. To optimize the egg production, a series of tests were and will be conducted under standardized conditions. Therefore, a total of 24 cages (60 x 60 x 90 cm) are accommodated in a room with climate control (at 30 °C and 80% relative humidity). A first test assessed a range of fly densities in a cage and determined its effect on the total and per female egg production. Density was varied by inserting varying amounts of pupae in the cages (with an individual weight of 111 mg per pupa) and could be as low as half a fly per dm³, up to 39 flies per dm³ of cage volume. Egg production was maximal at the highest fly density (51.8 g per cage). However, while lower densities up to 5.7 flies per dm³ still produced on average 13 mg of eggs per female, this decreased linearly with 0.12 mg per extra fly per dm³. Which could potentially be (partially) attributed to crowding of the oviposition site. This, but also other topics such as availability of water dispensers, pupae size, etc., are part of still ongoing research.

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Reproductive Performance in *Hermetia illucens* (Diptera:Stratiomyidae): The Role of Adult Body SizeY. Riabtseva¹, B. Grodzki¹, M. Tejeda¹, M. Bolard¹¹ Nasekomo AD, Saedinenie Str. 299, Lozen village, 1151 Sofia, Bulgaria

According to the Food and Agriculture Organization, insects like the Black Soldier Fly (BSF, *Hermetia illucens*) are a promising alternative protein source for the feed industry. Nevertheless, little is known about the biology of adult flies. Understanding these factors is crucial for optimizing production and gaining better insight into the drivers of reproductive outcomes. The objective of research was to investigate how adult body size influences the reproductive performance in BSF. Insects were reared under controlled conditions. Thorax length (TL) was measured as an indicator of adult body size in newly emerged flies. Mating enclosures were set up according to the 4 possible combinations between sizes (L=Large, S=Small) and sex with a 1:1 Male:Female sex ratio and labelled as LL, LS, SL and SS. Mating status (mated or unmated) and the ovarian development were assessed for females. Age was determined by evaluating daily cuticular banding. Results indicates that small females have a delay in ovarian development, irrespectively of the size of the mating partner. We also observed that size of female relative to size of male (size ratio) was positively correlated with reproductive performance. Increased mating activity and higher egg productions were recorded in enclosures where small males copulated with relatively larger females. Thus, in BSF, males with a smaller size appear to have a mating advantage (SS+SL > LS+LL), probably because of the better maneuverability of a small body compared with a large one. These findings could be practically applied in optimization of BSF industrial rearing.

Simulation of a black soldier fly breeding program considering production and inbreeding

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In recent years, insects, in particular black soldier flies (BSF), have become an important feed component for sustainable aquaculture and livestock production. Therefore, insect breeding programs need to be set up and improved. A key challenge in insect breeding is that the measured traits, cannot be easily traced back to a specific individual afterward, as no large-scale identification of individual insects is used in practical breeding and genotype information are usually not available. A simulation study, simulating the genome of every individual, was performed to propose a practically feasible breeding scheme for BSF to increase body weight at the second larvae stage and simultaneously decrease development time till emergence as adult while limiting the inbreeding rate. For the body weight different means for males and females were considered. For scenarios using threshold selection, in a first selection step, the body weight was considered, followed by a second selection step accounting for the development time. Scenarios using phenotypic threshold selection, both with a static threshold and an adaptation of the threshold, as well as the simultaneous application of two different thresholds for body weight to aim for a balanced sex ratio, were compared to random selection as reference scenario and phenotypic mass selection considering body weight and development time in an equally weighted selection index as a theoretical option. The simulation has shown that a continuous adjustment of thresholds is necessary to avoid a rapid depression in genetic gains and that a second threshold for body weight is feasible to have an acceptable sex ratio in the selected BSF. Using mass selection resulted in a drastic increase in the inbreeding level compared to all other scenarios. Consequently, in practice it is important to avoid strong preselection of larvae for weight based on visual perception. The current study highlighted the importance of monitoring the design of BSF breeding programs, especially when improving selection techniques.

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Threshold selection for larval body weight in Black Soldier Flies

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The growing demand for sustainable and efficient protein sources for animal feed, driven by the increasing global population and the pressures on traditional animal farming, is a key driver behind the rapid expansion of the black soldier fly industry. Black soldier fly larvae, rich in protein and essential nutrients, present an ideal alternative to traditional feed ingredients like fishmeal and soy. Selective breeding has emerged as a powerful tool to improve the efficiency, resilience, and overall value of Black soldier flies, enhancing their utility in waste management, animal feed, and other sectors. Selective breeding in insects is however not easy to implement. Larval body weight is an easy to measure trait with high heritability and relevant for the industry, therefore ideal to explore potential selection strategies. The goal of this study was to assess the impact of threshold selection on larval body weight and its practical applicability. Our simulations revealed that threshold selection offers a viable alternative to mass selection, with the added benefit of lower inbreeding rates. However, the practical feasibility and effects on other important traits were previously unknown. To investigate further, we conducted a selection experiment at PT Bio-Cycle Indo, applying threshold selection across eight generations. Initially, a single threshold was used, but due to sexual dimorphism in body weight, we transitioned to a two-threshold system to ensure adequate selection of males, based on a lower threshold, for sufficient breeding candidates and mating success. We also progressively increased the threshold over generations to maintain selective pressure. Our results showed a rapid increase in female larval body weight, with a slower response observed in males. We also monitored egg clutch numbers, fertilization rates, sex ratios, and development time across generations. Overall, threshold selection proved to be a practical and effective method for improving larval body weight, though close monitoring of mating success is essential to ensure continued progress.

Genetic parameters for larval size and dry matter content in the black soldier fly (*Hermetia illucens*) reveal potential for selective breeding

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Larval size and dry matter content in black soldier flies (*Hermetia illucens* L.; Diptera: Stratiomyidae) (BSF) larvae are important traits for the profitability of insect production. We estimated heritability (h^2) and genetic correlations (rg) for larval size and dry matter content assessed at an individual level and group level. A full-/half-sib design was used, where virgin males successfully mated with 1-4 virgin females ($\mu = 1.91$ females with larvae per male). For each female that produced larvae, 100 larvae were divided equally across two cups filled with chicken feed and water. Twelve days after egg hatching, phenotypes were collected from 30 randomly selected larvae from each cup. Individual larval size was measured as the individual's surface area (ISA) on 13470 larvae from 124 sires and 237 dams. Weight of the group of 30 larvae (GW) and the group's surface area (GSA) was determined for 449 full-sib groups. The larvae were freeze-dried, and the dry matter weight of individual larvae (IDM) and of the group of 30 larvae (GDM) was determined. The data were analyzed using uni- and bivariate sire-dam models with population average and batch (5 batches) as fixed effects and sire, dam and cup (only for ISA and IDM) as random effects. In the bivariate models, sire effects were assumed correlated. The h^2 estimates varied from 0.22-0.39 ($SE < 0.10$), and the common environmental effect 0.19 ($SE < 0.02$). Some of the bivariate models including group-traits (GW, GSA, GDM) did not converge. Yet, the rg that could be estimated were strong, e.g. between ISA and IDM (0.78, $SE = 0.09$), and GW and IDM (0.91, $SE = 0.05$). The results reveal promising potential for BSF breeders that want to increase larval size and dry matter content of BSF larvae through selective breeding programs.

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Theatre 6

The influence of yeast and glucose supplementation on *Tenebrio molitor* progeny

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In a closed mealworm farm without external egg or larvae input, maximizing egg production is key to efficiency. It ensures a steady supply of offspring that will later be harvested as larvae biomass. To optimize space utilization, it is beneficial to keep the number of adult beetles needed for reproduction to a minimum and keep the adults productive as economically feasible. This way, more of the facility can be dedicated to larval production rather than broodstock maintenance. While past research has explored beetle reproduction over time, this study explores feed additives to extend the beetles' productive lifespan. A study was done in order to determine the effect of the supplementation of glucose (G) and yeast (Y) (*Saccharomyces cerevisiae*) to *T. molitor* beetles on progeny production over time and beetle survival. Young beetles were collected one week after eclosing from their pupae. They were randomly allotted to experimental units consisting of 200 beetles. G and Y supplementation was done by dissolving it in the agar gel used as a moisture source for the beetles. A total of 5 treatments, Y, G, and G&Y supplementation were compared to a negative control, beetles that received no moisture source, and a positive control receiving agar gel. Each treatment was tested in threefold. Beetles were housed at 27 °C and 60% relative humidity. Every week the beetles were removed from their substrate and moved to fresh substrate. Dead beetles were counted and removed. The old substrate, containing the eggs, was incubated for five weeks, after which the number of mealworms in the substrate was determined. If less than 10% of the initial beetles were still alive, which occurred around week 21, the experiment was terminated. While beetles that received no moisture only produced an average of 1,180 mealworm offspring, agar control beetles produced 77,800 mealworms, Y supplemented beetles had 85,300, G supplemented beetles had 71,100 and G&Y supplementation yielded 86,700. Although the experiment took 21 weeks, 50% of offspring was produced within the first 5 weeks of the experiment and 90% was produced by week 11. These results also provide insights on the accuracy of short term reproduction experiments (e.g. 2 weeks) on the overall reproductive output.

Improving the reproductive output in *Tenebrio molitor* by using agar-agar as nutrient carrier

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Tenebrio molitor (TM) is among the most widely reared insects in the world for food and feed. So far, scientific efforts have focused on the capacity of the larvae to convert agro-industrial by-products into food, feed and to a wide range of other substances. However, to create successful breeding programmes, information on the adult stage is required. Studies on this topic are disproportionately few, compared with the published works for the larval stage. In artificial rearings, *Tenebrio molitor* feeds on dry substrates supplemented with wet feed, but there is still inadequate information on how the presence of wet feed may affect adults' fertility. This study aims at assessing the adults' requirements in wet feed and the possibility of improving their reproductive output by administering protein and sugar supplements through the wet feed. In this context, agar (20 g/l) was used as control moisture source, tested against agar enriched with a protein source and agar enriched with both protein and sugar. For each type of wet feed, the effect of three increasing quantities was tested (0.1, 0.2 and 0.3 g/individual/day). In order to evaluate these parameters, groups composed of five mating pairs (14 days old adults) were used. Adults were placed in plastic vials containing 60 g of white flour, as the oviposition substrate. The experiment lasted 4 weeks, eggs were collected at 7 days intervals, and left to hatch over a period of 10 days. It was observed that the total number of eggs laid was significantly influenced by the type of wet feed. Agar enriched with protein improved oviposition compared to the control and agar enriched with protein and sugar improved oviposition compared to protein alone and control. On the other hand, the quantity of moisture source provision did not significantly influence the number of eggs laid. Our data clearly demonstrate that the type of wet feed is determinant for the fecundity of *T. molitor* adults, a parameter of major importance in mass-rearing protocols of this species.

Session 46

Theatre 8

Pedigree reconstruction in honeybees using different sources of genotypic information

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Managing breeding populations includes improving their genetic value for the traits of interest while simultaneously managing genetic variability in the population to prevent unwanted changes in performance. Both of them require measuring relatedness among animals in the population. This is traditionally done through pedigree tracking, which is challenging in insect populations, especially for polyandric species such as honeybees. An alternative is to use genomic information to reconstruct the pedigree and compute relatedness. However, insect populations are numerous and genotyping can represent a substantial cost and time investment. We would thus like to optimise the amount of genomic information we acquire without compromising the ability and accuracy of determining kinship. In this work, we obtained data from a mating control experiment in honeybees and compared paternal pedigree reconstruction with three types of genomic markers with an increasing number of genotyped markers: microsatellites, SNPs acquired by SNP microarray genotyping, and SNPs acquired by genotyping-by-sequencing. Each method was associated with a different method of determining parentage. We additionally explored the limitations of pedigree reconstruction by assessing the effect of SNP density and the choice of reconstruction method via simulation. Our work provides valuable insight into mating control evaluation in honeybees that is also applicable to other species. Furthermore, the results will provide practical recommendations for population management by guiding the choice of method while understanding its limitations.

Effective population size in honeybees from SNP data in simulations with examples from Central Europe
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Central European honeybee populations have largely been shaped by human activity. On one hand, breeding efforts produced highly productive and easy to manage hybrids. On the other hand, conservation of native subspecies and maintenance of genetic diversity has increasingly gained attention. The German breeding population consists mainly of the pure-bred *Apis mellifera carnica*, which was imported during the last century, and the hybrid Buckfast-bee, which combines genetic material from different evolutionary lineages. In Switzerland, the native subspecies, *Apis mellifera mellifera* is still preserved, in a conservation and a breeding population. Effective population size is a key parameter for genetic diversity of a population, but honeybees pose unique challenges. By natural behavior, the diploid queen mates once in her life with about 12 haploid drones during flight. To ensure controlled mating, virgin queens are brought to isolated mating stations, such as islands or valleys. The only fully grown hives in this area belong to the intended drone producing queens. We conducted a forward in time simulation of a population under selection. Five settings were simulated over 55 years and each was repeated 100 times. In the standard setting, 1'000 breeding queens per year, and 50 mating stations were simulated. In the 4 other settings, the mating scheme was changed in year 30 by increasing or decreasing either the number breeding queens or the number of mating stations by 40%. The effective population size was estimated via the linkage-based implemented in GONE and the temporal method from NeEstimator_v2. Both methods were compared to estimates from inbreeding reported by the simulation software. A recently developed 70-k-SNP-chip (HONEYBEE_2021) was used to genotype about 3'000 bees from Germany and Switzerland. Estimates of effective population size were generated for both data sets and discussed in the context of simulation results.

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Theatre 10

Association Between Gut Microbiota Diversity and Varroa Mite Infestation in a Honey Bee Breeding Population

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Honey bees play a vital role in pollination, supporting biodiversity and agricultural productivity. However, their colonies are increasingly threatened by the ectoparasitic mite *Varroa destructor*, a key driver of colony losses. While selective breeding aims to enhance resistance traits, recent studies suggest gut microbiota may also influence *Varroa* infestation dynamics. In this study, we investigated the associations between gut microbiome composition and phenotypes related to *Varroa* infestation, in a breeding population that has been selected since 2015 for docility, honey production and hygienic behavior. In detail, the relationship between microbiota diversity and three *Varroa* phenotypes was analysed. The three traits were: initial phoretic *Varroa* load (June), final phoretic *Varroa* load (late July), and *Varroa* growth rate (VGR), calculated as the difference between the two timepoints over a 45-day period, corresponding to two brood cycles. The microbial community was characterized at three timepoints (June, July, and October) using high-throughput sequencing. Results showed significant association between microbiome diversity and the *Varroa* phenotypes. Colonies with higher initial phoretic *Varroa* loads in June exhibited increased alpha diversity at the same timepoint, with differences driven primarily by *Commensalibacter* and *Rhizobiaceae*. Similarly, colonies with a higher *Varroa* growth rate (VGR) showed significantly higher alpha diversity at October. However, no significant differences observed for final phoretic *Varroa* levels. These findings suggest that a more diverse and less conserved microbiota is associated with increased *Varroa* presence, potentially indicating a microbiome profile that reflects susceptibility. Understanding microbiome-phenotype interactions in honey bees provides valuable insights into colony health and opens new avenues for microbiome-based strategies in bee breeding and management programs.

Using geometry, maths and computers to better understand insect nutrition

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Understanding how insects regulate nutrient intake is essential for optimising mass-rearing systems, ecological research, and advancing precision nutrition. The Geometric Framework for Nutrition (GF) has revolutionised our understanding of dietary trade-offs by allowing multi-dimensional analysis of nutrient regulation and its effects on physiological traits. However, extracting meaningful insights from complex GF data remains a challenge. In this talk, I present novel approaches that integrate geometry, algebraic topology, and machine learning to enhance the analysis of nutritional landscapes. Specifically, I demonstrate how mathematical theorems such as Pythagoras' theorem can quantify nutrient trade-offs and rules of compromise in insect feeding behaviour. I also discuss how topological data analysis can reveal hidden structures in nutritional hypervolumes, providing insights into how dietary constraints shape insect life-history traits. These methodologies provide a computationally efficient and biologically meaningful framework to analyse insect nutrition, offering practical applications for sustainable insect mass-rearing, conservation biology, and evolutionary ecology. By bridging mathematics and biology, this interdisciplinary approach opens new avenues for understanding how insects navigate complex nutritional landscapes.

Session 46

Poster 12

Optimizing Black Soldier Fly Breeding: The Role of Humidity and Facilities

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Entomophagy presents a viable alternative to meet the increasing global food demand. Among edible insects, the black soldier fly (*Hermetia illucens*) stands out as a promising species. However, its production faces challenges in cold climates with limited sunlight. This study evaluated the impact of temperature and relative humidity (RH) on oviposition and adult population dynamics in experimental units. Adult black soldier flies were housed in two environments, both with two greenhouse cages built with PVC and greenhouse plastic. The indoor environment (IE) had the cages placed inside a shed, acclimated with two thermostats (turned on at 26°C and off at 30°C), two poultry heaters (150 Watts), one oil heater (1500 Watts), two colored LED lamps, and an ultrasonic humidifier used for moisture supply. The outdoor environment (OE) placed cages outside the shed, and the luminosity, temperature, and RH of the air were ambient, without mechanical influences. For both environments, temperature and RH of the cages were monitored using digital thermohydrometers. Correlation tests were performed between environmental parameters and the estimated adult population and egg mass in the two treatments, separately and simultaneously. The IE exhibited a higher adult population (520 ± 276) with an average egg mass of 15.7 ± 34.6 mg. In contrast, the OE showed a lower adult population (204 ± 128) with no oviposition observed. Among the environmental factors, RH displayed a strong correlation with the adult population in the IE ($r=0.8297$), where higher population levels were associated with increased humidity ($R^2=0.669$). Conversely, in the OE, all environmental parameters showed weak correlation indices, with a higher value of $r=0.233$ for maximum temperature. This is likely due to the lack of control over outdoor environmental conditions. These findings emphasize the impact of environmental conditions on fly reproduction, underscoring the need for facility-specific control measures. Since humidity has greater influence in oviposition and outdoor systems lack regulation over this parameter, an indoor system is recommended to optimize reproduction.

Evaluation of Black soldier fly larvae (*Hermetia illucens*) production under sub-optimal low-cost conditions M. Kerst¹, A. Raza¹, S. Pauling¹

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The rearing of black soldier fly larvae (*Hermetia illucens*) is a promising method for organic waste management and animal feed production. This study investigates the effects of temperature on the development and survivability of black soldier fly larvae in three semi-regulated temperature zones during February-March, in Kleve, Germany. One temperature zone was in a non-insulated, non-heated building (Z1), the second zone was in an insulated, heated but not temperature-controlled building (Z2) and the third zone was an insulated, temperature-controlled climate chamber (Z3). 5-day old neonates were reared on three different substrates for 7 days and the growth rate and survival rate of the larvae were observed. Z1 and Z2 represent typical infrastructure that is not optimized for larvae but can be suitable for the rearing process without further equipment or cost. The Z3 zone represented optimal growth conditions in an environment similar to industrial standards and served as an experiment control. The findings show the influence of substrate and temperature on the development and distribution of the larvae under sub-optimal conditions and provide valuable insights for improving small-scale black soldier fly farming with a low-cost approach.

Navigating Climate Challenges: Future Directions for Resilient Beekeeping Practices in Slovenia

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We monitored bee colonies in three apiaries utilizing different hive technologies (Alberti-Žnidaršič – AŽ 10-frame, AŽ 11+3 frame, Langstroth-Ruth – LR) from spring 2024. We implemented temperature sensors for measuring conditions within the hives and brood, complemented by electronic scales with remote data transmission for continuous weight tracking. A method for recording time and material usage was established at one location. Our preliminary results indicate that electronic scales are vital technological tools for real-time monitoring of apiary conditions, particularly regarding food consumption in spring and autumn, as well as during the foraging period. Initial measurements show some differences between the hive systems; however, definitive conclusions cannot yet be drawn as data monitoring must continue over multiple years. Monitoring temperature and humidity inside the hives has also proven beneficial, revealing how different hive technologies manage to maintain optimal brood temperature despite external weather changes. Fluctuations arise from thermal variations, with colonies managing these changes to varying degrees based on their living environment (hive and weather conditions). Continuous monitoring of these parameters throughout the year will yield significant insights into the characteristics of hive systems, the health status of colonies, and the energy expenditure of bee colonies in maintaining optimal conditions (considering the season, food consumption, survival, yield, and disease prevalence). The results of these measurements and observations will form a valuable foundation for calculating the economic viability of beekeeping in Slovenia, benefiting not only professional beekeepers but also allowing for accurate forecasting of future opportunities in beekeeping. Additionally, recommendations for sustainable and resilient beekeeping will be prepared considering the significant challenges posed by climate change and extreme weather events. This study is unique to our region, particularly due to the monitoring of beekeeping practices in two types of AŽ hives compared to LR hives. The results, arising from the efforts of the coming years, will be compared to similar studies conducted globally.

Genome-wide and Chromosome-wise Diversity Studies in Selected Goat Breeds from Asia, Africa, and Europe

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Goats (*Capra hircus*) are among the earliest domesticated livestock species, showing high genetic and phenotypic diversity due to natural adaptation and human-driven selection. This study examined the genetic diversity and population structure of 25 goat breeds from five geographic regions (West Asia, South Asia, West Africa, East Africa, Europe) using genome-wide and chromosome-wise analyses. Data from the AdaptMap project, including 53K SNP genotypes of 5,863 goats from 149 breeds, were analyzed. After quality control, 25 breeds (1,110 individuals) were selected, averaging 45 goats per breed (ranging from 9 to 159 individuals per breed). Principal Component Analysis (PCA) and ADMIXTURE assessed population structure, while F-statistics (F_{is} , F_{st}) and heterozygosity measures (H_e , H_o) quantified genetic diversity. Chromosome-wise ADMIXTURE explored ancestry proportions across autosomes, and TreeMix was used to infer phylogenetic relationships and migration events. The PCA revealed clear genetic differentiation among breeds, with European, African, and South Asian breeds forming distinct clusters. African breeds showed higher genetic diversity compared to European breeds, likely due to intensive selection for milk production. Chromosome-wise ADMIXTURE identified six chromosomes (1, 12, 16, 18, 19, 20) with unique ancestry patterns, suggesting historical introgression events. TreeMix analysis indicated three significant migration events, including gene flow from West African to South African breeds, from West Asian to East African breeds, and admixture between South Asian and West Asian breeds. The chromosome-wise ADMIXTURE results suggest that the six unique chromosomes represent a different timeframe for breed differentiation compared to the other chromosomes. These findings align with the migration events identified by TreeMix, highlighting the gene flow between South Asian and West Asian breeds. This study highlights the benefit of chromosome-wise analyses in uncovering fine-scale genetic diversity and historical admixture events in goat populations. The findings provide valuable insights for conservation strategies and breeding programs aimed at maintaining genetic diversity in goat breeds.

Genomic diversity and population structure of Nubian goats: Ancestry of Anglo-Nubian

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Nubian goats play a vital role in food security, providing milk and meat, and are widely distributed in Northeast Africa. In the 19th century, they were imported to UK and crossbred with Old English and Jamupari goats, forming Anglo-Nubian breed. This study examines the genetic diversity and population structure of Nubian goats, their relationship with Anglo-Nubian, and other African breeds. Anglo-Nubian genetic composition was analyzed to determine Nubian ancestry and identify selection signatures. A total of 2,750 animals were analyzed, including 429 with the SNP65K chip and 2,321 with GoatSNP50 chip. Genetic diversity and inbreeding were assessed using heterozygosity, and excess of homozygosity. Population structure was analyzed using FST, PCA, phylogenetic trees, and ADMIXTURE. Selection signatures were identified within RoH islands. Nubian from Algeria had the highest heterozygosity (40.39%), while Old English from Ireland had the lowest (26.98%), indicating reduced genetic diversity and higher inbreeding. Pairwise FST showed close relationships among Nubian populations but differentiation from Anglo-Nubian and Old English goats. Nubian from Egypt, Ethiopia, and Sudan clustered with North African goats, while Anglo-Nubian formed a separate cluster between Old English, African, and Middle Eastern breeds. ADMIXTURE identified five ancestral populations, with Anglo-Nubian forming a distinct group. Notable genes detected in RoH islands of Nubian populations include HSPA9, associated with heat stress resistance, and IL17D, involved in immune response. Future studies on whole-genome sequencing are crucial for deeper insight into the evolutionary history, selection patterns, and ancestry of Nubian and Anglo-Nubian goats.

Assessment of the connection between Manech Tête Rousse and Latxa Cara Rubia dairy sheep populations I. Granado-Tajada¹, E. Ugarte¹, J. M. Astruc², S. T. Rodríguez-Ramilo³

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Due to geographical proximity and common use of pastures in the Pyrenean Mountains, animal exchanges between Latxa Cara Rubia (LCR) and Manech Tête Rousse (MTR) sheep populations have been common over the years. Furthermore, the LCR breeding program has systematically imported semen from MTR since 2010. The availability of genomic information from both populations permitted the study of this connection at genome level. This work aimed at analysing how the genome of the LCR population has evolved after more than a decade of inseminations with MTR semen. Based on the LCR flockbook, 784 LCR and 765 MTR genotyped rams used for artificial insemination were identified. Coancestry between individuals was estimated on a SNP by SNP basis, and its evolution over time was assessed comparing old with young individuals. Population structure was studied with ADMIXTURE v1.3.0 while Runs of Homozygosity (ROH) were detected with detectRUNS software. The results over time showed an increase in coancestry within each population and between them (0.6% MTR, 1.5% LCR, 2% between them). Population structure analysis showed that MTR individuals have maintained their genetic background over time (20% LCR origin), while the LCR population has evolved (from 66% to 34% LCR origin), being nowadays closer to the MTR population. These results agree with the detection of one island of ROH on OAR6, which was previously reported in MTR, and it may be related with the presence of two candidate genes (NCAPG and LCORL) known to influence weight and height in sheep. In short, the constant use of MTR blood in LCR population can be detected at genome level, reflecting that currently both populations are more similar. The coancestry between LCR and MTR has increased more than within populations, and they share ROH islands. Therefore, these populations are getting closer, and their evolution tends to join in the same genetic line.

Session 47

Theatre 4

Exploring the genetic diversity of fat-tailed Chios and thin-tailed Frizarta dairy sheep

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Local sheep breeds possess unique resilience and adaptation potential. Monitoring their genetic diversity is a key contributor to sustainable sheep production. In this study we assessed (i) the genetic diversity of two Greek dairy sheep breeds, the fat-tailed Chios and the thin-tailed Frizarta, (ii) the genetic distance between them, (iii) their inbreeding levels and (iv) their relatedness to other breeds. We genotyped 1,033 sheep using Illumina's OvineSNP50 Genotyping Beadchip. Edits removed samples with call rate <90%, and single nucleotide polymorphisms (SNPs) with call rate <97% and minor allele frequency <2% (consistent with literature to retain rare SNPs) and those deviating from Hardy-Weinberg equilibrium ($P < 10^{-6}$). Post quality control 665 Chios and 362 Frizarta genotypes ($n=42,588$ autosomal SNPs) were used to assess within-breed observed and expected heterozygosity, nucleotide diversity, linkage disequilibrium, historic effective population size and Wright's inbreeding coefficient (FIS). Runs of homozygosity (ROH) throughout the genome were detected and inbreeding coefficient FROH was estimated. Genetic distance between breeds was assessed based on F_{ST} (Fixation index). Representative samples of Chios ($n=36$) and Frizarta ($n=29$), identified according to similarity threshold of 0.75 and 3 k-dimensions, as well as 364 sheep genotypes of 14 dairy breeds from Europe and Asia ($n=44,004$ SNPs) were used to assess genetic relatedness with admixture and Principal Component Analyses (PCA). Data were analyzed with Arlequin, PLINK, SNeP, R BITE and ADMIXTURE software. Results suggest that Chios and Frizarta breeds maintain adequate levels of genetic diversity and are well differentiated ($F_{ST}=0.071$). Chios population was less heterozygous with higher levels of homozygosity. Admixture and PCA showed that Chios is related to East Mediterranean fat-tailed breeds, whereas Frizarta to breeds of European origin. Results contribute to genetic diversity management in the studied populations. The work was funded by SMARTER (772787) and iSAGE (679302) Horizon2020 projects.

Genetic diversity and population structure of local goat breeds in Burkina Faso using SNP Markers

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Genetic diversity and population structure of Burkina Faso goats using SNP markers was investigated in this study. Neogen blood cards were used to collect blood sample from 561 animals in two agroecological zones: Sudano-Sahelian (SS) and Sudanian zone (SD). The animals were part of five Community Based Breeding Programs (CBBP) implemented in the areas. Genomic DNA was extracted using the GenElute™ Blood Genomic DNA Kit and genotyped with the Illumina goat SNP 50K chip, featuring 67,076 SNPs. The final sample included 545 animals and 51,932 SNPs after quality control. A Principal Component Analysis was performed on these 545 individuals, with 83 additional goats from three West African populations [Sahel goats (SAH) and Djallonke (DJA) from Burkina Faso, and Sahelian (SHL) from Nigeria], filtered by common SNPs. Results of principal component and admixture analyses confirmed the genetic background of two distinct ancestral populations (Sahelian and Djallonke) and levels of admixture in all five breeding populations. Inbreeding levels based on Runs of Homozygosity >4 Mb were low, population averages ranging from 0.023 (SS) to 0.029 (SD). Structure and degree of admixture of the Burkinabé goat population were assessed using STRUCTURE software and showed a significant introgression of the Sahelian goat, following a North-South cline. Although overall differentiation is poor ($F_{ST} = 0.025 \pm 0.035$), the current analysis clearly suggests that genetic variation in Burkina Faso goat follows this North to South cline. This may reflect the adaptation of goat populations to different agroecological environments, with marked genetic as well as phenotypic differences between the zones. The sustainable use of these adapted local goat genetic resources calls for the promotion of sustainable genetic improvement using participatory breeding approaches.

Session 47

Theatre 6

Identification of Functional SNPs Affecting Alternative Splicing in Sheep Challenged with Gastrointestinal Nematode Infections

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Gastrointestinal nematode (GIN) infections pose significant challenges to sheep health and productivity worldwide, driving the need for genetic strategies to enhance resistance as a sustainable solution. Alternative splicing (AS) has emerged as an important post-transcriptional process affecting host-pathogen interactions. However, its role in providing resistance to GIN infections in sheep remains poorly understood. This study aimed to identify potential functional single nucleotide polymorphisms (SNPs) associated with AS events using RNA-sequencing data contributing to natural resistance to GIN infections in sheep. We utilized publicly available RNA-sequencing data from the NCBI Gene Expression Omnibus database (Accession ID: GSE63547), comprising 20 abomasal lymph node samples collected from the most resistant (R; n=10) and most susceptible (S; n=10) Scottish Blackface lambs challenged with *Teladorsagia circumcincta* nematode. Quality control and sequence alignment to the Oar_rambouillet_v.3 reference genome were performed using CLC Genomics Workbench v.20.0.4. SNP discovery was performed using the fixed ploidy variant detection tool to identify functional SNPs with splice site effects (SSE) uniquely fixed in R and S groups. These SNPs were further explored for their association with phenotypic variation in response to GIN infections through AS. The Ensembl VEP was used to assess the functional consequences of the identified SNPs, while functional analysis was conducted using DAVID. A total of 202213 and 193198 unique SNPs were identified in R and S groups, respectively, and 369423 were shared between the two groups. Among them, 269 and 257 unique SNPs with SSE were identified in R and S groups, respectively. Primarily, metabolic pathways linked to essential cellular functions were significantly enriched in the R group, while immune response pathways were enriched in the S group ($FDR < 0.05$). These findings contribute to a better understanding of the mechanisms underlying the immune response of sheep to GIN infections.

Identification of Candidate Genes for Heat Adaptation in Hair Sheep: A Comparative Liver Gene Expression Study

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The ability to withstand high temperatures has become crucial for mitigating the negative impacts of heat stress, although this capacity differs between animals. Identifying candidate genes associated with heat adaptation traits is crucial for enhancing heat tolerance and selection strategies for livestock in hot climates. This study aimed to identify liver-specific candidate genes that play a vital role in the heat stress response of Santa Ines sheep, a hair breed that displays varying degrees of heat tolerance. Eighty sheep were placed in a climate-controlled chamber for 10 days and maintained at an average temperature of 36 °C from 10 a.m. to 4 p.m. and 28 °C from 4 p.m. to 10 a.m. To evaluate heat dissipation via rectal temperature, 14 animals showing extreme phenotypes, seven heat-tolerant and seven heat-sensitive animals, were chosen for liver sampling to conduct RNA sequencing and differential gene expression analysis. They were classified based on an individual's ability to dissipate heat after the heat-stress period. The heat-tolerant group had an average rectal temperature of 38.77 ± 0.019 °C, whereas the heat-sensitive group had an average of 39.10 ± 0.021 °C ($P < 0.01$). The significance threshold for identifying differentially expressed genes was set using False Discovery Rate (FDR) correction with a threshold of ≤ 0.05 . 14,491 genes were expressed in sheep liver samples, of which 7 genes were differentially expressed ($FDR < 0.05$). Heat-tolerant sheep exhibited higher expression of six genes GPx3, RGS6, GPAT3, VLDLR, IGHG1, and EVC, which are associated with the Hedgehog signaling pathway, glutathione metabolism, glycerolipid metabolism, and thyroid hormone synthesis. COL1A1, which is expressed at higher levels in the liver of the heat-sensitive group, contains instructions for producing a segment of the complex molecule known as type I collagen. The enhanced activity of these pathways in heat-tolerant Santa Ines sheep may help to alleviate the adverse effects of heat stress, resulting in greater resistance to high temperatures. Future research should examine these gene pathway mechanisms and assess their relevance across breeds using larger sample sizes.

Session 48

Theatre 1

Definition, interests and usages of serious games in agricultural context

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Serious games are increasingly recognized as valuable tools for learning, decision-making, conception, design practices, and fostering collaboration, particularly in the context of sustainability transitions. Despite the critical role of agriculture in these transitions, research on the use of serious games in this field remains limited, with even less focus on their connection to sustainability issues. This introductory communication presents some definitions of serious game, why they are suitable to use to deal with wicked problems and explore their usage in agriculture through time. French scholars have notably contributed to the definition of games by focusing on the experiential and cognitive dimensions of play, distinguishing between game mechanics and player engagement. Serious games are now recognized as a multidisciplinary research area, extending beyond digital formats to include board games, simulations, and other interactive experiences. Their applications range from education and collaborative ideation to knowledge sharing and problem-solving. Literature highlighted their value in overcoming barriers to stakeholder engagement but also their capacity to mobilize student in other ways than lessons. To explore their potential contribution to sustainability a systematic review has been done. The review encompassed 237 articles, including 182 empirical studies, revealing a growing global interest in serious games for agriculture. These games serve multiple functions, including education on specific topics, mediation and co-design, and research facilitation. They enable diverse stakeholders to collaborate effectively in addressing complex sustainability challenges. However, despite their promise, there is a need for more rigorous evaluation of their real-world impact. There is also a real need to raise awareness of the existing game in order to ensure better practices in their conception and utilization.

Tierschutz erLeben – Enhancing Animal Welfare Education through Gamified Learning

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Tierschutz erLeben is an innovative educational platform designed to enhance animal welfare education through a gamified approach, primarily for agriculture students. Traditional teaching methods often struggle to effectively convey the complexities of decision-making and their real-world consequences. Our platform leverages interactive storytelling, digital comics, and the ‘comic automaton’ to create a dynamic and immersive learning experience in English and German. The platform covers key topics related to cattle, sheep, and beef production, structuring them into subtopics that guide students through engaging narratives. The visual and relatable storytelling approach makes learning more accessible and engaging. A core feature is learning through active creation. The comic automaton enables students to construct their own comics, allowing them to explore different perspectives, develop empathy, and deepen their understanding of complex processes. A free mode for open-ended storytelling and a structured mode with task cards that provide creative prompts and guidance are offered. This approach not only fosters analytical thinking but also encourages users to reflect on their own decision-making. The platform has undergone continuous refinement with student involvement at various stages of development. Moderated workshops and feedback sessions have provided valuable insights, leading to improvements in usability, flexibility, and saving features. Students have responded positively, highlighting the platform’s engaging and interactive nature. Additionally, an integrated learning diary helps them document key insights, track progress, and reflect on their learning journey. By combining gamification, interactive storytelling, and hands-on learning, Tierschutz erLeben represents a novel approach to animal welfare education. It equips students with the knowledge and decision-making skills necessary to ensure sustainable animal care, bridging the gap between theoretical knowledge and practical application.

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Implementing circularity of agricultural products in a region: is it that easy? Raising awareness on its complexity with an educative serious game

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CircAgri is an educational serious game aimed to raise awareness on the complexity to implement circularity in an agricultural region. It was built for students in agricultural and environmental studies who may be confronted to similar cases in the future. We designed it for five players who, each, endorse the role and activities of a farmer (with a crop, livestock or mixed orientation), a biogas plant or a cooperative. Each player manages a combination of activities that require and produce given types and amounts of products. They monitor their individual level of treasury and three collective regional indicators related to environmental impacts (i.e. excess of fertilizers and manure), dependence to the external market, production of food and energy to match a regional demand. Each year is divided in four steps where players simultaneously engage in: i) planning and proceeding to local exchanges/sales of the biomass they produce and need; ii) buying and selling the remaining biomass to an external market; iii) updating regional indicators; iv) adjusting individual combination of activities. A game session lasts for about 2,5 hours and is composed of four years followed by a debriefing.

COWLEARNING - The complex world of dairy and beef supply – an Alternate Reality Game

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Urban young adults inevitably engage with food supply through daily shopping, yet often lack awareness of its complexities due to the spatial and cognitive separation between consumption and production. Reaching this target group requires innovative approaches that not only provide information, but also encourage critical thinking and reflective consumer behaviour. The interactive WhatsApp mystery game ‘Cow Chat Crime’ provides a playful way to explore the complexities and challenges of a sustainable dairy and beef supply. The game, an alternate reality game, highlights the interrelationships of the supply of animal-based foods and how individual consumption choices impact it and have far-reaching consequences for the environment, animals and people. The aim is to inspire young adults to take responsibility for a sustainable future. The game is the result of the research project COWLEARNING <https://cowlearning.boku.ac.at/> (funded by FWF CM 400B), in which scientists from BOKU University, the University of Veterinary Medicine Vienna and professional game developers and stakeholder worked together. By combining real-life elements with digital media, the boundaries between reality and fiction are blurred and the player becomes part of a gripping story. The interactive crime thriller presents players with choices and ensures a realistic gaming experience. Multimedia content keeps players engaged with intriguing challenges, videos, and secrets. The focus is on sustainability: the game shows how humans, animals and nature are connected (the game is based on the One Health/One Welfare approach). It can also be played flexibly – in one go or in stages. The player decides when and where to immerse himself/herself in the story. Click here to play the game: <https://waurl.me/vw70tba0f>

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The « farmer's and advisory journey », a tool for advisors to reflect on change in farming and their role as advisors

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Agricultural advisory services all over the world combine advisory interventions in order to support farm management, farmer's decision making and eventually behavior change of farmers in order to foster the adoption of practices for agro-ecological transitions, climate-smart farming or other agendas. In the frame of the ClimateSmartAdvisors project (2023-2030), we organized focus groups in 10 different European countries, to have advisors to reflect about their practices, tools & approaches used, and their impact on farmers' decision making in the context of Climate-Smart Farming. We observed important differences between countries, and used these results to design a tool with cards to support advisors in reflecting on their advisory practices. Called «the farmer's and advisory journey », this tool has cards representing the steps farmers engage in (like troubles, doubts, awareness, desire to explore, trials, dropouts...) and cards representing possible advisory interventions which can support the farmer in its process. It has been used again in different contexts: training for advisors, peer-learning in Communities of Practice (groups of advisors) and with students. Feedback of the persons who used the tool were collected, asking for the adaptation they made, their difficulties and the outcomes reached. For the advisors involved, this tool has been beneficial as it fosters discussion in a group about different views and roles of advisors, various interventions type, variety of farmers' types and objectives... Through the exercise, the advisors identified their strengths, training needs, capacities they would like to develop, needs for collaboration with other advisors and advisory services – and not least the motivation to work with the identified challenges. This work invites to reflection on the types of advisory roles and their articulations, and on the learning processes which better support sustainability positions and systemic change. The workshop format and tools can be adapted to many different contexts and purposes.

ARTIFICE, a serious game to boost crop-livestock integrations at local level

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In recent years, French agriculture has been marked by an increasing greening of land and livestock farming has declined in many regions. Nevertheless, to promote the agro-ecological transition, we could use the potential of circularity based on livestock farming to advance sustainable and healthier food systems. Many research and extension projects are being developed to help farmers close material cycles, add value to production and promote human contact and knowledge exchange. The FICELLE project focuses on the interactions between crops and livestock at local level in four agricultural areas characterised by archetypal farming systems: mixed cropping system, mixed cropping-livestock system, mountain livestock system, intensive livestock system. The stake of this project is to reconnect farmers, livestock breeders and local stakeholders through interactions based on local resources. To support A serious game, ARTIFICE, has been developed to improve the combination of available natural, economic, social and technical resources. The aim of the game is to examine all the types of resources available in a given area and to generate ideas on how to combine them to solve local problems. A pack of cards represents the different resources identified in the four zones studied. Players successively propose a resource card to activate, and another player proposes to activate it with one of his own. This is a collaborative game, in which the challenge is to succeed in developing crop-livestock interactions in the area, while earning points. These points are used to meet a common challenge, chosen at the outset and aimed at strengthening ecosystem services, territorial anchorage, economic added value or improving farmers' work. This game could be used in a participatory workshop with farmers to highlight the obstacles and levers to the development of crop-livestock interactions in the different project zones. The game highlights the possibilities for activating resources to stimulate local interactions. Players can be farmers, agricultural advisors, local stakeholders or political decision-makers who want to work together to set up a territorial synergy for sustainable food production.

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Dynamix serious game : a Swiss knife to co-design collaborations between neighbouring crop and livestock farmers?

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Crop-livestock integration beyond the farm level consists in exchanges of grain, fodder or manure between neighbouring farms. This is a relevant option toward agroecological transition as it limits inputs while addressing limiting work availability at farm level. However, such collaboration between neighbouring farmers is challenging because it requires collective redesign to address technical and organizational issues. We developed the serious game Dynamix to support the co-design scenarios of crop-livestock integration among neighbouring farms. The serious game Dynamix is based on a spatially-explicit board game associated to a need-offer balance for each type of grain, fodder, manure or animals which could be exchanged or sold between farms. A multicriteria evaluation of the scenarios is led at the individual farm and group levels using the simulation model included in Dynamix. The game session lasts about 2 hours but is part of a broader six-step participatory approach that includes i) before the game session, an initial group meeting to define the problem and farmer interviews to identify motivations and collect technical and economic farm data and ii) after the game session, a group meeting to discuss the results and monitoring of selected scenario implementation. We applied this whole methodology with two groups of farmers in southwestern France. The Dynamix game includes an explicit collective map to co-design the flows and logistics of integration. Beyond discussing need-offer balances of grain, fodder and manure, the map allows to discuss the needs for storage or transportation and new actors to involve in the scenarios. Considering explicitly trust and equity between farmers in the scenarios is a key aspect to avoid hitting a brick wall. The serious game Dynamix was easily scaled-out to various agricultural contexts across Europe, but the right timing in the participatory approach required particular attention. Dynamix was initially developed to assist the collective design with farmers, but has been adapted successfully as a role-playing game for students or advisers to learn how to reconnect crop and livestock systems.

Highlighting the services of cattle farming in Madagascar central highlands through a serious game

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In Madagascar Central highlands, cattle farming plays a strategic role on family farms, both for its economic value and for the services it provides to the territory, e.g., organic fertilisation production, soil preparation, animal traction or household capital. In the DINAAMICC project, a serious game representing agricultural activities in a typical landscape of the highlands has been built. As part of a participatory approach, it has been used as a tool for analysing and discussing different dynamics on a territorial scale through sessions involving various stakeholders: farmers, stockbreeders, local authorities, technicians. This game highlights the stakes between cattle farming and other agricultural activities in terms of resource allocation, while raising awareness of its importance in the sustainability of agro-sylvo-pastoral systems. A key aspect of the game is the integration of contextual elements specific to local realities, in particular the threat of the Dahalo (cattle thieves). Often regarded as a tradition, livestock theft is a major constraint that weakens farming systems and has a cascading effect: by reducing livestock cattle, it leads to lower supplies of organic fertilizers and a weakening of household capital. By simulating these disruptions, the game enables participants to gain a better understanding of the issues involved in securing livestock production and its effects on the whole farming system. If it is not a tool for political decision-making, the game enables to enlighten debates about importance of livestock farming in family farming and rural areas sustainability. Keywords: Serious game, cattle farming, biomass management, territory, sustainable

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La Grange (The Barn) - A serious game to reveal trade-offs and synergies among stakeholders in livestock farming areas

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Livestock farming systems have received particular attention in the news and scientific media. However, do these considerations ever come to a real debate? Nothing is less certain. There is much attention on the negative impacts of livestock farming on the environment, and it remains difficult to have a fruitful dialogue on the desirable developments of livestock farming areas. This is also true at a local scale, where antagonisms among stakeholders can be strong. We present La Grange®, a generic serious game that reveals trade-offs and synergies in livestock farming areas. Its aim is to promote horizontal knowledge and dialogue among stakeholders on locally-adapted transition for livestock farming. After presenting the conceptual model underlying the game with the different game components and phases, we report the outputs of four case studies with groups of farmers or students in agriculture. We show that La Grange® fills a gap in the toolbox for understanding livestock farming, making it possible to integrate generic and local knowledge and incorporating its biotechnical, environmental, economic, and sociocultural dimensions. In each case study, La Grange® not only revealed trade-offs and synergies between these dimensions, but also allowed new cooperation and strategic roadmaps to be set up for a livestock farming area. Both farmers and students perceived the game as a less top-down way of learning. When played with citizens, two of them joined the steering committee on an experimental farm after the game session. As a low-tech, easy-to-access game, La Grange® can be easily used by extension services and agricultural teachers. Outputs from the four case-studies makes it possible to classify La Grange® as a “Game for Change” due to its impact on supporting sustainability.

Guardians of diversity: The role of traditional livestock breeds in sustainable grassland farming

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During the last century, livestock farming has seen an enormous increase in livestock productivity – mainly caused by output-oriented breeding. Today, the vast majority of livestock population consists of specialized dairy or beef breeds. On the contrary, livestock numbers of traditional, dual-purpose breeds have declined – some breeds even disappeared. However, traditional breeds also have an underestimated potential in delivering ecosystem services beyond the production of milk and meat. Based on several own observational studies and grazing experiments, and on a broad literature review, we illustrate how modern breeding unintentionally changed grazing-relevant animal characteristics. Selection for high output modified animal anatomy such as body weight and claw. Moreover, breeding altered foraging and movement behavior. These trends impair biodiversity and sustainability of pastures – especially under challenging environmental conditions, such as steep slopes, low forage quality and harsh weather. In these environments, modern breeds struggle to cope with their genetically pre-defined potential of productivity. In this case, traditional breeds can be even more productive than so-called highly productive breeds. Moreover, the elevated nutritional demands, increased body size and decreased robustness of modern breeds lead to an unsustainable use of heterogeneous pastures. By clearly preferring the most attractive places of a paddock, modern breeds cause an overuse of these spots, whereas less attractive areas are underused. Both over- and underuse have negative long-term consequences, including loss of pastureland and biodiversity, shrub-encroachment, erosion and a reduced attractiveness of the landscape. We demonstrate, how traditional breeds maintain these important services for society and allow a sustainable use of low-productive grasslands.

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Evaluating the Influence of Climatic Variables on Growth and Adaptation of Podolica Bulls in Pre-Performance Phases

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The Podolica breed (PO) is well known for its adaptability to the challenging environmental conditions of Southern Italy, where extensive farming plays a crucial role in land preservation. Despite its recognized resilience, the early growth phases of this breed remain understudied, particularly in relation to climatic stressors. This study aims to assess the influence of environmental factors on the pre-performance growth of Podolica bulls. A dataset of 21 environmental variables was examined using linear mixed models, incorporating age in days as a fixed effect and herd-year as a random effect. Among the climatic parameters tested, relative humidity (RH) emerged as the most significant factor affecting entry weight and average daily gain (ADG). Specifically, calves experiencing higher RH levels (>80%) had entry weights up to 20% lower (~240 kg vs. ~300 kg) compared to those raised in drier conditions. Similarly, ADG showed a ~24% reduction under high RH, dropping from 0.92 kg/day to 0.70 kg/day, suggesting increased thermoregulatory stress. Conversely, the temperature-humidity index (THI) showed a complementary effect, with moderate values (68-72) favoring metabolic efficiency and improving growth rates. Seasonal trends revealed that most births occurred in winter (January-March, ~65% of total), while weight assessments started in autumn, coinciding with cold and humid conditions that may have hindered early development. These findings highlight the necessity of adjusting breeding schedules, optimizing housing ventilation, and monitoring THI thresholds to mitigate climatic stressors and enhance animal performance. This study reinforces the importance of considering environmental adaptation strategies in the sustainable management of Podolica cattle, particularly in marginal areas affected by climate variability.

Residual feed intake of Fleckvieh dairy cows on two farms with different concentrate levels

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Feed efficiency is influenced by several factors, including genetics, diet composition and management. Cows fed a ration with a high proportion of concentrates tend to produce more milk, which might result in higher gross efficiency. The objective of this study was to compare the feed efficiency, defined as residual feed intake (RFI), of two farms with different concentrate levels. Performance data were available for 54 Fleckvieh cows from research farms in Germany (Achselschwang, DE; n = 37) and Austria (Raumberg-Gumpenstein, AT; n = 17). The German farm had an average concentrate percentage of 41.3%, compared to 19.2% at the Austrian farm. Weekly RFI was calculated between 60-180 days in milk. Statistical analyses, including a t-test and a mixed model, were conducted to examine the differences in RFI. Additionally, energy-corrected milk (ECM) and dry matter intake (DMI) were compared between the two farms. The mean RFI for the German and Austrian farms was 0.43 kg DM/d and 0.44 kg DM/d, respectively, with no significant difference between them (P = 0.94). The mixed model also showed no significant effect of the farm on RFI (P = 0.88). However, there were significant differences in mean ECM and mean DMI between the two farms (P < 0.001). The German farm had a higher mean ECM (39.9 kg/d) and DMI (25.9 kg/d), compared to the Austrian farm (22.5 kg/d and 17.8 kg/d, respectively). In conclusion, despite significant differences in feed intake and milk yield, the farms did not differ in their mean feed efficiency.

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Carcase results of intensively vs extensively fattened dairy-beef crosses in Switzerland

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Switzerland is a pioneer of commercial channels for the extensive fattening of dairy-beef crosses. As part of the EU Horizon Project INTAQT, we collected feeding and slaughter information of 24 extensively (EF) and 30 intensively fattened (IF) dairy-beef crosses. Additionally, 19 out of the 24 EF carcasses and 28 of the 30 IF carcasses were assessed at the 10th thoracic vertebrae using the Meat Standards Australia (MSA) grading scheme. Rations of EF farms (n=5) were composed of over 95% of fresh or conserved grass, less than 5% whole-plant maize silage, 0.3% minerals and 0% concentrates. Intensive fatteners (n=5) fed on average 43% concentrates, 41% whole-plant silages or sugar beet, less than 15% conserved grass, 1.1% buffers or straw, and 0.6% minerals. EF animals were composed of 67% steers and 33% heifers, against 90% bulls and 10% steers for IF. The average carcass weight and slaughter age with EF was 289 kg at 739 days, versus 288 kg at 393 days with IF. Despite a marked difference in slaughter age, average MSA ossification scores of EF animals were similar to those of IF animals (151 vs 146). EF carcasses showed lower levels of subcutaneous fat, characterized by lower average fatness scores (2.7 vs 2.9) and less total rib fat (5.4 mm vs 6.6 mm), than intensively fattened animals. Yet, MSA marbling scores were higher for EF than IF animals (316 vs 239). Lastly, the Eye Muscle Area of EF animals (67 sq cm) was smaller than of IF animals (79 sq cm). Our study suggests that extensive fattening systems of dairy-beef crosses are particularly adapted to Swiss premium beef markets, as the carcasses promise limited levels of subcutaneous fat, higher levels of marbling and smaller steak sizes.

Greenhouse gas emissions from pasture-based beef production - should they only burden the meat?

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Beef production supplies nutrient-dense human foods, but at a high climate cost. However, many ruminant systems are multi-functional also providing other ecosystem services (ES) than food to the society, e.g. nature conservation of biodiverse habitats and maintenance of open landscapes. Traditionally, carbon footprint (CF) of beef production determined using life cycle assessment (LCA) has been attributed only to the meat produced whereas other ES have been overlooked, which may risk that positive contributions of beef production to the society are ignored. Economic allocation, using revenues from agri-environmental payments and supports as a proxy for the economic value of the ES, is one way to partition the CF of beef production on other ES in addition to the meat. In this study, CF was allocated in this way for crossbred heifers born to a dairy cow and a beef breed sire, raised at either moderately high (21 mo. slaughter age) or low (27 mo. slaughter age) intensity, grazing one or two summers on semi-natural pastures, respectively, in three regions of Sweden. Quantification was done using LCA, attributing the CF to meat and other ES by economic allocation. The results revealed that 13% (min) to 47% (max) of the CF of production could be attributed to ES other than food, depending on production model and region. For 27 mo. heifers, taking ES into account reduced their CF by 9.3 ($\pm$ 2.0) CO₂e/kg carcass weight (CW), whereas for 21 mo. heifers the reduction was only 3.0 ($\pm$ 1.2) CO₂e/kg CW. The 27 mo. heifers still had higher CF than the 21 mo. heifers after allocation to ES, but the difference between the intensities was reduced from 10.0 ($\pm$ 1.8) to 3.6 ($\pm$ 1.0) CO₂e/kg CW. Hence, low intensity of production and large area of semi-natural pastures were essential for decreasing the proportion of CF of the meat. Although emissions still remain, using ES-corrected climate impact puts CF of beef in a new perspective, which can be most valuable when communicating with consumers and in political decision-making.

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Associations between vaginal discharge score and health status, milk yield, and reproduction in seasonal-calving pasture-based dairy cows

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This study aimed to evaluate the associations between vaginal discharge score (VDS) and health status, milk yield, and reproduction in spring-calving pasture-based dairy cows. At 3 $\pm$ 1, 6 $\pm$ 1, and 14 $\pm$ 1 days in milk (DIM), blood samples and rectal temperature data were collected from 262 lactating cows. Vaginal discharge score was recorded at 14 DIM (0: no mucus; 1: clear mucus; 2: clear mucus and flecks of pus; 3: mucopurulent with < 50% pus; 4: mucopurulent with $\geq$ 50% pus; 5: watery, brown/reddish and fetid). Cows were grouped by VDS [$\leq$ 2 (VDS2: n = 129), 3 (VDS3: n = 63) or 4+ (VDS4: n = 70)]. Presence or absence of a corpus luteum (CL) was assessed using ultrasonography at 21 $\pm$ 3, 35 $\pm$ 3, and 49 $\pm$ 3 DIM. Binary data were analyzed using logistic regression and continuous data using linear regression. Compared with VDS2 cows, VDS4 cows had a higher ($P < 0.01$) frequency of fever ($>39.3^{\circ}\text{C}$; 24 vs 4%). Cows in the VDS4 group had greater ($P < 0.01$) blood concentration of haptoglobin (Hp) at 3 and 6 DIM compared with cows in the VDS2 and VDS3 groups. Overall, cows in the VDS4 group had lower concentrations of Mg ($P < 0.01$) and Ca ($P = 0.06$) compared with cows in the VDS2 group. The association between VDS and NEFA was conditional to DIM ($P < 0.01$), whereby cows in the VDS4 group had higher NEFA at 3 DIM compared with VDS2, but concentrations were similar across groups at 6 and 14 DIM. Cows in the VDS3 and VDS4 groups had a lower likelihood of having a CL present at 21 DIM [$P < 0.01$; VDS3 (OR = 0.4, 95% CI = 0.2-0.7); VDS4 (OR = 0.4, 95% CI = 0.2-0.7)] and 49 DIM [$P = 0.03$; VDS3 (OR = 0.3, 95% CI = 0.1-0.7); VDS4 (OR = 0.4, 95% CI = 0.2-1.0)] compared with cows in the VDS2 group. Pregnancy to first artificial insemination was similar ($P = 0.18$) between VDS groups. The association between VDS and milk yield was conditional to DIM ($P = 0.07$), whereby milk yield tended to be lower for cows in the VDS4 group compared with the VDS2 group at 6, 9, 11, and 18 DIM. In conclusion, higher VDS is associated with impaired health, delayed resumption of cyclicity and reduced milk yield in spring-calving pasture-based dairy cows.

Fattening of German Holstein steers in a silvopastoral system

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Dairy farming produces not only milk and heifers but also male calves as a by-product. A key issue is the fate of male dairy calves, which often lack sustainable fattening options. In addition, the current handling of these calves, in particular their export to other European and non-European countries, is increasingly the subject of criticism. However, data on organic fattening procedures for male dairy offspring of the currently used Holstein genetics is rare. Therefore, we are evaluating a grass-based fattening system in a silvopastoral environment with German Holstein steers under organic conditions. The trial is part of a larger experiment that will be completed in 2025. The study was conducted with 26 steers in the first fattening run, divided into two groups: one with access to a fodder hedge and one without. Animals underwent two grazing seasons with winter housing in between. Live weight gain was recorded weekly, and carcass traits and meat quality parameters were analyzed after slaughter. Statistical analyses included a linear mixed model and ANOVA to assess treatment effects. Fattening performance was within expected ranges (weight 599 ± 47 kg and age 629 ± 29 d at slaughter). Even without concentrate supplementation during grazing, steers achieved weight gains comparable to Holstein-Friesian bulls. Hedge access had no significant effect on relative weight gain ($p > 0.05$). Meat quality parameters met high standards, with steers exhibiting a high intramuscular fat ($4.4 \pm 1.4\%$) deposition. The access to the fodder hedge showed no significant effect on meat quality ($p > 0.05$). The results suggest that high performance and excellent meat quality can be achieved with German Holstein steers in a pasture-based system using limited concentrate feed. This approach provides a sustainable alternative to conventional beef production, meeting societal expectations for improved animal welfare and environmental sustainability.

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Theatre 8

Milk yield and Composition in Organic High-Input and Low-Input Feeding Systems

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Organic dairy farming is expanding due to the demand for sustainable, animal-friendly products. Feeding strategies impact milk quality, cow health, and efficiency. High-input (HI) systems are characterized by the use of corn silage and high concentrate ratios to increase milk yield, while low-input (LI) systems rely on pastures and minimize corn silage feeding to reduce competition with crops for human consumption. This study, conducted at Gladbacherhof Experimental Farm (JLU Giessen) with Holstein cows in an organic dairy system as part of the GreenDairy project, investigates the impact of HI and LI feeding systems on milk yield and composition in organic dairy farming. Cows were divided into two feeding groups: HI, receiving corn silage and high concentrate ratios, and LI, without corn silage and with a lower concentrate ratio. Both groups received grass and alfalfa silage, plus pasture access from April to October. They were housed separately in a loose-housing barn equipped with an automatic milking system (AMS) and an automatic feeding system. Milk yield, fat, and protein content were recorded by the AMS from January 1 to December 31, 2024. Milk production was standardized to a 305-day lactation period, and yield was adjusted to energy-corrected milk (ECM) using the standard formula. Statistical analyses were conducted using SAS (MIXED procedure) after normality testing. The REML model included feeding group, month, days in milk, lactation number, and their interactions. ECM yield of HI cows was over 40% higher than that of LI cows (32.4 vs. 22.7 kg/d), likely due to the higher energy intake from corn silage and concentrate. LI cows had higher fat (4.43% vs. 4.26%) and protein (3.27% vs. 3.19%) content, reflecting a forage-rich diet. In conclusion, the feeding strategy influences the balance between milk yield and composition in organic dairy farming.

Improving the nutritional quality of ewe milk through animal feeding practices in grassland systems

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Grazing is often highlighted for its positive effects on the quality of dairy products. However, this has been little studied on French dairy ewe grassland systems. In Occitanie (southern France), grass represents over 80% of animals diet, and grazing is widespread. In this context, we studied the links between the various feeding strategies developed by these farms and the nutritional quality of fat in ewe milk. To this end, 41 surveys were carried out on dairy ewe farms, enabling to identify different feeding strategies implemented by farmers, using clustering. Bulk milk was also collected from each farm on the 120th day of lactation, during the animals grazing period, and analyzed by gas chromatography to determine its fatty acid (FA) profile. An analysis of variance was used to compare these profiles between feeding strategies. Five strategies were identified: diets based on rangeland grazing without complements (D1); diets based on legume hay and pasture with complements (D2); diets based on grass hay, silage and pasture with nitrogen complements (D3); diets based on legume hay with high level of complements (D4); and diets based on legume silage with complements (D5). The results showed that 4 of these strategies improved significantly the nutritional quality of ewe milk, but in a different manner. For instance, D2 strategy provided a better $\omega 6/\omega 3$ ratio in milk (1.64 vs 1.89 in other diets). D1 and D3 strategies promoted trans-11 FA way of biohydrogenation over trans-10 (trans-11/trans-10 ratio respectively of 12.57 and 12.04 vs 8.88 in other diets). D5 strategy promoted the activity of $\Delta 9$ -desaturase (C14:1/C14:0 ratio of 0.024 vs 0.020 in other diets). Strategy D4 gave the worst results in terms of nutritional quality of milk. These results show that the agroecological practices involving the use of grass for animal feeding are likely to improve the nutritional quality of milk fat at various levels.

Session 49

Poster 10

Study regarding effects of climatic conditions on milk production in dual-purpose cows

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The aim of the study was to assess the influence of climatic conditions (air temperature and humidity) on the milk production of dual-purpose cows. Temperature-humidity index (THI) was used for climate expression, calculated with data from a local weather station, using the following equation $THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$. Six classes of THI were used in analysis: 21-30 (G1), 31-40 (G2), 41-50 (G3), 51-60 (G4), 61-70 (G5), and above 70 (G6). The value of THI was calculated with the values of temperature and humidity in the test day and associated with test day milk yield (TDM) and test day fat and protein percentage (TDF and TDP, respectively). A number of 11869 records obtained from the dairy cows at the Research and Development Station for Bovine in Arad, during seven years (2018-2024), from two breeds (Romanian Spotted and Romanian Brown) were used in this study. The statistical model was Main Effects ANOVA in STATISTICA software, using as independent factors THI, Breed, Year, Season, Lactation order and as dependent factors TDM, TDF and TDP. Also, days in milk was used as a continuous covariate. Generally, all independent factors had a significant influence ($p < 0.001$) on the milk yield, as well as fat and protein percentages. TDM increased with the THI class ($p < 0.001$) and took values as follows: 14.5 ± 0.401 kg, 15.3 ± 0.139 kg, 16.1 ± 0.124 kg, 17.5 ± 0.133 kg, 17.3 ± 0.096 kg, and 17.5 ± 0.149 kg for G1, G2, G3, G4, G5, and G6, respectively. Milk fat and protein percentages decreased as THI increased. Thus, the values obtained were: $5.08 \pm 0.068\%$ and $3.92 \pm 0.048\%$ for G1, $4.49 \pm 0.025\%$ and $3.75 \pm 0.013\%$ for G2, $4.47 \pm 0.020\%$ and $3.76 \pm 0.011\%$ for G3, $4.14 \pm 0.019\%$ and $3.69 \pm 0.011\%$ for G4, $3.92 \pm 0.05\%$ and $3.57 \pm 0.009\%$ for G5, and $3.85 \pm 0.021\%$ and $3.42 \pm 0.012\%$ for G6. In conclusion, we observed a strong relationship between TDM, TDF and TDP and THI, even though we did not have extreme values of THI during the eight years of the study.

Exogenous fibrolytic enzyme utilization on young bulls receiving two levels of supplementation under grazing condition

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The aim of this study was to evaluate the effect of adding exogenous fibrolytic enzymes to the supplement offered to stocker beef cattle on pasture during the dry season and its impact on cattle performance. For this purpose, 80 Nellore young bulls, with an average age of 12 months and an average initial body weight (BW) of 274.5 kg, were used. The animals were housed in 16 paddocks of *Urochloa brizantha*, 1.0 hectare each, provided with supplement bunks and drinkers, with five animals in each paddock. The experiment was arranged as a 2 x 2 factorial design, with two supplementation levels (0.1 x 0.3 % of BW), with and without the addition of exogenous fibrolytic enzyme (xylanase) to the supplement. The experiment was carried out during the dry season and lasted 120 days. The animals were weighed under fasting conditions at the beginning and end of the experimental period, and were weighed every 28 days to adjust the amount of supplement provided throughout the experiment. All statistical procedures were conducted using SAS® software. Analysis of variance was performed and treatments were compared by orthogonal contrast. There was no interaction ($P>0.05$) of supplementation level and exogenous fibrolytic enzyme on any variables measured. By design, the animals receiving the 0.3% BW supplementation level consumed a higher amount of supplement (0.92 kg/day) than the animals offered 0.1% BW (0.32 kg/day). The addition of fibrolytic enzyme did not impact supplement intake. The higher supplementation level (0.3% BW) resulted in higher ($P<0.05$) average daily gain (0.44 x 0.35 kg/day) and higher final BW (317.98 x 303.88 kg) than the lower supplementation level. Exogenous fibrolytic enzyme did not improve ($P>0.05$) cattle performance. In conclusion, increasing the supplementation level of young Nellore bulls from 0.1 to 0.3% of BW resulted in an additional body gain of 94 g/head/day during the dry season, reducing the length of the production cycle, with positive productive and environmental effects.

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Poster 12

Dynamic assessment of feeding and nutrition practices implemented by the new generation of small- and medium-sized family livestock keepers in Camagüey (Cuba)

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Tropical livestock keepers face major challenges to match nutritional herds' requirements, due to forage seasonality and climate change. The objective of this work was to get updated insights on feeding and nutrition (F&N) practices implemented by Cuban livestock keepers, for whom access to supplements are limited, little is known and literature remains outdated. Eight farms belonging to current small- and medium-sized private farmers in Camagüey province (21°N-77°W) were evaluated. Semi-structured surveys were conducted with farmers, relatives and local stakeholders. Quantitative and qualitative information was collected from different sources and databases were built to characterize F&N systems according to season, available feed resources, herd mass per month or other management strategies (e.g., reproduction). Using the INRA feeding system (2018), dynamic nutritional balances were performed in each farm, herd category and physiological stage throughout a typical year. The availability of dry matter (DM) and requirements of protein truly digestible in the small intestine (PDI) and net energy forage unit for lactation (FUL) were considered. Results show that farm' available DM is not enough and PDI, FUL requirements are not matched in the dry season and almost the entire rainy season (most abundant biomass period). Some farmers apply judicious strategies e.g., allocating resources according to prioritized stages and performances. Differences between farmers were identified, mainly related to contrasting practices in terms of reproduction management or F&N policies. There is room for improving the nutritional status of herds without necessarily requiring major investments (e.g., reproduction applying male effect to concentrate calving peaks, pasture management, adding value of by-products, etc.).

Characterizing residual feed intake in Girolando cattle across growth and lactation phases

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This study aimed to characterize residual feed intake (RFI) in 27 Girolando heifers, classified into three feed efficiency groups—high (HE), medium (ME), and low (LE)—across the calf, growing (150 and 450 days), and first-lactation phases. RFI was evaluated during both the growth and lactation phases and across three lactation stages (early, middle, and late). No significant differences were observed among the groups regarding average body weight or average daily gain. However, the HE group demonstrated significantly lower dry matter intake (DMI) compared to the LE group, with reductions of 23.9% (Calf), 14.7% (150 days), and 12.3% (450 days) during the rearing and growing phases. HE cows consumed 12.6%, 9.3%, and 12.1% less DMI during the early, middle, and late lactation stages, respectively, compared to the LE group. RFI did not influence energy-corrected milk yield (ECM), indicating that feed efficiency improvements did not compromise milk production. Spearman correlation analysis revealed moderate to high correlations between RFI during the growth and lactation phases, suggesting the potential for the early identification of feed-efficient animals. These findings highlight the utility of identifying Girolando heifers with low RFI to predict and enhance lactation feed efficiency.

Commercialization of Eggshell Powder-Based Feed Additives for Improving the Robustness and Productivity of Hanwoo and Dairy Cattle

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Main purpose of the current study is a commercialization of novel functional feed additives utilizing egg shell powder (ESP) and its derived microorganisms and enzymes for improving the productivity of Hanwoo beef cattle and dairy cows. This study involves the development of a functional feed additive using ESP, beneficial microorganisms isolated from ESP (*Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus subtilis*, *Lactobacillus plantarum*), and enzymes to enhance the health and productivity of livestock. Materials and methods include the isolation and cultivation of beneficial microorganisms from ESP, in vitro efficacy testing to confirm their antimicrobial and physiological properties, and the development of a complex probiotic formulation for inclusion in ruminant feed. The survival rates of probiotics in ESP and their viability in the rumen environment are tested under various conditions. The formulation is optimized in terms of the ratio of ESP, microorganisms, and enzymes to create a highly functional feed additive. In vivo and in vitro experiments on Hanwoo beef cattle and dairy cows assess the effects of the feed additives on production parameters, meat quality, metabolic stability, and rumen fermentation characteristics. The feed additives' impact on ammonia concentration, pH changes, volatile fatty acid (VFA) levels, and microbial populations in the rumen are analyzed. The feed additives effectively improved key production parameters, including meat quality, metabolic stability, and rumen fermentation. Notably, the additives led to favorable changes in ammonia concentration, pH levels, volatile fatty acid (VFA) profiles, and microbial populations in the rumen, suggesting enhanced feed efficiency and overall productivity. These results highlight the importance of the ESP-based feed additive in improving livestock health and optimizing production in a sustainable manner and this feed additive may contribute to sustainable animal production by enhancing livestock productivity while reducing environmental impact.

Quality and floristic composition of dryland pastures in Montado: effects of liming and different types of grazing
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The Montado is an agro-silvo-pastoral ecosystem, characteristic of the Mediterranean region. In Montado, soils tend to be acidic with manganese (Mn) toxicity, which leads to pastures with low productivity, poor quality and floristic composition. The application of dolomitic limestone contributes to the reduction of soil acidity and Mn toxicity, possibly improving pasture productivity and quality. This study aimed to evaluate the effect of application and non-application of dolomitic limestone (unapplied, U and applied, A) and two sheep grazing systems (continuous, CG and deferred, DG) on quality and floristic composition, in dryland pastures under Montado. The stocking rate was of 7 sheep/ha in CG (traditional stocking rate in Montado) and 16 sheep/ha in DG (intensified stocking rate). In 4 plots of 1ha each, 4 treatments were constituted: T1 (UCG); T2 (UDG); T3 (ADG); and T4 (ACG). The study was carried out at the University of Évora, between Nov 2019 and Jun 2020. In each treatment, 12 sampling points were identified, representing the existing botanical communities. Pasture samples were collected on every point and throughout the vegetative cycle, constituting a composite sample for each treatment (composed of 12 individual samples). These samples were dehydrated and ground, and the crude protein (CP) and neutral detergent fiber (NDF) were subsequently determined. At the end of the pasture's vegetative cycle (May), botanical species to the Poaceae and Fabaceae families were identified. Statistically, there were no significant differences ($P > 0.05$) between treatments for CP. For NDF, significant differences were found between all treatments ($P < 0.05$). Treatment T4 presented the highest NDF value, followed by T3, T2 and T1. In total, 20 botanical species from the Poaceae family and 12 from the Fabaceae family were identified. The T1 treatment had the most botanical species identified and T4 had the fewest. In T4, the fewest botanical species belong to the Fabaceae family. The results show: i) CP was not influenced by the treatment; ii) NDF was different between all treatments, being higher with the application of dolomitic limestone and GC; iii) the T4 treatment led to lower botanical biodiversity, with a reduction in the number of species of the legume family.

Session 49

Poster 16

Spatial heterogeneity of rumen microbial diversity in dairy cows

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Accurate characterization of the rumen microbiome requires an understanding of spatial variability. This study investigates microbial diversity and composition across different rumen locations in dairy cows. Six rumen-fistulated Holstein-Friesian cows (66 ± 18 days in milk) were maintained under controlled conditions in climate respiration chambers and fed a consistent diet. Rumen fluid samples were collected in duplicates from the front, middle, and back of the rumen. Microbial DNA was extracted, and 16S rRNA gene sequencing (V4 region, Illumina) was performed. Alpha diversity indices (Observed and Chao1) revealed significant differences between front-middle ($p = 0.015$) and front-back ($p = 0.03$, $p = 0.02$) comparisons, while middle-back differences were non-significant ($p = 0.19$, $p = 0.79$). Beta diversity analysis, assessed through PCoA and PERMANOVA, indicated no significant differences in overall microbial community structure across locations (PERMANOVA $p > 0.05$). In conclusion, our findings reveal significant spatial heterogeneity in rumen microbial alpha diversity, with anterior regions exhibiting distinct diversity compared to posterior sites. This spatial variation underscores the importance of considering sampling location to accurately represent rumen microbial communities.

Fermentation kinetics, nutritional profile and conservation efficiency of different ensiling methods to preserve calabash tree (*Crescentia cujete*) fruits to feed livestock in tropical sylvopastoral systems

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Nothing is known about the best method for conserving *Crescentia cujete* (calabash tree, CT) tree fruits, highly appreciated by farmers to feed livestock in the dry Colombian Caribbean region. We compared the effectiveness of different ensiling methods in an experiment with five treatments: T0: control (intact, unground and not ensiled fruits); T1: ensiled ground whole fruit without additives or T2: adding salt at 1.5%; T3: ensiling a mixture of ground fruits and fresh foliage of CT (30:70 ratio); and T4: idem to T3 but mixing (50:50) with angleton hay instead of CT foliage. For 90 days, we monitored effects on fermentation profile, conservation efficiency and nutritional quality of the final product. The feasibility of ensiling CT fruit was demonstrated, for which nutritional quality was affected by treatments. When comparing initial vs final (90 days) ensiled mass, dry and organic matter contents decreased in all treatments, except in T4 for which crude protein decreased, whereas fibrous components increased and gross energy remained stable. Most of the fermentation parameters, excepting lactic:acetic ratio, ammonia nitrogen and acetic acid concentrations, were affected by the interaction treatment × conservation time. However, we failure to confirm the accepted myth conserving CT fruit in its natural form or the benefits of adding salt to improve fermentative quality. Conserving CT fruit alone, without other ingredient and under shade-fresh environmental conditions, demonstrated to be the best ensiling method, which is recommended for supplementing ruminant diets during the dry seasons' shortage in tropical farming systems.

Session 50

Theatre 1

Human centric design as a pathway to PLF integration in Pastured Poultry production systems

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The re-imagination of livestock production systems emphasizes sustainability, digital integration, and resilience. Pastured poultry production is increasingly popular among small-scale farmers due to its benefits for soil health and farm income diversification. However, the high variability in infrastructure and management practices limits the adoption of innovation and digitalization, leaving small-scale farmers outside the scope of technological advancements. Addressing these challenges requires standardized infrastructure that supports both traditional practices and future digital integration. A human-centric design approach was adopted, focusing on standardization to simplify the integration of digital tools. The coop was developed for standardized dimensions, mobility, and biosecurity protocols. Key features included support for electric fencing for predator protection, optimized space for bird welfare, and infrastructure readiness for digital tools like environmental sensors and automated feeders. The design's modular structure allows for easy scalability and is supported by CAD assembly manuals which significantly enhances the scalability of the system. The standardized mobile coop provided similar or better service and ease of use compared to existing models. The slightly higher production costs could be recovered within 1.6 years through increased productivity and reduced operational expenses. The coop's design supports hygiene and safety standards and prepares for progressive adoption of digital tools, enabling small-scale farmers to transition to data-driven management without extensive infrastructure changes. A human centric design approach offers a practical solution for integrating innovation and digitalization into small-scale pastured poultry systems. By addressing the barriers posed by infrastructure variability, this approach enables efficient and sustainable management while preparing for future technological upgrades. The findings suggest that standardized, cost-effective designs can play a crucial role in the evolution of livestock systems.

AI-Powered Acoustic Diagnostics: Advancing Respiratory Health and Animal Welfare

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Bovine Respiratory Disease presents a significant global challenge, necessitating innovative diagnostic solutions for early treatment to protect animal health and welfare. Acoustic biomarkers offer an exciting and non-invasive method for assessing respiratory functionality. In this study, 28 healthy, unweaned calves were divided into four groups to represent a range of respiratory dynamics: (1) antibiotic-treated and infected with influenza D virus (IDV), bovine coronavirus (BCoV), and *Mycoplasma bovis*; (2) non-antibiotic-treated but infected; (3) antibiotic-treated and non-infected; and (4) non-antibiotic-treated and non-infected. The animals were monitored for clinical symptoms from 15 days before infection to 21 days after infection. The study aimed to develop an AI-driven diagnostic tool that identifies cough sounds as early biomarkers for respiratory diseases using continuous acoustic data. Of the 2,728 hours of recordings collected (~7 hours per sensor per day), a preliminary subset was processed for feature extraction using Wav2Vec2 without extensive preprocessing. A HuBERT-based model (ls960) classified 1,045 cough and non-cough events from 86 hours of recordings, achieving an impressive 92% accuracy. Precision and recall values were 0.94/0.95 for non-coughs and 0.87/0.87 for coughs. To validate these results, a Gaussian Generalized Additive Model was applied to compare AI-derived cough scores with veterinary assessments of clinical symptoms. The analysis revealed no significant difference, demonstrating that the AI-powered approach is on par with veterinary evaluations. This method is non-invasive, cost-effective, and capable of providing autonomous real-time data, enabling prompt interventions to safeguard animal health and welfare.

Session 50

Theatre 3

Seeing Double: Dual Camera 3D Localisation and Tracking of Dairy Cattle Using Monocular Depth Estimation

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Accurate identification, localisation, and tracking form the basis of an automated visual animal welfare monitoring system. In this work we demonstrate a scalable approach for inter-camera tracking and 3D localisation of dairy cattle within a straw-bedded calving barn environment, utilising low-cost RGB CCTV cameras. Our method applies deep learning-based monocular depth estimation to video feeds to estimate the camera-to-cattle distances. Building on our development of 3D barn reconstruction and quasi-3D bounding box generation (Dickson et al, 2024), we further apply this method for cross-identification of cattle in a dual camera system. We automate inter-camera image transformations using a small sample of segmented and labelled cattle, eliminating the need for costly and time-consuming calibration and reducing setup complexity in real-world deployments. This allows commodity cameras to function as a cost-effective 3D tracking system, requiring minimal set-up and user input. Our results demonstrate that the inter-camera transforms, validated through the ID matching of eight cattle from the dual camera views over a 15 minute period, are robust to changes in cattle position, posture, and motion within the barn environment. This work provides an important step towards flexible multi-camera tracking systems, in addition to improving the accuracy and accessibility of animal welfare monitoring through the incorporation of large foundational depth estimation models and automated inter-camera matching techniques. Dickson, L. T., Davison, C., Michie, C., McRobert, E., Atkinson, R., Andonovic, I., Ferguson, H., Dewhurst, R., Briddock, R., Brooking, M., Pavlovic, D., Marko, O., Crnojevic, V., & Tachtatzis, C. (2024). Augmenting cattle tracking efficiency through monocular depth estimation. Paper presented at IEEE Conference on AgriFood Electronics, Xanthi, Greece.

A Consensus-Based Computer Vision Approach for the Quantification of Sow Activity and its Effect on Piglet Crushing

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In the Project ZISSAU, we investigate the relationship between sow body repositioning activity, the provision of different nesting materials, and their effect on piglet crushing losses. Since previous studies have highlighted the impact of sow posture changes on these losses, we intended to analyze the frequency of sow posture changes to gain more insights into sow behavior and crushing events. Therefore, the aim of this study was to propose an objective approach for the automated assessment of sow body repositioning activity. As part of this approach, we trained a YOLOv11 object detection model to directly compute sow body posture and compared its performance to an image classification model that processes the cropped bounding box of the sow. Although both models independently yield good results, identifying false positive predictions is crucial to prevent a biased activity assessment. To address this, we employed a consensus-based method that focuses on matching results from both approaches, enabling more efficient filtering of true positive predictions. This refinement improved overall performance, achieving precision, recall, and F1-score values of 0.92, 0.93, and 0.92, respectively. This enhanced classification allows us to quantify sow posture changes across 3,000 hours of video footage, facilitating the assessment of potential correlations between sow repositioning activity and the provision of organic nesting materials. This work is funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number 28N305603).

Session 50

Theatre 5

CT measurements obtained by Computed Tomography can predict commercial cut weights in pork carcasses

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Within the pork industry, accurately determining the percentage of saleable meat yield (SMY%) enables more sophisticated payment systems which would improve profitability of the supply chain. In the Australian lamb industry lean meat yield percentage (LMY%) prediction technologies are trained to predict computed tomography (CT) measurements of lean, fat and bone %, providing a more robust reference standard than manual dissection. The CT value is then linearly transformed into the commercial cut weights relevant to each abattoir. In pork, similar LMY% technologies predict CT measurements; however, the equations to convert these measurements into pork commercial cut weights have not been established. Therefore, this study assessed the ability of CT to predict cut weights in pork carcasses from a commercial abattoir. In this experiment, 109 pig carcasses were split into 4 sections and CT scanned to estimate fat, lean and bone %. Each section was then manually boned-out into commercial cut weights by a team of 4 boners. Models were trained to predict boneless rindless, trimmed collar butt, eye of the loin and topside cut weights using carcass weight as an independent variable with different combinations of CT lean, fat and bone% from the whole carcass or sections. The precision of models predicting cut weights using CT lean% demonstrated the best precision for half of the cuts analysed, and CT bone% the worst across all cuts, and there was no difference between acquiring this measurement from the whole carcass or from the leg section of the carcass. The model with the greatest precision was that using carcass weight and section CT lean % ($R^2 = 0.72$, $RMSE = 0.043$), however this was only marginally better than the model using carcass weight and whole carcass CT lean % ($R^2 = 0.71$, $RMSE = 0.044$). The weakest precision was for models using CT bone %, which when used with carcass weight had markedly lower precision ($R^2 = 0.39$, $RMSE = 0.110$). This will enable technologies trained to predict carcass CT lean% to also predict commercial cut weights.

An automated AI image analysis system for in-line green ham assessment

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The classification of green hams for PDO product processing is currently performed by expert evaluators, but the process is influenced by subjectivity. This study aimed to develop and optimize an automated image analysis system for green hams, integrating 2D and 3D imaging technologies for in-line image acquisition and ham objective classification. The system, deployed at a commercial slaughterhouse, included two 2D cameras, one 3D time-of-flight (TOF) volumetric camera, a sensor-based positioning system using a microcontroller and a set of depth-sensors to ensure consistent sample detection, and diffused light sources to enhance image quality and eliminate overexposed areas. Two 2D images (top and cross-section views) and one 3D image (top view) were acquired for each of 26,000 trimmed green hams over five days. A Python-based software framework was developed for synchronized, low-latency, multithreaded image acquisition, integrating calibration routines and real-time sensor triggering. For image analysis, algorithms were designed to process 2D and 3D data simultaneously. Both 2D images were pre-treated using an Implicit Segmentation Neural Network (ISNet) to isolate the region of interest from the background. The top-view 2D image allowed the detection of skin surface defects (bruises, veins, discolorations, redness) by isolating the region of interest (skin) and quantifying the area and color intensity of each defect. The severity of veining was assessed by measuring the extent of the visible vein pattern by a ridge detection filter and the color intensity of the vein network. The cross-section 2D image enabled quantification of subcutaneous fat depth and intramuscular fat area as a percentage of the cross-section. The 3D models provided volumetric metrics, including ham roundness and shape assessment. Acquisition and computation times of the optimized system are suitable for in-line deployment. These advancements allow to automate green ham quality assessment, providing a scalable solution for industrial applications.

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Theatre 7

Real-Time Monitoring of Bovine Metabolic Disease through Dermal Biosensing

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Wearable biosensors have the potential to serve as vital tools in precision livestock farming, enabling continuous monitoring of animal health and behaviour. These devices collect real-time data on vital signs and activity levels, facilitating early disease detection and improved welfare management. Such technologies offer maintenance-free solutions, providing farmers with actionable insights to optimize livestock care and productivity. This study had two primary aims: (1) to evaluate the histopathological effects of smart-patch insertion into the dermis and (2) to determine whether a skin smart-patch can monitor biomarkers in real time. For the histological study, a Holstein cow and calf, euthanized within one hour before testing, were used. A 4 × 4 cm area was clipped and shaved on the calf's neck, the cow's neck, and the caudal udder of the cow. Methylene blue dye was applied to the skin and microneedles before inserting the smart patch for one minute, with manual pressure to ensure adherence. After patch removal, the skin was recoated with dye, dissected, and fixed in formalin. Each site was processed identically, and a pathologist evaluated tissue sections for evidence of microneedle penetration and microtrauma. In the second phase, a smart patch was applied to adult dairy and beef cattle (n = 5) for up to eight hours to measure intradermal glucose levels in real time. A subset of cattle received an intravenous infusion of 0.9% saline with or without 5% dextrose. Preliminary results suggest that the smart patch causes minimal microtrauma, with no histological evidence of microneedle insertion. Furthermore, real-time biomarker monitoring, including glucose detection, appears feasible, highlighting the potential for non-invasive metabolic disease monitoring in cattle. Microneedle technology has the ability to measure multiple biomarkers simultaneously, such as calcium, ketones, and lactate. This will be the focus of future studies.

Using machine-learning as part of an enhanced bone-detection method for chain-speed DXA in Australian lamb abattoirs

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Dual Energy X-ray Absorptiometry (DXA) technology has been installed into six Australian sheep meat abattoirs, predicting carcass composition at chain-speed to enable trading, enhanced producer feedback, and real-time sorting of carcasses pre-fabrication based on fat % and lean %. These devices have been accredited by AUS-MEAT – the national body responsible for approving objective measurement technologies – with the DXA image processing algorithm developed by Murdoch University. This algorithm detects bone pixels using a function of the R-value and thickness, and thresholding either side of the mean of all these pixel values to allocate them as either soft tissue or bone. This process successfully isolates bone and enhances the precision and accuracy of predicting carcass fat and lean%, but it cannot isolate individual bones or bone-regions for further analysis. To identify individual bones (femurs, pelvis, lumbar spine, thoracic spine, ribs, forelimbs, and neck), a machine-learning algorithm has been trained on 8-bit images of individually isolated bones from the low-energy DXA image as positive prompts, with the other bones representing negative prompts. This trained cascade algorithm can then correctly identify individual bones from lamb carcasses. Once the individual bones are highlighted and demarcated from the surrounding soft-tissue, the dimensions and count of individual bones were predicted from the DXA images of 600 phenotypically and genotypically divergent lambs, which was verified by dimensional measurement through computed tomography scanning. Finally, the bone-containing pixels within the DXA images were separated into their bone-only, and soft-tissue-only components, through a more accurate calculation of the soft-tissue R-value and thickness values immediately adjacent to each bone. This enhanced bone detection is likely to improve the prediction of eating quality from bone containing pixels and enable phenotyping of hard-to-measure traits such as pelvic size, which influences dystocia rates.

Session 50

Theatre 9

Improving Broiler Transport Efficiency Using CFD: Heat Stress Analysis and Mitigation Measures

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Heat stress during broiler transport to slaughterhouses poses significant challenges to animal welfare and meat quality. Prolonged exposure to high temperatures, coupled with inadequate ventilation and high density, can lead to physiological distress and increased mortality rates. Monitoring heat stress during broiler transport can be costly and logistically challenging, making it difficult to assess. This study addresses this issue by employing Computational Fluid Dynamics (CFD) as a cost-effective and practical alternative to predict and analyze the thermal microenvironment within transport vehicles. The model incorporates key environmental factors such as temperature, humidity, and airflow dynamics, alongside broiler-specific parameters like metabolic heat production and stocking density. By solving the governing equations for heat transfer and fluid flow, the model provides a detailed spatial analysis of heat distribution and stress levels experienced by broilers during transit. Field data, including real-time temperature and humidity taken at different location, were used to validate the model under varying climatic conditions. Validation against field data demonstrates that the model provides accurate predictions of temperature and humidity. The effective temperature (ET), a combined metric of temperature, humidity and air velocity, is calculated to assess the thermal comfort of broilers. From ET, the model predicts the temporal increase in animal body temperature over time, providing insights into the physiological response of broilers to transport conditions. Using this framework, the study evaluates the efficiency of adaptation measures, such as reducing stocking density and leaving empty drawer to improve airflow and reduce heat production. Simulation results reveal that leaving empty drawer in key location might be the most efficient solution in hot weather conditions. These findings underscore the potential of CFD modeling as a predictive tool for optimizing transport conditions and enhancing animal welfare. The results provide actionable insights for stakeholders to implement cost-effective and climate-resilient strategies in broiler transport.

Measuring broiler activity using computer vision

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An important percentage of the resources used by a growing animal is spent on physical activity. It has been shown that selection for reducing residual feed intake has led to less active animals. We developed a computer vision system to track and quantify the physical activity of chickens. We have used MaskR-CNN library to perform the instance segmentation to detect all the visible chickens in frame, MaskR-CNN produces a table with the coordinates of each chicken. In a second step, the detected chickens were tracked across frames using Simple Online and Real-Time Tracking (SORT). The neural network was trained using photos from four 2560 x 1440 pixel cameras placed on the roof of four pens. Each camera recorded the entire surface of a pen (21 chickens per pen) measuring 1,8 m on each side, and the image of each pen had 1440 pixels on each side. The images for training were collected from a production batch and randomly represented different fattening stages (0-35 days). The mean Average Precision of pixels assignment to the chicken class in the validation set was 0.95. Tracking images were recorded at 25 frames per second. Animal activity was measured as the sum of the Euclidean distances between the positions of the same animal in the different frames. Noise produced by the positioning of animals at different points even when there was no movement was corrected by accumulating only the distances greater than half the length of the chickens. Results indicate that broilers move at an average of 31,2 meters per hour during the first week and the physical activity dropped as the broilers get older to 15,1 meters per hour at 35 days. This overall activity is the sum of small movements that are longer, faster and straighter during the first week. When the broilers are heavier, there is more deviation from the moving average that can be an indicator of leg disorders.

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Poster 11

PointNet-Based Non-Contact Pig Weight Estimation System Using 3D Point Cloud Processing

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Accurate livestock weight estimation is crucial for farm management, aiding in growth monitoring, feed optimization, and animal health. Traditional scale-based methods are labor-intensive, costly, and stressful for animals. To overcome these issues, this study proposes a non-contact sow weight estimation system using the PointNet model and 3D point cloud data, providing a scalable solution for modern livestock management. Our method employs top-view 3D imaging to capture point cloud data of sows, which is then processed using a deep learning approach based on the PointNet framework. A dataset of 289 sows with varying body weights was used for model training and validation. To enhance estimation accuracy, a segmentation-based technique was incorporated into the PointNet model. Specifically, 3D point cloud data were first converted into depth images for segmentation, and the extracted 2D coordinates were then remapped to reconstruct segmented 3D data. This process improved the precision of weight predictions by refining the feature extraction process. As a result, the Mean Absolute Percentage Error (MAPE) was reduced from 13.19% to 11.25%, representing a 1.94% improvement over the baseline model. The proposed AI-driven system offers significant benefits over traditional methods. By eliminating direct animal handling, it reduces stress, lowers labor costs, and enhances efficiency. Seamless integration into farm environments enables continuous and automated weight monitoring, improving herd management. Our findings highlight the potential of deep learning-based 3D point cloud processing in livestock applications. The proposed method demonstrates that non-contact weight estimation can be both accurate and practical, paving the way for future advancements in precision livestock farming.

Relationships between back angle and reproductive performance in sows

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Backfat thickness is commonly used to estimate sow reproductive performance. This study investigated whether back angle, which has a high correlation with backfat thickness at P2, affects key reproductive traits, including weaning to estrus interval (WEI), total piglets born (T. born), stillborn piglets (S. Born), mummified piglets (Mummy), piglets born alive (Born Alive), total piglet birth weight (T. BW), and average piglet birth weight (Ave. BW). A total of 283 gestating sows (parity 0–8) were analyzed for reproductive traits in relation to back angles before farrowing and after weaning. A negative correlation ($r = -0.33$) was found between back angle before farrowing and T. born. However, back angle before farrowing showed a very low correlation ($-0.2 < r < 0.2$) with other reproductive performance. Segmented regression analysis identified a breakpoint at a back angle of 17 before farrowing, indicating an optimal threshold. When the back angle exceeded 17, the total number of piglets born tended to decline. This suggests that maintaining a back angle of 17 may be beneficial for sow reproductive performance. This study serves as a preliminary experiment for AI-based sow body shape management using back angle measurements. While back angle alone has limited predictive power, determining an optimal threshold could enhance automated sow management. Further research on different body locations for back angle measurement is needed.

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Poster 13

Non-Invasive Assessment of Pig Intramuscular Fat using Ultrasound Imaging and Advanced Deep Learning Techniques

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In South Korea, pork remains a popular meat choice, with consumers valuing intramuscular fat (IMF) for its influence on taste and tenderness. Traditional IMF assessment methods are invasive and limited to post-slaughter analysis, restricting real-time quality management. This study proposes a non-invasive approach using ultrasound imaging combined with advanced deep learning models, including Vision Transformer-Huge (ViT-H) and Xception. ViT-H uses a transformer-based architecture, effective in capturing global image context, while Xception leverages depthwise separable convolutions to efficiently learn spatial features. AutoAugment, a data augmentation technique, was applied to improve model generalization by generating diverse variations of the training data. We also incorporated KB-Net, a noise-reduction model, to enhance image quality by removing ultrasound noise, further improving IMF prediction accuracy. Our results show that ViT-H, particularly when enhanced with Auto-Augment and KB-Net, significantly outperforms other models, reducing the root mean square error (RMSE) to 0.3559%. This methodology demonstrates the potential for real-time IMF monitoring, offering a scalable solution for improving meat quality assessment in livestock management.

Haplotype-based GWAS in Swedish Warmblood Horses (SWB)

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The Swedish Warmblood Association's breeding goal is to produce competitive horses in terms of rideability, performance, and temperament, able to meet the new challenges in the horse breeding sector. Achieving this requires a good understanding of the genetic background of the traits under selection, which is currently lacking. This is further complicated by the polygenic nature of these traits. Our aim was to better understand the genomic architecture behind performance-related traits. In this study, we conducted a haplotype-based genome-wide association study (GWAS) for 37 phenotypes recorded during young horse evaluation tests involving 380 SWB horses. The horses were evaluated by expert judges using both traditional and linear evaluation systems, including three main categories of traits: conformation, free jumping, and gaits. All samples were genotyped using a high-density SNP chip and remapped from EquCab2 to EquCab3. The haplotypes were first phased using Shapeit v.2, and then haplotype blocks were calculated in the R package GHP 1.2.2 for a total of 78,000 haplotypes. To assess the association between the haplotypes and studied traits, a linear mixed model was used, correcting for sex and the date-location in which the evaluation took place. In the single-trait analysis, a total of 11 haplotype blocks were found to be significantly associated with a total of six traits: height at withers, two conformation traits (hooves and correctness in movement), and three free jumping traits (technique: haunches, carefulness, and distance estimation). In the proximity of those haplotypes (windows size $\pm 500\text{kb}$), 36 protein-coding genes, 27 lncRNAs, and three miRNAs were found. The haplotype-based GWAS approach proved to be a noteworthy advancement over traditional single SNP-based methods to overcome the limitations of small sample size and the polygenic nature of performance traits. Future studies with larger sample sizes and using novel tools for objective phenotyping will be essential to further investigate the genetic mechanisms behind sports performance.

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Theatre 2

An increased number of heterozygous calls in the Axiom™ Equine Genotyping Array

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Genotyping arrays are commonly used in livestock research to investigate complex traits including genome-wide analysis and fine mapping, genomic prediction, genetic diversity and selection signature analyses. In the context of a European Equine diversity study, we analysed the 670K Axiom™ SNP genotype data from 4,520 horses across 21 breeds. While assessing genome-wide runs of homozygosity (ROH) patterns, we observed an increased number of heterozygous calls in 70 purebred horses, which exhibited fewer ROH segments compared to F1 crosses. To further investigate this, we conducted a 4-fold genotype concordance comparison of replicate pairs on the same batch, between two different batches, between Illumina EquineSNP50 BeadChip® and between Illumina paired-end HiSeq 2000 whole genome sequencing data. Additionally, we used SNPisherr™ classification to evaluate the genotype performance of the 670,783 genome-wide SNPs. Including the overlapping SNPs between the different genotyping platforms, replicated pairs within the same batch showed the highest average genotype concordance (98.81%), followed by Illumina 50K (97.88%) and whole genome sequencing (96.84%). In contrast, re-genotyped horses with few ROH segments (i.e., replicates on different batches) showed the lowest concordance (93.52%). A lower pass rate was observed in one batch, suggesting a processing performance issue that contributed to the reduced concordance between batches. According to SNPisherr™ classification a total of 120,828 genome-wide SNPs were not recommended for reproducibility. Calling genotypes of the two different batches together in accordance with Axiom Best Practice (e.g. removing failed samples before the final genotyping) and excluding non-recommended SNPs, genotype concordance improved in all comparisons: same batch (99.84%), Illumina 50K (98.33%), whole genome sequencing (97.81%), and different batches (98.59%). Based on these findings, we recommend excluding horses exhibiting an unusually high number of heterozygous calls, using only SNPs with validated genotype performance, and accounting for batch effects when analysing 670K Axiom™ SNP data from different batches.

Microsatellite imputation using a 60K SNP Chip for parentage control in French horse breeds

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Currently, equine parentage control relies on 14 Microsatellite (MS) markers to compare foals and parents' genomes. However, the industry is shifting to Single Nucleotide Polymorphism (SNP) markers. As MS and SNP are not directly comparable, a transition plan is needed to avoid costly dual testing or systematic SNP re-typing of parents. To this end, the imputation of MS alleles from a 60K SNP Axiom Chip was tested in Thoroughbred horses and French Saddle Ponies (FSP). A panel of 1087 FSP and 1090 Thoroughbred horses with both MS and SNP markers was used. The study focused exclusively on SNPs located on chromosomes with MS. A quality control (minimum allele frequency ≥ 0.05 , Hardy-Weinberg equilibrium $\geq 1e-9$) per breed resulted in 24,460 SNPs for FSP and 22,080 SNPs for Thoroughbreds. FIMPUTE and BEAGLE softwares were tested for imputation and performances were measured by 5-fold cross validation repeated 10 times. Performance was measured by the proportion of correct alleles per horse and per MS. Parentage control was performed using imputed MS and MS of parents from the official database (SIRE, IFCE) with 12 MS. Performances of FIMPUTE and BEAGLE were close. Only results from the BEAGLE software are presented. MS scores ranged from 0.967 to 0.993 for FSP and 0.987 to 0.998 for Thoroughbreds. The repeatability over the 10 replicates was superior in Thoroughbreds. Current parentage controls rules allow one incompatible MS out of at least nine verified MS for validation. Among the 987 FSP with 9-12 MS initially compatible with their parents, 78% had fully compatible imputed MS, 19% had one incompatible, and 3% had 2 or more incompatible MS. In the Thoroughbred study, among 1075 horses with 9-12 compatible MS, the respective results were 95.6 %, 3.6 % and 0.98 %. We conclude that MS imputation from SNPs is a cost-effective transition strategy. To confirm these results, the method was applied to other breeds and we intend to use it routinely in the near future.

Session 51

Theatre 4

Extended vs. new imputation systems for supporting transfer to SNP-based parentage control in horses of different breeds

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When the demand for riding horses for young people and children increased in the 1970s in European countries, the German riding pony (GRP) was established originating primarily from crosses of English riding ponies with Arabian and English thoroughbreds. The breeding regulations still allow crosses with a wider range of horse breeds including Warmblood riding horses which has facilitated breeding progress in dressage- and jumping-related traits. With the growing genomic toolset for horses, the studbooks' motivation to replace traditional laboratory analyses of microsatellite markers (MS) for parentage testing by routine genome-wide single nucleotide polymorphism (SNP) genotyping. For efficient transfer to this new system, SNP-based MS imputing has been implemented for German Warmblood horses (GWH) already in 2021, but not yet for pony breeds. Depending on similarity and genetic exchange between breeds, MS allele frequencies can differ significantly, and breed-specific alleles can occur. Accordingly, collection of reference data for imputation development for ponies was required, resulting in N=2,118 GRP with available SNP (medium density array, 80k+) and MS data by the end of 2023. Aim of this study was evaluating best use of this data, so three test scenarios of SNP-based MS imputing differing in composition of the reference population were run: GRP only (A), GWH only (N=12,417; B), and GRP and GWH (C). Average MS imputation accuracies for the target population were lowest in B ranging between 0.89-0.98 for 13 MS markers. Corresponding accuracies in A were higher (0.97-0.99) and very similar to those in C (0.98-0.99). No detrimental effects on MS imputation accuracies for GWH were seen in C. These results allowed the decision to integrate the GRP reference data into a joint reference population with GWH through which full coverage of all occurring MS alleles is ensured. The extended system for SNP-based MS imputing is now the basis of routine parentage control in GWH and GRP in Germany facilitating pony breeding to benefit from genomic developments.

Identification of candidate causal variants on ECA1 for withers height in horses

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Withers height is an important selection criterion in horse breeding. Besides influencing overall appearance, it is associated with health and performance and is essential for the classification and marketability of horses. Therefore, deciphering the genomic background of withers height is of great interest. In a previous study, we imputed medium-density SNP array data from 2709 German Warmblood mares to sequence level using a reference panel of 175 sequenced horses. A subsequent GWAS for withers height based on the imputed data identified two genome-wide significant association signals on ECA1 and ECA3. While the prominent QTL on ECA3 close to the LCORL and NCAPG genes has been detected and investigated in several studies, the QTL on ECA1 at ~56-59 Mb has rarely been described. However, as this QTL explained a considerable proportion (~6%) of the phenotypic variance for withers height, its further characterisation is of great interest. Therefore, we applied a fine-mapping approach based on LD structures between the significantly associated variants on ECA1 to identify potential causal variants for withers height underlying this QTL. Sixty-three variants were in high LD ($R^2 > 0.8$) with the highest associated SNPs from GWAS. They were predicted to affect 16 different genes, 11 of which have previously been associated with body height in humans. While all other variants were annotated with modifying effects on the respective genes, one missense variant was predicted to have a moderate impact on the TET1 gene. Variants in this gene have previously been associated with body height and various body mass traits in humans, and reduced body size has been observed in TET1 knockout mice. Therefore, the missense variant in TET1 represents a plausible candidate causal mutation for withers height in horses.

Session 51

Theatre 6

Transcriptomic characterisation of recurrent exertional rhabdomyolysis in Thoroughbred horses

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Recurrent exertional Rhabdomyolysis (RER) is a syndrome characterized by repeated episodes of exercise-induced muscle soreness and cramping. It involves skeletal muscle fiber necrosis, increased serum activity of the muscle enzymes creatine kinase and aspartate aminotransferase, and, on occasion, myoglobinuria, renal failure and death. Horses are particularly susceptible, with a 5-7% prevalence in Thoroughbreds (TBs). ER has a genetic component ($h^2=0.39-0.49$) and is considered a complex disease influenced by genetic and environmental factors. We have shown previously that RER has a distinct complex polygenic genetic architecture. In this work we have investigated the transcriptomic profiling of RER by comparing cases and controls using RNA-seq in blood samples from 26 TBs (13 cases, 13 controls) and semimembranosus muscle biopsies (3 cases confirmed by histology diagnostics and ten controls). RNA was extracted from muscle (the local tissue of interest) and blood (for systemic signatures) and mRNA-seq analysis performed to identify differentially expressed genes (DEGs). We identified 97 DEGs in blood and 1056 in muscle. Functional enrichment analyses of both tissues revealed enrichment of transposons and retrotransposition. 17 DEGs overlapped the two tissues and were enrichment for transposons and retrotransposition. These elements have been previously associated with skeletal muscle ageing and adaptation to stress. We hypothesised a role of transposable elements in RER susceptibility through genomic adaptability to exercise induced stress. Understanding these mechanisms would facilitate tailored training programs. Further understanding of heritable components influencing this mechanism would provide a framework for future targeted breeding strategies to reduce the incidence of RER in the equine population.

Identification of a SNP in eca-mir-449b and Its Potential Impact on miRNA Processing and Gene Regulation in Horses

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MicroRNAs (miRNAs) regulate gene expression by binding to mRNA 3' untranslated regions, leading to degradation or repression. In horses, miRNAs influence traits like muscle development, spermatogenesis, immune regulation, and endurance adaptation. This study aimed to identify SNPs in horse miRNA genes and assess their potential functional impact. 413 horses of Slovenian breeds were genotyped using the GGPTTM Equine SNP microarray (Neogen). Variant Effect Predictor (VEP) was used to identify miRNA SNPs. miRNA target genes were predicted based on the reverse complement of the miRNA seed region from miRBase and aligned with Ensembl transcripts. Functional analysis was performed using g:Profiler, potential target genes associated with phenotypes were identified using VEP, and miRNAs secondary structure was predicted using RNAfold. SNP (TBIEC2_582461; C/A) is in pre-miRNA, within the terminal loop of eca-mir-449b, a region crucial for Drosha/Dicer processing. The SNP has a high conservation score (3.16), indicating potential functional relevance. miR-449b has 3,986 potential targets, enriched in parathyroid hormone regulation pathway. Moreover, it potentially targets genes linked to horse traits, including GYS1 (polysaccharide storage myopathy), PLOD1 (Ehlers-Danlos syndrome), SLC5A3 (immunodeficiency), SCN4A (hyperkalemic periodic paralysis), B4GALT7/ACAN (dwarfism), MITF/STX17 (coat color variations), MUTYH (cerebellar atrophy), and HOXD3 (atlanto-occipital fusion). The SNP microarray also contains a variant within predicted miR-449b target site of PRSS53 (BIEC2_214737; C/T), a gene associated with hair morphology, metabolism, immune function, and neurological traits in mice and humans. Genotyping of miRNA SNP showed breed-specific variation, with AC predominant in Lipizzan and Posavje but rare in Slovenian Cold-blooded horses. In contrast, the SNP in the predicted miRNA target PRSS53 was monomorphic (CC) across all breeds. These findings suggest breed-related differences in miRNA regulation. Further research with functional validation and broader population studies is needed to clarify the role of selection in shaping miRNA-related genetic variation in horses.

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Poster 8

Genomic diversity patterns in Noric of Muran horse: Implications for population management

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The Norik of Muran horse breed represents a unique genetic resource playing a key role in traditional forestry, mountainous agriculture, and local biodiversity conservation in Slovakia. This study tested its intrapopulation variability and genetic connectedness to other historically related horse breeds. The gene pool of Noric of Muran horses represented 123 animals (48 stallions and 75 mares) that were genotyped by GGP Equine 70k chip. The group of historically connected breeds included genomic data of Hucul (N=34), Norwegian Fjord (N=21) and Belgian (N=30) horses, while Shagya Arab was used as an outgroup (N=47). Intrapopulation variability was quantified by calculating heterozygosities (Ho and He), a genomic-based indicator of inbreeding (FHOM), principal component analysis using IBD matrix, and constructing a neighbour-joining tree derived from genetic distances. Interpopulation genetic connectedness of Noric of Muran to other breeds was then estimated based on discriminant analysis of principal components, phylogenetic trees derived from genetic distances, Wright's FST index, admixture proportion and migration rate between breeds. Noric of Muran mares (Ho=0.33±0.17, He=0.31±0.17) and stallions (Ho=0.31±0.15, He=0.30±0.16) showed comparable levels of heterozygosities. Moreover, the FHOM (-0.05 both in mares and stallions) values indicate sufficient intrapopulation genetic variability. All the applied approaches pointed to the differentiation of animals into genetic groups reflecting maternal families and paternal lines. As expected, further analyses of relationships between Noric of Muran and other breeds showed its strong genetic connectedness to Fjord and Hucul breeds used in its grading-up process. The obtained results have practical implications not only in the population management of Noric of Muran in Slovakia but are also valuable for optimising long-term conservation strategies for this unique breed. This study was supported by the Slovak Research and Development Agency (grant no. APVV-17-0060 and APVV-20-0161) and by the projects GACR 24-14325L and QL25020022.

Genomic Selection Evaluation of Neck Morphology in Pura Raza Española Horses: A Comparative Analysis C. Ziadi¹, M. Ripollés-Lobo², M. Valera², N. Laseca^{2,3}, A. Molina¹

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Ewe neck, neck length, and head-neck junction (very poorly marked to very marked) are essential morphological variables in horses, with a direct impact on the animal's biomechanics, balance, and functionality. In breeds such as the Pura Raza Española (PRE), these variables have both aesthetic and functional value, playing a key role in morphological selection. Moreover, the presence of disease reduces the market value of horses during sales and is considered a disqualifying morphological defect in the studbook for high scores. This study aimed to assess the impact of incorporating genomic data using single-step restricted maximum likelihood (ssGREML) on reliability (R²) of neck morphological traits in the PRE horse breed, compared to traditional pedigree-based REML. The analysis involved two morphological defects (Ewe neck (EN) and head-neck junction (HNJ)) and one morphological trait (neck length (NL)) with 64,810 animal records. Genomic data were obtained from 6,752 animals with the Axiom EQUIGENE 90K SNP array. Genetic parameters were estimated using a multivariate model. Results showed that heritability estimates were similar between the two approaches, ranging from 0.17 to 0.38. A significant increase in R² was observed for ssGREML compared to REML, with overall gains ranging from 13.61% (NL) to 28.27% (HNJ). Remarkably, the gain in R² ranged from 17.96% to 31.4% in genotyped animals, and was lower in non-genotyped animals (12.96% to 27.5%). Interestingly, individuals with low R² values in REML demonstrated the largest R² gains in ssGREML (18.77% to 34.49%). In addition, the increase in R² was similar for mares and stallions. However, this improvement was much greater (from 12.57% to 25.09%) when only considering stallions with less than 40 controlled foals. These findings demonstrate that the application of genomic selection can contribute to improving the R² of mating decisions in a large horse breeding program such as the PRE breed.

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Theatre 1

One size does not fit all : from a theoretical monolithic livestock future to anchored levers for agroecological transitions in a diversity of landscapes

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Livestock farming generates high environmental impacts at a global scale, what is calling for a major change. Circular economy approaches call for recycling nutrients, optimizing feed production and incorporating innovative practices. Such general assumptions are related to a theoretical monolithic vision of livestock, whereas livestock systems are as diverse as the territories where they are anchored, thus providing a diversity of services and impacts. Here, we aim at enlightening the debate illustrating livestock agroecological transition forms in the lens of a diversity of landscapes. For this, we combine landscape agronomy and transitions studies frameworks considering both i) the central role of farmer motivations, practices and biotechnical aspects in a landscape approach and ii) the socio-technical systems including actor networks and the broader market and policy factors over the long term. We explore a diversity of landscapes from high density livestock areas (Brittany) to low density livestock areas (Parisian Bassin and South-Eastern France) passing by areas where crop and livestock are balanced and infrastructures and knowledge still exist (South-Western France). This transversal analysis led us to conclude on the need for livestock agroecological transition studies to consider jointly i) the looping of geochemical cycles through crop-livestock interactions not only at farm level, but also at regional level, considering the need for down-scaling models and ii) the importance of the local ecosystem of innovation and the motivations for change of a large diversity of actors beyond farmers. Environmental stewardship could be key through mechanisms such as rural development, including coupled innovations with food, tourism and cultural services, and managing commons for limiting the risk of wildfires or water pollution.

Advancing Circular Economy in Insular Regions: Insights from Crop-Livestock Integration in Réunion Island
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The circular economy (CE) is gaining attention as a strategy for sustainable transitions, particularly in island regions facing isolation and resource constraints. Crop-Livestock Integration (CLI), have emerged as practical application of CE principles. This study examines CE objectives according to local stakeholders and the barriers to CLI implementation. Research was conducted on Réunion Island in 2023 in 3 phases: (i) stakeholder mapping identified relevant actors; (ii) 38 semi-structured interviews were conducted with plant and animal sector representatives, authorities, and cooperatives; (iii) a participatory workshop with 30 stakeholders to complete and correct collected information. Key CE objectives was mentioned by the stakeholder: waste reduction and resource efficiency (9 mentions), reconfiguring territorial metabolism (8 mentions), reducing biomass imports by optimizing local biomass (5 mentions), and converting waste into resources (5 mentions). 11 CLI initiatives were identified, such as organic fertilizers from livestock effluents, using sugarcane straw as poultry litter, and developing arenas for exchanging and facilitating collaboration between stakeholders. 3 main barriers were identified: governance, economic, and technical constraints. Governance challenges, the most cited, included overly restrictive, locally misaligned regulations and difficulties coordinating actors for collective projects. Economic challenges primarily stemmed from the high levels of uncertainty associated with these initiatives, which hinder the access to private funding. Additionally, the limited availability of locally adapted human and material resources represented a significant technical constraint. In conclusion, stakeholders acknowledge the critical role of CE in facilitating sustainable transitions within Réunion Island. Despite the existence of multiple barriers, CLI initiatives continue to be developed. However, the increasing number of CE initiatives raises concerns regarding competition for biomass resources. Further research is needed to assess the long-term viability of these initiatives and develop policy recommendations to reduce biomass competition.

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Theatre 3

Characterising territorial crop-livestock relationships of farms in PDO cheese areas: a methodological approach

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Integrated crop-livestock system (CLS) is seen as a way of designing more sustainable systems, by promoting farm autonomy. Initially studied at farm level through the analysis of matter flows and the assessment of crop-livestock coupling using the NiCC'El method, attention is now being focused on crop-livestock integration at territorial level (TCLI), by considering interactions between stakeholders. Cheese appellation areas, where feed self-sufficiency is a requirement, illustrate this territorial social coordination. This internship, in collaboration with the RMT Fromages de Terroirs and the RMT SPiCEE, has two level analysis : 1) at the national scale, it aimed to understand the role of TCLI for PDO cheeses and 2) at the farm scale, it assessed the effect of cheese PDOs on crop-livestock relations using a methodological adaptation of the NiCC'El crop-livestock coupling assessment to integrate the matter flows between farms and their productions and an analysis of interactions made by the farmers. Surveys of 28 farms in the Langres and Camembert de Normandie PDOs were used to establish a typology based on crop-livestock interactions. The results show that: (1) the cheese sectors prioritise feed self-sufficiency over global autonomy, (2) territorial integration reduces the coupling score due to dependence on external inputs, (3) there is no correlation between coupling and the number of interactions, (4) four groups according to the number of interactions and the intensity of fees self-sufficiency, (5) membership of the Langres PDO has no significant effect on coupling, and (6) the intensity of coupling and crop-livestock interactions differs between two cheese PDO zones. Finally, this internship set up a methodology that emphasises the role of local purchases in CLS and had to be tested in an area where there are complementarities between livestock production and crop production like in Picodon area.

Fostering circularity through livestock in small-scale territories of Madagascar highlands: a preliminary study of their protometabolism

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Increasing agrifood systems' circularity is a sustainable transition pathway in which livestock can play a major role. This study addresses the protometabolism of two small territories (fokontany) from Madagascar highlands: Malaza (MLZ) and Miarinarivo (MRN). This assessment is based on quantification of matter inflows, outflows and circulating between four components: crops, livestock, households and natural areas. Data farming systems management from 30 farms per fokontany has been harvested in 2023. This provides a better understanding of biomasses management and circularity within the two territories with a special focus on role of livestock. MLZ relies more on intensive agriculture, notably irrigated rice, and dairy production. This intensification leads to dependence on external inputs and increased pressure on natural resources. MRN is more focused on household subsistence, relying most on local resources and limiting inflows and outflows. Its agriculture is less intensive, based on rainfed crops and less productive, mainly extensive, forms of livestock farming. Livestock plays a major role in both areas. In MLZ, dairy systems are highly integrated with crops, enabling nutrients to be recycled through the consumption of residues and organic manure deposits. In MRN, while crop-livestock integration (CLI) also exists, notably family pig farming systems, extensive zebu rearing partly ensures nutrient transfers from natural areas through daily grazing. These results illustrate different potential solutions for livestock-related circularity. In MLZ, improving manure management and developing agroforestry systems in association could optimize the use of local resources and thus reduce the use of external inputs. In MRN, while CLI remains an issue, the low availability of irrigated land limits agricultural intensification. The improvement of security conditions (zebu theft) are therefore a challenge to ensure territory sustainability.

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Theatre 5

Multi-actor initiatives in the adaptation of livestock farming practices to societal challenges: Case-study of Italian beef sector

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Livestock farming faces growing societal challenges, particularly in urbanized societies increasingly disconnected from agriculture. The Entr'ACTES project analyzed how controversies around livestock farming impact stakeholder practices and engagement in four European countries, including Italy. This study focuses on meat sectors in the Pô Valley, a highly urbanized region with diverse livestock systems. A Master's 2 class from Institut Agro, supported by local academic partners, conducted 26 semi-structured interviews with stakeholders (farmers, researchers, unions, banks, consumer associations...) to assess their perception of how citizens view livestock farming, and how they address these perceptions. Stakeholders perceive a widespread lack of knowledge among Italians, particularly urban dwellers (23/26 interviewees). In their view, we could classify citizens into three groups: (i) critics, misinformed; (ii) indifferent consumers; (iii) interested consumers, eager for information—on whom efforts should focus. They identify animal welfare, meat quality, and trust in labels as key concerns. Four main strategies emerge: (i) Enhancing local/national biotechnological production to improve livestock conditions and image; (ii) Strengthening local communication (fairs, events) to boost product image and trust through traceability; (iii) Preserving traditional systems and breeds via strict European standards (DOP, PGI, but not organic); (iii) Developing product marketing, culinary valorization, and distribution via national/international networks, such as Slow Food, with a strong economic focus. Compared to France, where the first three strategies are mostly managed by national institutions facing the challenges of international and economic valorization, Italy seems to stand out for promoting synergies between different actors. Local initiatives target consumer expectations, while national/international actions strengthen the economic dimension.

Sustainability Typologies of Austrian Dairy Farms: An Integrated Clustering and Expert Assessment Approach
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This study analyzed 162 Austrian dairy farms using key indicators per “kg milk” and “hectare” to assess sustainability in animal health, economic performance, and environmental impact. The goal was to classify farms’ sustainability performance using an integrated approach: unsupervised clustering on key features followed by expert qualitative assessment. Moving beyond binary classifications like organic vs. conventional, this method transforms complex data into insightful farm typologies, ensuring practical relevance for sustainable dairy farming. Four distinct clusters emerged: Cluster 1 (Intensive Alpine): High milk production via external concentrate feed in challenging conditions, with moderate cow longevity, below-average revenue, and medium environmental impact. Cluster 2 (Intensive Favorable Location): High milk yields from on-farm feed, economically efficient but with shortest cow longevity, highest udder health issues, and high environmental impact per hectare. Cluster 3 (Extensive/Organic Favorable Location): Lower milk yields, average cow longevity, lower nutrient surpluses, and reduced environmental impact compared to conventional lowland farms. Cluster 4 (Extensive/Organic Alpine): Longer cow longevity, lower milk yields, and low environmental impact per hectare, well-adapted to alpine conditions. Findings highlight synergies and trade-offs between production intensity, economic performance, environmental impact, and animal welfare, shaped by local conditions. Results suggest that beyond location-specific optimal intensity levels, increased production can lead to health risks, higher costs, and greater environmental burdens, particularly in lowland regions.

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Theatre 7

Reorganizing territorial resources management towards an agroecological transition: from improving self-sufficiency to boosting integrated crop livestock systems

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Livestock farming is heavily impacted by climate change, economic instability, social expectations and ecological issues, so the future of livestock farming requires a major redesign of livestock farming systems (LFS). A number of conceptual approaches suggest different ways of redesigning livestock production systems. Agroecological transition is a promising approach based on ecological mechanisms as the key to improving LFS. In this way, self-sufficiency can reduce dependence on feed purchases and limit the risk of pollution by chemical elements; interactions between crops and livestock can improve the looping of geochemical cycles. In many research projects, self-sufficiency has been studied at farm or regional level. One of the weaknesses of this approach could be the increase in nitrogen and pesticide purchases used by farmers to achieve self-sufficiency. The development of approaches based on the combination of local resources by stakeholders is also an interesting way of analysing the anchoring and natural biological component of LFS. Our studies show that increasing the anchorage and use of natural resources improves the agroecological potential of LFS. Finally, the development of projects aimed at increasing crop-livestock interactions is a way of challenging the circularity of livestock-based food systems. But in order to rethink LFS, we need to bridge the gap between the benefits of LFS for circularity and the disadvantages denounced by the public; a number of opportunities could be devised to improve animal product quality, preserve landscapes and biodiversity, and strengthen exchanges between stakeholders.

Social and environmental impacts of milk production – comparison of dairy cows' diet scenarios

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Dairy cows' diets in Finland consist mainly of grass silage (GS), remaining part being concentrates (CO), including imported rapeseed meal (RS). Here, diet scenarios were used to assess the environmental and social impact of substituting RS with domestic faba bean (FB). Also, GS was substituted by half with clover grass (CG). Carbon footprint (CF) and social impacts were assessed using Life Cycle Assessment (LCA; Hietala et al. 2021; UNEP/SETAC 2009; UNEP 2020). Social LCA (SLCA) deviated from environmental LCA by focusing only on required feed production. Collected data for scenarios incl. feeding, milk yield (MY) and composition. Milk production and diet responses were based on Kokkonen et al. (2025). Feed production, nutritive values and precrop effects were as in Hietala et al. (2021, 2023), Kuoppala et al. (2024). Fat and protein corrected MY was used as functional unit (FPCM, IDF 2015; FU). The n of calvings, slaughter age and weight were constant (Hietala et al. 2021). The RS+GS diet included 4413 kgDM GS year⁻¹, RS amounting 469 kgDM. In FB+GS and FB+CG diets, the isonitrogenous amount of FB substituting RS was 595 kgDM. In RS+CG, FB+CG, the amount of forages, GS and CG, was 2409 kg. All diets included barley, oats (1:1) and molasses. In comparison to RS+GS diet, FB+GS reduced MY by 2%, whereas RS+CG increased MY by 2%. MY was unchanged for FB+CG. The lowest CF per FU resulted from RS+CG, -7%, and FB+CG -6%, in comparison to RS+GS. FB+GS diet performed worst with CF increased by 2%. SLCA showed the highest risk values for diet with highest share of imported RS and lowest MY (RS+GS). Substituting RS with Finnish FB together with higher MY had lowest risk values (FB+CG). Results indicate that the best combined environmental and social performance of Finnish milk is achieved when clover grass and domestic legumes are included in dairy cows' diets.

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Theatre 9

A novel farmer-centric above-ground biodiversity framework assessment for New York dairy farmers

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The term 'Biodiversity' is becoming a hot topic for agricultural producers and corporations as part of a larger sustainability initiative. Similar to greenhouse gas emission assessments, many corporations are trying to capture scopes of their biodiversity impact on the planet. However, biodiversity is difficult to efficiently capture accurately or precisely on-farm and there are several ongoing debates on how to best approach biodiversity conservation in agricultural settings. Furthermore, the status of biodiversity on integrated crop-livestock systems, such as New York (NY) dairy farms, is unclear. Hence, there is a demonstrated need for an accessible approach to measure agricultural biodiversity. Here we present a review of current literature related to above-ground biodiversity and dairy farming using the Web of Science search platform. A literature review covering research on biodiversity indicator species, tools for biodiversity measurements, impacts of agriculture on biodiversity, and biodiversity conservation framework development was conducted, and potential applications to a NY dairy biodiversity framework were assessed. In addition, a landscape review of the current biodiversity practices of corporations connected to the dairy industry was developed, including dairy cooperatives, processors, and sustainability programs. Company practices were analyzed for effectiveness where possible, and for their potential to be implemented into the proposed NY biodiversity framework. Current work includes discussion of feasible options with NY dairy farmers before development of a novel, informative, outcome-based above-ground biodiversity framework relevant to NY dairy producers.

Consequences of alternative proteins on the environmental sustainability of broiler production

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Replacing soybean (SBM) in broiler diets is crucial for environmental sustainability. A previous review identified microalgae (MA), wheat DDGS (DD), and processed animal proteins (PAP) as potential alternatives. This study assessed their environmental footprints using life cycle assessment (LCA) in SimaPro. System boundaries included broiler production stages. Environmental impacts were measured per 1,000kg of carcass weight. Inputs included feed and energy, while outputs were broilers and manure. Diets were formulated to meet Ross 308 broiler requirements. A total of 21 diets were evaluated: SBM, DD at 15% (DD15) and 20% (DD20), MA at 10% (MA10) and 15% (MA15), and PAP at 10% (PAP10) and 15% (PAP15). SimaPro databases provided background data, except for MA. The study assessed global warming potential (GWP), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), land use (LU), and water consumption (WC). SBM diets contributed 3,226kg CO₂-eq to GWP, 22.6kg SO₂-eq to TA, 1.2kg P-eq to FE, 2.6kg N-eq to ME, 5,036.8m²a crop-eq to LU, and 28.2m³ to WC. Alternative protein diets reduced FE, ME, and LU. DD decreased all impacts, with greater reductions at DD20. MA increased GWP (16.1%–27.2%), TA (18.7%–23.6%), and WC (49.0%–77.4%). PAP significantly reduced GWP (34.3%–45.0%), FE (30.5%–40.2%), LU (33.6%–44.5%), and WC (23.3%–30.7%) but slightly increased TA (7.2%–8.3%). Manure emissions from SBM contributed 176 kg to GWP, 16.9kg to TA, 0.06kg to FE, 0.35kg to ME, 256.9kg to LU, and 1.44m³ to WC. Alternative protein diets reduced manure LU impacts. DD increased manure emissions, except for GWP, which decreased by 4.1% at DD20. PAP reduced most manure-related impacts, though WC (3.3%) and FE (0.8%) increased slightly with PAP10. MA significantly increased manure GWP (14.3%–23.5%) and WC (49.2%–74.2%) but reduced TA (16.1%–15.8%) and ME (21.2%–22.6%). PAP emerged as the most sustainable alternative to SBM, followed by DD, while MA posed sustainability concerns due to increased GWP and WC.

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Theatre 11

Enhancing ecosystem services in cattle farming: insights from the PRESENCE project

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Cattle farming, crucial to the agricultural economy and landscape multifunctionality in northwestern France, has declined over the past two decades. This study, part of the PRESENCE project (ImProve Ecosystemic Services of bovine livestock in tErritories), synthesizes findings from three interconnected phases to assess and enhance the multifunctionality of cattle farming at the territorial scale. Semi-structured interviews (n=20) done during phase 1 (2023) across three areas revealed that, while ecosystem services (ES) concepts are not always explicitly recognized by stakeholders, their valuation depends on territorial and ecological contexts. Public-private collaborations, supported by policy tools (such as labels), help structure ES provision and aligning cattle farming with local sustainability goals. In phase 2 (2023), a survey of 65 cattle farms across the three areas identified 11 farm types (4 dairy, 5 suckler, 2 mixed) and 5 ES bundles using factorial analysis and hierarchical clustering. Dairy and mixed systems significantly contribute to food production and employment, while suckler and grass-based systems enhance biodiversity and rural vitality. These findings underscore the need for refined methodologies to characterize ES provision across diverse farming systems. Phase 3 (2025), a targeted survey of 30 farms in one area analyzes the links between feeding strategies, agrobiodiversity, and land-use configurations. It also stresses out the impact of climate and economic pressures on feeding strategy. This research improves understanding of how cattle farming systems interact with socio-ecological environments, informing policies that support both productive and ES-oriented practices. By integrating feed management, farm diversity, and territorial dynamics, this work aids the transition to sustainable cattle production.

Fatty acid profile of milk from Murciano-Granadina goats fed white grape pomace silage during a complete lactation

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As the first-largest wine producer worldwide, Europe generates approximately 14 million tons of grape pomace annually. Utilizing these by-products in animal feed aligns with the sustainability goals of the European 2030 Sustainable Development Goals (SDGs). This practice lowers dairy production costs and supplies bioactive compounds beneficial to animal health, enhancing the production of high-quality and safe animal-derived food for human consumption. However, the nutritional quality of milk needs to be assured under these production conditions. This work aimed to evaluate the effect of white grape pomace silage (WGP) in dairy goats diet on fatty profiles during a full lactation period. 40 dairy goats were selected and divided into 4 groups (2 treatments x 2 plots). The treatments consisted of a standar dairy goat diet and an isoenergetic and isoproteic diet adjusted to include 15% DM of WGP. At the beginning, middle, and end of the full lactation period (8 months), the fatty acid profile of milk yield from both treatments was analyzed. Milk fatty acids were extracted by the Folch procedure and were methylated following the Nudda et al. (2005) method. The identification was by a GC-MS/FID. Nutritional and health indexes for assessing fatty acids were calculated according to Chen & Liu (2020). The effect of the treatment was determined using a general linear model (Proc. Glm, SAS v9.4), considering 2 treatments, sampling (4 levels: pre-experimental and 3 experimental) and the interaction between them. UFA and LCFA were significantly higher in WGP milk. SFA were significantly higher in the standar milk. WGP milk had the highest n6/n3 ratio; however, CLA was also significantly predominant in this treatment. The sum of TFA was significantly lower in the standar milk. The health human indexes indicated a positive effect of WGP intake, as reflected in lower IA, TI, HPI. Keywords: Circular economy, byproduct, bioactive compounds, health. This study is supported by the MCIN with funds from the UE (NextGeneration-PRTR-C17.I1) and the Generalitat Valenciana (AGROALNEXT/2022/062-SOSCAPRI).

In vitro evaluation of olive leaves as a sustainable feed ingredient for ruminants: effects on nutrition and methane emissions

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The incorporation of agro-industrial by-products into ruminant diets is gaining attention as a sustainable approach to reduce feed costs and environmental impact, while maintaining animal productivity. Olive leaves, a by-product of olive oil production, are widely available in Mediterranean regions and could serve as an alternative feed resource. This in vitro study evaluated the nutritional value and fermentation characteristics of olive leaves preserved by different methods: fresh, dried and ensiled with 2% urea and 2% molasses. Chemical composition, in vitro digestibility (DOM), metabolizable energy (ME), total gas production and methane (CH₄) emissions were evaluated. The results indicate that the inclusion of dried olive leaves in a standard concentrate reduces total gas and CH₄ production in vitro, with the most significant effects observed at a 10% inclusion level. This suggests that dried olive leaves could be used as a dietary strategy to reduce enteric CH₄ emissions without compromising energy supply. In contrast, ensiling slightly improved in vitro DOM and maintained a similar ME value to fresh leaves, but resulted in an increase in CH₄ production. These differences may be attributed to differences in the chemical composition and fermentability of the preserved material. These results highlight the potential of olive leaves as a sustainable feed ingredient for ruminants, especially when dried, due to their methane mitigating properties. However, further in vivo research is required to validate these effects under practical feeding conditions, to assess their influence on animal performance and to explore the long-term implications for feed efficiency and environmental sustainability.

Potential of red grape pomace silage as an alternative ingredient for dairy goat diets

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Within the framework of the UN's 2030 SDGs, the valorization of by-products and the reduction of inputs in the production of high biological value foods are a priority. The aim of this work was to study the effect of 15% (DM) inclusion of red grape pomace silage in dairy goats diet on their productive indexes during a full lactation period. 40 dairy goats were selected and divided into 4 groups (2 treatments x 2 plots). The treatments consisted of a standard diet (control) and an isoenergetic and isoproteic diet adjusted with respect to the control to include 15% DM of red grape pomace silage (RGPS). After a one-week adaptation period to the plot, a pre-experimental sampling was conducted, followed by an 8 month experimental period. During the experimental period, the evolution of body weight, milk yield (Lactocorder, WMB AG, Switzerland), macro-composition, milk urea content (mg/L) and SCC (log 10³ cells/mL) (CombiFossTM 7 DC, Foss, Denmark) were determined every 6 weeks. Additionally, dry matter intake (DMI) and water intake (WI) were recorded for 3 consecutive days every 2 weeks. The variables were analyzed using a mixed linear model (Proc. Glimmix, SAS v9.4), considering the effect of the covariate in the pre-experimental sampling, the treatment, experimental sampling, and their interaction, and the plot nested to the treatment. The animal was considered a random effect. The effect of the covariate and sampling were significant in all variables ($P < 0.05$). The treatment was significant ($P < 0.05$) on DMI and WI; useful dry matter content (UDM), urea; and SCC of milk. The interaction between sampling and treatment was only significant for DMI and WI ($P < 0.05$). DMI between treatments equalized after the first third of lactation. WI was significantly lower in the RGPS treatment, and the body weight was significantly lower than the control during the last third of lactation. The best energy efficiency (kg milk/ufL) of RGPS treatment significantly improved standardized milk production (FPCM 3.7 MJ). It is concluded that the inclusion of red grape pomace silage in the goat diet maintains their productive indexes, representing an economical and sustainable nutritional alternative for ruminants.

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Poster 15

Evolution of perceptions of French high school students on livestock farming between 2014 and 2025

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In many Western countries, people are increasingly disconnected with farms realities, and their knowledge about agricultural practices is steadily declining, while their expectations about production, animal welfare and environmental impact are rising. In 2014, we conducted a survey on the perceptions and concerns about livestock farming on 1,083 French high school students. As significant societal and environmental changes occurred since then, a similar survey was undertaken in January 2025, among 503 French high school students. A similar questionnaire to the 2014 one was used, including closed and open-ended questions to evaluate the perceptions, consumption habits, knowledge, and interest in livestock farming and practices of these young people. From 2014 to 2025, the percentage of students who experienced contact with farm animals or visited a farm decreased by 22% and 15%, respectively, meaning that only 25% of them have been in contact with farm animals 2025. This might be due to a lower proportion of students enrolled in agricultural studies in 2025's sample, as well as stricter biosecurity measures that have restricted public access to farms. The results also showed that in 2025, 36% of students had a positive perception of livestock farming, compared to 44% in 2014. Students' main concerns regarding livestock farming remained consistent over time, with animal welfare (87%), environmental impacts (57%) and health crises (22%) being the most important in 2025. Over 90% of the students considered that livestock production is of importance in France, especially for its role in providing local food. Information regarding livestock farming mainly originated from social networks and TV news, and many students indicated that they would like to be better informed, mainly via professional interventions and social networks. It was concluded that, despite changing contexts, the main concerns of higher school students still remained similar from 2014 to 2025, and they were still in favour of preserving livestock farming in France.

A survey on feed autonomy in dairy buffalo farms in southern Italy

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This study aims to generate reference values for feed autonomy in dairy buffalo farms located in the PDO mozzarella-producing area. Between June and December 2024, twenty commercial dairy buffalo farms were surveyed. Farmers were interviewed using a questionnaire covering herd characteristics, milk yield, and general management practices, as crop production and feeding strategies. For each crop, dry matter (DM) production was assessed in terms of both quantity and nutritional value. Feed autonomy (FA) was determined as the proportion of self-produced DM consumed relative to the total DM intake at the herd level. Additionally, energy feed autonomy (FAe) and protein feed autonomy (FAp) were calculated by multiplying the amount of consumed DM by its energy and crude protein content for both purchased and self-produced feeds. The collected data underwent descriptive statistical analysis. The farms surveyed had an average usable agricultural area (UAA) of 25 ha, entirely devoted to forage production. Maize silage, often grown in rotation with winter grasses – either as pure crops or in legume mixtures – is the predominant forage. Regarding herd composition, an average of 337 (± 188) animals was recorded per farm. The average milk yield per cow was 2.395 ± 275 kg. Consequently, the average stocking rate was 19 ± 17 heads per ha of UAA. Regarding feeding practices, 35% of farms relied exclusively on hay as their forage source, while the remaining farms used varying proportions of maize silage and hay. Compared to herd requirements, 85% of the forage and 100% of the straw and concentrate feed were purchased externally. As a result, the average FA level was estimated at 15%, ranging from 2.5% in dry herds to 23% in lactating herds. Expressed in terms of consumed energy, FAe was 14%, while FAp was slightly lower at 12%. Our preliminary findings reveal a significant dependence on off-farm feed in dairy buffalo farming, with limited forage self-sufficiency and high stocking densities indicating that scarce arable land is a major challenge to economic and environmental sustainability.

Session 52

Poster 17

Livestock and orchard farmers perceptions on the impact of grazing in almond and olive fields

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In rainfed tree crops such as almond and olive orchards, livestock grazing on sown or spontaneous vegetation cover as an alternative to tillage is beneficial for soil structure and fertility, supports feed autonomy for the flock and promotes circular economy. To assess its potential interest, the perceptions and attitudes of farmers, livestock breeders and advisors towards this practice were analysed through direct surveys ($n=71$) conducted in Teruel (Spain). The questionnaire addressed farm structure and management, the perceived benefits and drawbacks of livestock grazing on orchards and its effects on the use of inputs. Respondents were fruit growers (40%), mixed farmers (21%), livestock breeders (4%) and advisors (35%). Most farmers cultivated both almond and olive orchards (47%), whereas mixed and livestock breeders were mostly sheep farmers (57%). Only 51% of fruit farmers had previously used cover crops, but up to 80% were favourable to using them and allowing grazing by livestock, preferably sheep. Current users managed them by mechanical mowing (65%), grazing (33%), or cutting and leaving them on the ground as mulch (30%), rarely using herbicides (7%). Respondents considered grazing to be slightly to highly beneficial for multiple agronomic, environmental and social aspects. However, fruit farmers were more sceptical than livestock breeders regarding young tree survival (57% rated it as slightly to highly detrimental), tree productivity (18%), pest control and soil quality (11%). Grazing was considered to reduce herbicide (87%), fuel (74%) and fertilizer (67%) use, reduce (29%) or maintain machinery requirements (47%), but increase fencing needs (37%). While opinions were divided regarding the impact on labour for livestock breeders, orchard farmers believed it would reduce labour demands (60%). Overall, strong support was shown for grazing cover crops in tree fields, with greater perceived benefits for livestock breeders, suggesting that familiarity with mixed farming systems positively influenced their assessment. The reduction in reliance on inputs highlights the sustainability of this practice that enhances economic and environmental efficiency while alleviating labour demands.

Best practices for animal welfare in dairy cows: compilation of current knowledge in the framework of a EUPAH&W project evaluating impact of animal welfare on sustainability

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Keywords: Animal welfare; sustainability; EUPAHW; best practices; dairy cows. Ensuring animal welfare (AW) is a crucial aspect of the transition towards sustainable agriculture. The present study was conducted within the framework of the European Partnership on Animal Health and Welfare (EUPAHW) Set of Actions 17 on “Sustainability aspects of animal welfare-promoting livestock systems”. A comprehensive review of existing projects, initiatives, and relevant literature – including mainly EFSA opinions, results from the Care4Dairy project, and animal welfare transport guides – was undertaken to select relevant AW topics and then identify best practices that enhance AW for dairy cows, heifers and calves at various stages of the livestock production chain (on-farm, during transport and, at slaughter). Based on these best practices and EFSA opinions, several “AW-promoting” production systems were identified including cow-calf contact systems, free stall with access to pasture, systems performing extended lactations. Additionally, training of staff and systems that emphasize a strong human-animal relationship were highlighted as crucial in improving AW. The next phase of this project will include a literature review on the impacts of these best practices and AW-promoting systems across environmental, economic and social sustainability pillars. This will help identify potential synergies and trade-offs between AW and sustainability, while highlighting existing knowledge gaps, the broader sustainability impacts of AW promoting practices and systems. This work was co-funded by the European Union’s Horizon Europe Project 101136346 EUPAHW.

Session 52

Poster 19

Toward an interdisciplinarity method to explore the local embedding of organic pig farming systems in France

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Although currently weak, the organic pig farming sector under Organic Farming (OF) specifications offers potential for development and for the agroecological transition of livestock farming systems in Europe. This project is based on the main barriers and levers identified in French pig production. It aims to promote a systemic approach to adding value in pork chain, and to consider the complementarities between animal and plant production at farm level, and between organic and non-organic farming sectors at regional level. The question addressed is how the local anchoring of organic pig farming contribute to the transition of local agri-food systems. The objectives of the project are to establish a method for analysing the role of organic pigs in localized food systems, integrating both the farm and regional levels and to test it in contrasted French territories. The project brings together researchers and academic staff with expertise in technical sciences as well as human and social sciences. Different dimensions will be emphasized, such as natural environments, interaction and cooperation between stakeholders, territorial resources, valorisation of products and coproducts and the link between animal and plant production for human nutrition. Three stages will be followed: (1) definition of conceptual and theoretical frameworks to build interdisciplinarity, (2) design and testing of the approach in three regions of France, and (3) synthesis and dissemination of the results. The approach will be communicated to students, stakeholders from both organic and non-organic sectors, policymakers and scientists.

Exploring perceptions of sustainability issues in Italian PDO small dairy chains in the LoST-EU project

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Italian small dairy chains play a vital role in the social and economic fabric of the regions where they operate, contributing to local communities and society overall. However, these production systems are facing a decline due to several factors, including a lack of generational turnover, depopulation of marginal areas, and the broader challenges posed by globalization. At the same time, today's increasingly conscious market demands products that embody and certify sustainability. In response to this trend, this research aimed to draft the guidelines for the identification of best practices for sustainability in dairy production considering the environmental, economic and social dimensions focusing on eight small PDO cheeses from five Italian regions. To achieve this aim, a structured questionnaire was administered to 87 supply chain stakeholders a survey was conducted to 1) define the principles and criteria of sustainability through a survey with the stakeholders of the cheese chains involved; 2) choose criteria and assign weights to the chosen criteria based on the results that emerged from the stakeholder discussion; 3) develop indicators and measure the performance of production practices employed in the considered supply chains. First results about the importance (measured using a 7-point Likert scale) given by the stakeholders towards the selected sustainability issues – categorized into environmental, social, and economic dimensions – revealed an overall positive perception of sustainability, with average scores no lower than 4. The highest ratings were assigned to aspects of social sustainability, while lower scores were observed for certain economic and governance-related issues. Additionally, differences in perception emerged based on variables such as age group, education level, supply chain sector, and stakeholder category. These preliminary findings will inform the next phases of the project, guiding efforts to address the most critical issues while enhancing the sustainable aspects that stakeholders prioritize. Support: Reg.UE 1144/2014 Convenzione n.101095047 – LOST -UE- WP 8

Session 53

Theatre 1

Food culture and cell-culture: technical, ethical and social frontiers

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Maintaining quality standards is crucial for the success in the food industry. The concept of “food quality” encompasses multiple dimensions, including safety, nutritional value, sensory attributes, and needs of consumer. As cell-based foods enter the market, it is critical to assess how consumer perception align with these novel products. This review aims to identify the main research gaps that must be filled to better understand how quality standards and expectations can be met with respect to production of cell-based foods (also known as “cultured meat,” “cultivated meat,” “in vitro meat” or “lab-grown meat”), particularly in economic and social sciences. First, although some of the materials, inputs, and equipment used for cell-based food production may be new, the approaches to food safety assessment and risk mitigation are very similar to those used for conventional food. Second, many technical challenges still stand in the way of large-scale production and commercialization of this new product (scalability, cost-effective and animal-free culture media). There is also a need for scaling-up to validate the sustainability claims (potential low resource use and low environmental impacts). Concerning social issues, mixed consumer perception has been observed around the world. Further cross-cultural studies in social sciences are needed to assess real consumer attitudes and the factors influencing acceptance or rejection of these products. Although regulatory approval of cell-based food products has been granted in three countries, consumer experience with these new products is minimal. In many countries, particularly in Europe, debates are still ongoing (about food production model, food culture, ethics, religion, etc). There is also a need to establish a clear nomenclature for cell-based food and hybrid products as well. These barriers highlight the key areas where progress is needed for the cell-based food industry to grow.

Thinking the future with citizens: foresight approach for assessing Portuguese beef consumption patterns
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Currently there is an uncertain environment where predicting consumers' choices has grown increasingly challenging. Forecasts do not adequately explain the large variety of potential outcomes or capture the range of uncertainty in the future. It is desirable to understand potential futures and how they will impact different key factors, improving the ability to handle upcoming challenges. This research aimed to develop a set of relevant and plausible future scenarios of cattle meat consumption, in the Portuguese population, in order to inform the industry and potential decision-makers. In this study, a foresight participatory scenario planning (PSP) approach, with large citizen involvement, was developed and applied to identify key drivers of change and generate future scenarios for beef consumption in the Portuguese population until 2050. A group of 141 Portuguese participants took part in this stage of the project. By analysing the results, sixty drivers were firstly identified as most relevant/impacting in influencing the future of beef consumption and were used to build a first approach with two opposite scenarios: "Beef deal" (describing a future with high, yet plausible, consumption) and "No deal" (describing a future with low, yet plausible, consumption). In a further analysis on key drivers, three scenario formats were then formulated: the "Best-Case" portraying optimistic realities, the "Worst-Case" depicting pessimistic realities, and the "Business-As-Usual" painting a future in line with current trends. To highlight specific adverse 'end states', the "Worst-Case" was divided into four formats: "Economic Crisis", "Climate Crisis", "Borders Closed" and "Social Crisis". This process, complemented by a manual analysis facilitated the extraction of six scenario configurations which were later finalised with stakeholder input. These informed the construction of six scenario narratives with 6 potential futures for beef consumption in Portugal in 2050.

Session 53

Theatre 3

How much meat do we really eat? Assessment of meat consumption in Portugal

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Meat consumption in Portugal has increased (excluding the pandemic years), contrary to the trend seen in the European Union. Whether this is due to consumption by the resident population (RP) or due to increased tourism is less clear. It has also been criticized based on the environmental and sustainability problems associated with its production. Meat capitation data indicate excessive consumption and a clear dependence on imports. However, sampling methodology, data treatment and variables considered are not clear, often contradictory between different sources of information. The attempt to compare national values with those of other countries or the European average is even more complex, for the reasons mentioned. This study aimed to evaluate the real meat consumption in Portugal, considering the RP and tourists (number and length of stay: 47,598 tourists/day; Turismo de Portugal, 2024). Using this data, all possible waste that could be quantified throughout the food chain was assessed, from slaughter to the consumer's plate. Based on the values provided by INE (2023) from meat production (1.233 million tons; 109.2 kg/capita/year), were quantified the edible parts (INSA, 2023) and losses along the food chain, i.e., in retail, food services and households, respectively 2%, 5% and 11% (Foodwaste, 2021), in total meat and by animal species (beef, poultry, pork, sheep and goat meat). Other meats and offal were excluded from this study due to a lack of information regarding waste throughout the food chain. From this analysis of the information, it was concluded that daily meat consumption per capita in Portugal is 197 g/day, which represents only 64.6% (vs. 305.5 g/day; INE 2023) of the capitation values presented by INE. This fact suggests that real meat consumption (total meat and by species) is much lower than that presented in national statistics, as such, the methodology for collecting and processing statistical data should be reassessed.

Consumer expectations and satisfaction for beef in the French region Auvergne-Rhône-Alpes

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Beef consumption has been decreasing in France for various reasons: (i) its high price, (ii) its controversial image (mainly related to the protection of animals and of the environment), (iii) the changes in consumption patterns (less time for cooking and eating), and (iv) its variable sensory quality. The project OABov-AURA (2022-2025) was carried out to study consumer expectations and satisfaction regarding the intrinsic and extrinsic qualities of beef in the region Auvergne-Rhône-Alpes (AURA), the 2nd largest beef cattle breeding region in France. From an on-line survey (740 respondents with 62% females, 46% less than 40 years of age and 51% with a good knowledge of farming), it was shown that 51% of respondents reported having reduced their meat consumption for different reasons: health (24% of answers) and ethics (25%) issues. The majority of consumers (56%) eat beef for pleasure and are not disappointed with raw (82%) or cooked (51%) beef. The most important extrinsic criteria are: origin (44%), proximity (37%) and the presence of signs of quality and origin (SIQO) (33%) (% of answers "very important"). The most important intrinsic criteria are: taste (63%), tenderness (60%) and freshness (58%). Sensory analyses (on sirloin steak and premium beefsteak) confirmed the primary importance of flavor liking and tenderness. These quality traits are the highest for cooked beef from cull cows (for the sirloin steak) and heifers (for the beefsteak) compared to young bulls. However, raw beef from heifers is preferred for its color and global satisfaction, compared to young bulls for which the color is too pale. Among the reasons why consumers did not want to purchase again the cooked meat, are: lack of taste, hard beef, not tender and not juicy. These findings were consistent with the literature, where consumers showed that they were willing to pay more for a beef that is tender, and that flavour was the priority for the consumers. In conclusion, consumers in the AURA region may wish to have access to a varied supply of meat in butcher shops, of French origin and under quality signs.

Session 53

Theatre 5

Application of Rapid Evaporative Ionization Mass Spectrometry (REIMS) for the Classification and Prediction of Carcass Traits and Beef Quality

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Rapid Evaporative Ionization Mass Spectrometry (REIMS) technology enables real-time molecular analysis of intact beef tissues, offering potential for predicting carcass traits and beef quality. The 10-day aged rump (m. gluteus medius, RMP) and striploin (m. longissimus dorsi et thoracis, STR) muscles of 66 Limousine cow were studied using REIMS, and data were used to develop classification and prediction models using orthogonal projection to latent structures-discriminant analysis (OPLS-DA). Muscle type, EUROP Grid, carcass weight, Meat Standards Australia marbling score, intramuscular fat (IMF), Warner-Bratzler shear force, compression, and untrained consumer sensory scores for tenderness, juiciness, flavor liking, overall liking, and the calculated meat eating quality score (MQ4) were evaluated. REIMS accurately classified RMP and STR (classification and prediction accuracies 98.9%-100%) and was also strongly related to carcass evaluation and lab measurements (R^2Y : 75.1%-87.1%, Q^2 : 50.5%-79.8%). Model performance varied by muscle, with stronger RMP models for carcass weight, conformation, IMF, and compression, whereas STR models performed better for marbling. Sensory models showed validation and test accuracy of 93.3%-99.2% and 60.6%-77.4%, respectively. RMP models had stronger predictive ability for flavor liking, overall liking and MQ4, while STR models was more accurate for tenderness. These results highlight REIMS as a potential tool for rapid carcass and beef quality evaluation, with muscle-specific predictive abilities.

Investigating the performance of Charolais and Limousin young bulls and heifers in north-eastern Italian fattening units

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The Italian specialised beef fattening system depends mainly on the importation of young bulls and heifers from France, with Charolais (CHL) and Limousin (LIM) being the predominant breeds. Exploring the performance of such breeds is crucial to optimize meat yield and quality, and economic returns. The present study aimed to investigate the performance of young bulls and heifers imported from France and reared for the finishing phase in fattening units of the Veneto region, north-eastern Italy. The dataset consisted of 42,018 young bulls and heifers slaughtered between October 2021 and December 2024, namely 28,245 CHL (14,297 males and 13,948 females) and 13,773 LIM (6,577 males and 7,196 females). Live weight (kg), carcass weight (kg), and carcass yield (%) were analyzed through a mixed linear model that included the fixed effects of sex, breed, season of arrival, and the interactions between sex and breed, and breed and season of arrival. Slaughter batch and slaughter date were included as random factors. The season of arrival affected live weight and carcass weight ($P < 0.05$) but not carcass yield. CHL young bulls had higher live weight (733.50 ± 1.16 kg) and carcass weight (451.20 ± 0.71 kg) than LIM young bulls (617.50 ± 1.49 kg and 392.70 ± 0.92 kg, respectively). The same trend was observed for the heifers, while LIM animals performed better in terms of carcass yield. No significant differences in live weight and carcass weight were observed across seasons of arrival for LIM. Oppositely, CHL animals had the highest live weight and carcass weight in winter (653.60 ± 1.68 kg and 392.70 ± 1.04 kg, respectively) and spring (654.40 ± 1.55 kg and 393.10 ± 0.95 kg, respectively). The results provide insights into the factors influencing the performance of beef cattle imported from France in the Italian context, and put the basis for future research on sustainable farming, resource utilization, meat quality, and animal health and performance.

Session 53

Theatre 7

Impact of sires' breed on calving ease, resulting fattening performance and carcass quality parameters in fattening bulls from Holstein Friesian herds in North-Eastern Germany?

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Historically, cattle were bred for various purposes, including milk and meat production as well as labour. As breeding has become increasingly specialized over the last decades modern dairy breeds have mainly been focussing on milk production, producing bull and surplus female calves with low economic value. To address this dilemma, the present study, conducted in Holstein Friesian test herds in North-Eastern Germany, evaluates how crossbreeding with beef bulls and pure breeding affects calving ease and subsequent fattening performance, and carcass quality. For the study pedigree information, calving information as well as fattening performance and carcass quality parameters of 5,217 fattening bulls were considered. Results showed that crossbred calves had higher birth weights and better carcass conformation ratings than purebred dairy calves, reflecting beef breeds' superior muscle development. However, crossbreeding also increased the frequency of difficult births (dystocia), which was shown to have a negative effect on the fattening performance of affected animals and was previously stated to negatively impact calf health and productivity and fertility in dairy cows, thereby reducing the overall performance and economic benefits of dairy operations. The effect of parity was not relevant for fattening and carcass quality parameters but highlighted the present breeding practice of using beef sires in multiparous cows rather than in primiparous ones. Seasonal variations were also observed, with spring and summer-born bulls exhibiting faster weight gain, likely due to better forage availability and warmer temperatures. To sum up, the study underscores the need for careful management, such as sire selection and targeted mating, to balance the benefits of crossbreeding while minimizing the risk of dystocia and subsequent poor fattening performance.

Selection of genetic lines for crossbreeding of dairy beef calves and its impact on performance and meat quality

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A total of 144 Holstein x Angus bulls (155 ± 2.3 kg of BW, 120 ± 1.1 days of age) were used to evaluate the effect of different genetic lines of Angus semen to improve performance and carcass and meat quality of dairy beef bulls fed high-concentrate diets. Calves were randomly allocated in 8 pens of 18 bulls/pen and distributed into two genetic lines of semen: a genetic line specifically selected for off-spring with increased body mass and fleshing ability (A1; n=4 pens, 72 bulls), and another genetic line selected to maximize profitability and conception rate, especially in dairy cows to produce dairy beef off-springs (A2; n=4 pens, 72 bulls). The study lasted 272 days. Body weight was recorded monthly, and pen concentrate intake daily. At the end of the study, bulls were slaughtered, and hot carcass weight (HCW), carcass conformation, and fatness were recorded. A subset of 36 bulls/treatment were used for meat quality analysis, where instrumental color, Warner-Bratzler shear force, and intramuscular fat content (IMF) were analyzed. Data were analyzed with mixed models and a Chi-square test. Although no differences were observed in the final BW, HCW ($P = 0.01$; 284 ± 3.40 kg vs. 306 ± 3.40 kg for A1 and A2, respectively) and the number of carcasses with a higher degree of conformation was greater ($P = 0.01$) for A2 than A1 bulls. Regarding meat quality, A2 bulls had a greater percentage of IMF ($P < 0.01$; 2.09 ± 0.124 % vs. 2.69 ± 0.124 % for A1 and A2, respectively) and were less tough ($P < 0.01$; 6.67 ± 0.115 kg vs. 6.19 ± 0.115 kg for A1 and A2, respectively) than A1 bulls. Results from this study showed the importance of the selection of genetic lines for crossbreeding dairy beef bulls to maximize performance and meat quality.

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Theatre 9

Genetic Relationships Among Carcass, Growth and Size Traits in Nordic Red Dairy Cattle

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The beef-on-dairy approach, where beef bulls are used on dairy cows to produce crossbred offspring, has gained popularity in many countries. This approach allows to produce more meat from surplus dairy calves while emitting less greenhouse gases per kg meat produced. Inseminating dairy cows with beef semen improves the meat traits of dairy-origin beef. However, the focus on crossbreeding often overlooks the role of the cow's genetics on meat traits. This issue is further compounded in the Nordic countries, where a saved feed index is included in the Nordic Total Merit (NTM) index. This index lowers maintenance energy requirement by applying a negative index weight on metabolic body weight. While this improves feed efficiency, its impact on carcass traits remains unclear. To address this, our study estimated genetic parameters for three trait groups: cow carcass traits (carcass weight, carcass conformation, carcass fat), bull calf growth traits (daily gain, carcass conformation, carcass fat), and cow size traits (metabolic body weight, stature). The analysis included data from 18,757 Nordic Red dairy cows and 42,307 bull calves. Variance components were estimated stepwise, beginning with multivariate analyses for each trait group, followed by a comprehensive analysis. Multivariate animal models were applied in DMU software to estimate variance and co-variance components using the restricted maximum likelihood (REML) method. Preliminary results showed that heritability estimates range from 0.25 to 0.46 for cow carcass traits, 0.19 to 0.31 for growth traits, and 0.63 to 0.64 for cow size traits. Genetic correlations were strongest between the cow size traits (0.70), followed by cow carcass traits (0.48 to 0.67), and growth traits (0.01 to 0.41). Ongoing analyses are examining genetic correlations across all three trait groups. Further research will assess how incorporating cow carcass traits in the NTM index shapes the genetic framework and determine the optimal weighting to enhance economic and environmental sustainability in the dairy sector.

Variation in carcass and retail cuts value of beef $\times$ dairy crossbred heifers produced in mountain farms

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Interest in beef-on-dairy production is increasing, but knowledge about variation in economically relevant carcass characteristics remains limited. This study investigated this variation using data collected in collaboration with the Breeders Federation of Trento (BFT, Northern Italy) on 3,443 crossbred heifers slaughtered weekly from September 2019 to December 2023. The heifers were sired by Belgian Blue (BB) bulls on dams of various dairy breeds: Brown Swiss, Holstein Friesian, Simmental (Si), Alpine Grey (AG), Rendena, and Crossbreds. The heifers were born in 441 mountain dairy farms, weaned in 7 specialized weaner farms, and fattened in the same intensive finishing farm. For each heifer, the weight of whole carcass and 17 main retail cuts was recorded. The value of the whole carcass and of each retail cut was calculated considering the weight and the selling price at the BFT retail store. Total retail carcass value was determined as the sum of all retail cut values. It was divided by the whole carcass weight to obtain a deboned retail price per unit of carcass weight. Data were analyzed using a mixed model with dam breed, slaughter season, and year as fixed effects, and birth and weaning farm, and slaughter date nested within season and year as random effects. BB $\times$ Si heifers had the highest whole carcass, retail cuts, and total retail carcass values, due to their heavier carcass and cuts weight. However, BB $\times$ AG heifers outperformed the other crossbreds in deboned retail price, because of a higher proportion of first-quality cuts and less trimming losses, which enhanced the marketability of heifers from this local breed dam in terms of saleable meat. These findings reinforce the interest towards beef $\times$ dairy crossbreeding and emphasize the relevance of local breeds such as AG in supporting the economic sustainability of dairy farms in mountain areas. This study is part of the INTAQT project funded by the EU's Horizon 2020 research and innovation programme (grant agreement N°101000250).

Session 53

Theatre 11

Comparing the profitability of different selection strategies during drought in Australian Cattle

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Droughts force farmers to make critical decisions about which animals to sell. Selecting which breeding cows to sell has implications on herd characteristics when re-breeding following drought. We investigated the consequences of different criteria for culling livestock on herd characteristics and profitability during and following drought years. To explore these consequences, we simulated a commercial Bos Taurus beef herd in Australia. Simulated traits included weaning percentage, live weight, body condition score, fat and muscle. The selection criteria for culling included weight, body condition score and genomic breeding values using an index with positive emphasis on weaning percentage, sale weight, fat and muscle and negative emphasis on mature weight. Herd characteristics and profit were evaluated over a 10-year period post drought, using climate conditions based on 70 years of historic Standardised Precipitation Evapotranspiration Indexes (SPEI). We randomly selected 10 years of SPEI values with the first year always from the severe drought category (<-1.2). The number of animals that could be managed on the farm area was scaled using (i) SPEI values relative to a median year (SPEI=0) and (ii) estimated energy requirements using Australian feeding standards. Profitability was calculated from national price data adjusted for SPEI category, regionality and inflation. We found that selecting on a genetic index was most profitable. This selection was ~6% more profitable than random selection in the first year and ~3% more profit accumulated across the 10 years. This benefit was from improvements in the herd across the 10 years for economically important traits. Selecting for cow condition and weight improved productivity and profitability initially. These benefits, relative to index selection however, diminished over time. Benefits of selection were low because selecting cows had a low selection intensity relative to bull selection. This highlights the importance of selecting high quality bulls to improve herds over time.

Effects of trace mineral source on performance of beef heifers during nutrient restriction and starch challenge

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This study assessed the impact of trace mineral source (sulfate vs. hydroxychloride) during nutrient restriction followed by starch challenge on heifer performance. On day 0, 40 Nelore heifers (238 ± 38 kg; 21 ± 1 months) were allocated into individual drylot pens. The study was divided into periods of washout (day 0 to 27), nutrient excess (day 28 to 55), nutrient restriction (day 56 to 83), and starch challenge (day 84 to 112). Heifers were given free choice access to Tifton hay (*Cynodon* sp.) and salt from day 0 to 27. From day 28 to 112, 20 heifers per treatment were offered protein supplementation at 0.10% of body weight, which added with sulfate (SUL) or hydroxychloride (HYD) sources of Cu, Mn, and Zn. From day 56 to 83, heifers were limit-fed 50% of estimated hay intake. From day 84 to 112, each protein supplement was included into a high-starch diet (starting with 35% concentrate and 65% hay on day 84, reaching 80% concentrate and 20% hay from day 106 to 112). Data was analyzed using the MIXED procedure of SAS. Heifer average daily gain (ADG) from day 28 to 56 was lower ($P = 0.05$) for SUL vs. HYD heifers. Plasma insulin-like growth factor 1 (IGF-1) levels were higher ($P \leq 0.05$) for HYD vs. SUL heifers on days 56, 70, 77, 84, and 91. Plasma haptoglobin level was higher ($P = 0.05$) for SUL vs. HYD heifers on day 63. Treatment $\times$ day and treatment effects were not observed ($P \geq 0.35$) for plasma levels of cortisol, β -hydroxybutyrate, and non-esterified fatty acids. Therefore, Nelore heifers provided hydroxychloride sources of Cu, Mn, and Zn displayed higher plasma IGF-1 levels and a short-term improvement in ADG during the nutrient surplus phase compared to those given sulfate sources. Although hydroxychloride supplementation reduced the acute phase response early in nutrient restriction, it did not impact heifer performance during nutrient restriction and starch challenge periods.

Session 53

Poster 13

Development of a point of care rapid test for semiquantitative detection of bovine viral diarrhea antibodies in bovine blood

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Key words: BVDV, Serology, Point-of-care testing, Vaccination Bovine viral diarrhea virus (BVDV) is a highly contagious pestivirus responsible for many production-limiting symptoms in cattle worldwide. There is no cure for BVDV, and management is highly dependent upon preventative care, immunization, and establishment of herd immunity. A point of care (POC) BVDV antibody detection test would allow producers to quickly and effectively determine their herd's immunity and infection status, greatly aiding BVDV management programs. Thus, this project aimed to develop a novel, accurate, and affordable rapid POC test for the detection of BVDV antibodies in bovine blood. Conjugated HRP detectors and recombinant BVDV capture antigens were utilized to develop a vertical flow rapid immunoassay. Optimization testing was performed to determine the best conditions for detecting the target BVDV antibodies, yielding a test that could detect antibody titers as low as 1:4 in bovine plasma in under 10 minutes. Once optimized, 195 positive and 42 negative plasma samples from both beef and dairy cattle were run on the vertical flow test (VFT) and a reference gold standard ELISA will be used to determine the test's diagnostic sensitivity (DSe) and specificity (DSp). The VFT had a DSe of 98.3% and a DSp of 95.1%. The newly developed VFT has a diagnostic accuracy exceeding other reported rapid antibody diagnostic tests. Furthermore, the VFT DSe is comparable to an in-lab ELISA, suggesting that the test could be used to provide highly accurate results pen side. The shortened performance time, ease of use, and ability of this test to be performed outside of a laboratory could greatly improve BVDV surveillance and management practices worldwide, reducing disease incidence and improving profit outcomes for beef and dairy producers alike.

Improving meat tenderness using papain injection and ultrasounds: the consumer response

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Meat tenderness is the most important palatability trait of beef influencing the consumers' acceptability who are also willing to pay a higher price to have tender meat. In the last years, several promising methods have been investigated in order to achieve an adequate level of tenderness reducing costs, time and its variability among different commercial cuts. The present study aims to investigate purchase intention for meat obtained through a tenderization process based on a treatment with exogenous proteolytic enzymes. Particularly, perceived risks and perceived benefits on the consumer acceptance of tender meat produced through this emerging technology have been evaluated. A survey was conducted on a national representative sample of Italian consumers (N = 1006), who received information about the traditional and the emerging tenderization processes. Principal Component Analysis and Structural Equation Model were applied to the collected data. Results showed that consumer purchase intention for meat treated with exogenous proteolytic enzymes was strongly influenced by perceived benefits and weakly influenced by perceived risks. Particularly, perceived benefits are mainly influenced by trust in institutions involved in scientific research and food regulations which, consequently, plays a relevant role in the intention to purchase meat incorporating such process. The positive attitude has been found for a wide segment of consumers (35% of the sample) who could act as early adopters of the product obtained by such innovative technology. As a consequence, it could be relevant the role of an effective communication strategy by highlighting positive attributes as the sustainability of the treatment, its effectiveness, the absence of health risks and the role played by scientific institutions. Finally, the study allowed to profile consumer segments on the basis of socio-demographic characteristics in order to provide information useful for promotion activities aimed to support the introduction of the new product on the market.

Session 53

Poster 15

Detecting the role of the quality logo '100% Raza Autoctona' among Spanish beef consumers through a cluster analysis: Preliminary results

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Beef consumption is undergoing a major transformation, driven by consumers who are increasingly concerned about sustainability, animal welfare and food quality. Extensive livestock farming with local breeds plays a key role in biodiversity conservation, rural development and high-quality meat. However, their competitiveness depends to a large extent on the recognition of their differentiating attributes. Different certification labels, such as Protected Geographical Identification (PGI) and organic certification (OC), have proven to be highly relevant in Europe. Recently, in Spain, the Ministry of Agriculture, Fisheries and Food has implemented the '100% Native Breed' Logo (NB), which recognises farmers who protect Spanish native breeds. This study seeks to determine the relative importance and willingness to pay (WTP) of NB compared to other certification systems, using an approach based on a set analysis. An online survey based on a conjoint analysis was carried out. Three attributes were included in the evaluation: OC, PGI and NB, each with two levels: 'presence' or 'absence'. In addition, price was set at three levels. The survey was completed by 300 individuals. An orthogonal design was carried out; WTP was calculated by dividing the utility of each non-price attribute by the price coefficient. Data analysis was performed using the statistical software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The results showed that the most determining factor in the purchase decision was price (33.83%), with a clear preference for the lowest level (18 €/kg). After price, OC (22.6%) and NB (22.5%) followed by PGI (21%) were the most valued. With respect to WTP, the consumer sample expressed a similar willingness to pay for the attributes assessed. In particular, the consumer reported a WTP of 0.91 EUR/kg for the NB label, which is lower than the other labels, but promising. These results suggest that the '100% Raza Autoctona' logo has a competitive potential in the Spanish market, especially in those areas where other labelling alternatives are not available.

Features of digital technologies and data-driven solutions for livestock tracking

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This study aimed to assess digital tools and datasets for livestock tracking through stakeholder interviews. In total, 52 interviews were conducted with software developers, technology providers, industry, public authorities and research institutions from Greece, France, Netherlands, Poland, Denmark, Finland, Germany, Belgium and Austria. A structured interview protocol was used to assess user requirements, maturity, standardisation, conformance, cost, data sharing, legislation and strategy. Major user requirements included internet access (97.4%), account creation (94.8%), data input (76.9%) and documentation (71.8%). Most digital solutions had a high maturity level; plans focused on updates (85.7%), automation (77.1%) and language support (61.1%). The majority complied with regulatory standards (86.5%) and efficient conformity assessment (76.3%). Key standardisation needs included compatibility (39.4%), compliance (26.3%) and security (26.3%). The biggest initial and recurring costs were for software development (56.4%) and maintenance (48.7%), respectively. Data collection automation, financial incentives and trust were considered prerequisites to increase data sharing and use. Digi4Live project is funded by the European Union.

Session 54

Theatre 2

Associations between body weight measured with 3D cameras and milk yield in Nordic dairy cows

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Body weight (BW) is an important indicator of dairy cow performance, directly influencing milk production and farm profitability. This study investigates the associations between BW, estimated using 3D cameras, and milk yield in Holstein, Red Dairy Cattle (RDC), and Jersey cows from Danish commercial herds. The 3D images were captured using Microsoft Xbox One Kinect V2 cameras and analyzed with a partial least squares regression model calibrated against scale measurements, achieving a prediction accuracy of 0.94. Data were collected from 15,609 cows (6,724 Holstein, 4,675 RDC, and 4,210 Jersey) between 2020 and 2024, resulting in over 5.4 million daily BW records. Smoothed BW data were used to derive BW traits, including calving weight, minimum BW, average BW during the first 305 days in milk (DIM), and weight changes across lactation. BW traits were regressed on energy-corrected milk (ECM) yield, and Pearson correlations were calculated. BW at calving was positively associated with 305-day ECM yield in first-lactation cows, with the strongest effects in Jersey (13.0 kg ECM/kg BW, $r = 0.33$), followed by RDC (5.85 kg ECM/kg BW, $r = 0.30$) and Holstein (4.7 kg ECM/kg BW, $r = 0.19$). However, this association diminished or even reversed in later lactations. Average BW during the first 305 DIM also showed positive correlations with ECM yield in first-lactation cows, but this correlation weakened or became negative in multiparous cows. BW loss from calving to 100 DIM was negatively correlated with ECM yield, particularly in Jersey (-14.29 kg ECM/kg BW, $r = -0.23$) and RDC (-14.18 kg ECM/kg BW, $r = -0.39$), highlighting a trade-off between milk production and energy balance. Additionally, time to reach minimum BW increased with parity, suggesting prolonged negative energy balance in older cows. These results suggest that continuous BW recording with 3D cameras is useful for on-farm management, providing a non-invasive alternative to scales. Real-time BW tracking offers valuable insights for optimizing nutrition, supporting high-yielding cows while minimizing excessive BW loss.

A randomised controlled trial evaluating the impact of an automated lameness detection system on dairy cattle foot health and reproductive performance

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We evaluated the impact of an automated lameness detection system (CattleEye) on reproductive performance and foot health in a large dairy farm. Multiparous cows (n = 419) were enrolled within 30 days-in-milk (DIM) and randomly assigned to treatment (T) or control (C) groups, both following standard farm lameness management protocols. T cows received additional foot trims based on automated mobility scores; cows scoring ≥ 50 or increasing by ≥ 20 points within two weeks, if not trimmed in the past 28 days, were flagged for trimming. Scores were generated by an AI algorithm analysing overhead footage. Reproductive performance outcomes were analysed using Cox proportional hazards and binary logistic regressions. The number of foot trims, wooden block applications, and lesion cases per trim were assessed using Poisson regressions. All cows underwent routine trimming at 180 DIM and were classified into four categories: no lesions, mild, moderate, or severe lesions. Differences between groups were assessed using a chi-square test. The hazard of receiving the first artificial insemination (1st AI) by 150 DIM did not differ ($P = 0.680$). However, T cows had significantly greater odds of conception at 1st AI (odds ratio = 7.6, 95% CI: 1.6-35.6, $P = 0.010$) and a higher hazard of conception by 150 DIM (hazard ratio = 3.1, 95% CI: 1.7-5.7, $P < 0.001$). T cows underwent more foot trims than C cows (estimated marginal means: 2.7 vs. 1.8, odds ratio = 0.68, 95% CI: 0.6-0.8, $P < 0.001$), but wooden block applications ($P = 0.246$) and wooden block applications per trim ($P = 0.842$) did not differ. However, at the 180 DIM routine trim, the T group had a greater proportion of cows with no lesions (18.7% vs. 10.3%, $P = 0.015$) and fewer cows with moderate lesions (13.4% vs. 21.7%, $P = 0.042$) compared to the C group. Findings suggest that automated mobility monitoring facilitated earlier lameness treatments, leading to improved reproductive outcomes and better lesion resolution by mid-lactation.

Session 54

Theatre 4

Advancements in ketosis management strategies for dairy cows: a decade of experience with the KetoMIR system

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After a decade of using KetoMIR important outcomes and developments can be underlined. KetoMIR has improved the early detection of ketosis based on mid infrared (MIR) spectral data allowing farmers from the Cattle Network (RDV GmbH) to take preventive measures before health issues arise. Farmers and advisors were trained and the significance of metabolic health in cows was highlighted to understand the connections between nutrition, health, and performance. In Baden-Württemberg (BW), about 65% of ~3,600 farms use the KetoMIR within the Herdenmanager or RDV App. KetoMIR provide a user-friendly traffic light system that helps farmers quickly identify cow health issues. Red (class 3) and yellow (class 2) indicators signal potential problems, allowing for prompt preventive measures, while a green (class 1) light indicates not in danger. KetoMIR shows monthly herd monitoring and farmers ability to respond to health concerns effectively. Statistical analyses indicated that KetoMIR class 3 was associated about 17% to secondary diseases (e.g. acidosis, mastitis). KetoMIR and various MIR parameters showed higher than 0.4 pearson correlations for both negative (e.g. protein, lactose, deNovo fatty acids (FA), blood: IGF-1 and glucose, energy balance) and positive associations (e.g. fat-protein quotient, pre-form FA, acetate, milk BHB, blood: BHB and NEFA, feed efficiency). KetoMIR has significantly influenced the management of dairy herds in RDV and beyond over the past ten years, effectively addressing ketosis challenges while promoting cow health and productivity. KetoMIR index may contribute: to the estimation of breeding values or selection of cows that are less susceptible to ketosis. KetoMIR shows economic advantages on reduction of losses in milk yield or treatment costs for secondary diseases.

Assessing the Economic Impact of Digital Technologies in Dairy Farming

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The increasing adoption of digital technologies in dairy farming, such as connected sensors, data analysis software, and robotics, raises crucial questions about their profitability and techno-socio-economic impacts. A recent survey in France involving 856 dairy farmers (Nicolas et al., 2024) reveals that cost and lack of return on investment are the main barriers to adopting these technologies. Their economic gain is perceived only as the fourth advantage, far behind time savings and work comfort. Understanding the real benefits for farmers is essential to promote sustainable adoption and informed investment. Some technologies increase production while saving time, whereas others only generate additional costs. Therefore, it is crucial to rely on reliable evaluation methods. This presentation offers an analysis of existing evaluation approaches, using various classic dairy farming technologies as examples (heat detection collars, milking and feeding robots). Several methods exist, each with its advantages and limitations. Stochastic simulation models (Allain et al., 2016, Adenuga et al., 2020) allow for numerous hypotheses, testing different scenarios and performance thresholds, and quantifying return on investment over time using commonly used economic parameters (NPV, BCR, IRR, PBP). However, while these models can study many variables, they do not always accurately represent the reality of farms and can be very complex to use. A more pragmatic approach involves comparing similar farms, equipped and non-equipped, as in the European project Quantifarm or the Inosys network in France. The European project CODECS proposes comparing the performance of the same farm before and after equipment installation. Finally, another simple and effective method compares two types of investments (a new technology vs. a more classic equipment) for the same farm, all other things being equal (Rubin et al., 2022). Regardless of the approaches used, the results greatly depend on the farm's performance before equipment installation, the performance gain provided by the technology, and the consideration of time saved in the calculation.

Session 54

Theatre 6

Added value of individual pig monitoring on commercial farms using RFID: first experiences

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The PigID project investigates the added value of individual monitoring of pigs from birth to slaughter on commercial farms, using UHF RFID ear tags and linking farm data with slaughterhouse results. The project spans four years and involves 24 pig farms in Flanders, collecting data on birth details, genetics, management, health, and slaughter outcomes. By applying RFID tags, farmers can experiment with different breeding and management strategies, compare sow and boar lines, and monitor health issues and pre-slaughter mortality. Currently, data from nine farms supports internal and external benchmarking, identifying variations in growth, health, and slaughter traits. These farms use different commercial solutions and exchange tips and tricks and experiences. One case study involved looking at the effect of birth weight and fostering on the end results, to help the farmer decide on fostering strategies and management strategies for the smaller piglets. In total, 834 piglets were tagged. For 461 of these pigs the slaughterhouse results could be collected. A linear mixed effects model was built with slaughter weight as outcome variable, birth weight, gender, fostering, sow parity, live and dead born piglets and age as fixed factors and sow as random factor. Birth weight had a significant positive effect ($p < 0.01$), while female pigs had a lower final weight ($p < 0.05$). Other factors, such as fostering and sow parity, were not significant ($p > 0.05$). Beyond weight prediction, the project explores missing data and ear tag loss throughout the pigs' life-cycle and models total carcass weight per litter to summarize genetic and management effects. This data-driven approach strengthens farm decision-making and provides a solid foundation for best practices in commercial pig farming. The PigID project demonstrates the feasibility of UHF RFID ear tags for detailed piglet tracking, supporting accurate record-keeping and evaluation of management changes. With extensive data collection and close collaboration with the farmers, the project aims to deliver valuable insights for the pig farming sector and suppliers of solutions for individual monitoring.

Real-time detection of calving in extensive beef production systems using GNSS tracking

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Calving is a critical event in extensive beef production systems, but direct monitoring of calving cows is limited due to the size of pastures. Undetected calving difficulties can lead to increased calf mortality, reduced fertility and economic losses. This study developed a real-time monitoring system to detect parturition using GNSS tracking collars based on the identification of behavioural changes associated with this event. Data were collected from cows on two extensive farms in Spain, with position fixes recorded every 30 minutes. Movement and social interaction indicators were extracted to assess movement patterns and herd dynamics. A total of ten behavioural indicators were calculated at 30-minute intervals using a rolling 24-hour window. These included trajectory length, sinuosity, number of trajectory segments greater than 25 m, distance to nearest neighbour, relative trajectory length, relative distance, relative trajectory straightness, relative sinuosity, relative maximum step length and relative number of segments greater than 25 m. The values of each indicator were first transformed using a delta-self approach and then standardised using z-score normalisation to identify behavioural anomalies (z-score greater than 2.5). The performance of the detection system when considering different numbers of indicators was evaluated. The results showed that using a single indicator to set a calving alarm provided the best balance between precision (76.2%) and recall (69.8%), achieving an F1 score of 72.9%. Increasing the detection threshold to a minimum of two indicators improved precision to 92.9% but reduced recall to 32.5%, missing a large proportion of calving events. Beyond three indicators, detection sensitivity was very low, failing to identify more than 10% of calving events despite achieving near perfect precision. These results highlight the trade-off between false alarms and accurate early detection. By adjusting indicator thresholds, farmers can tailor detection settings to their specific farm conditions, optimising calving monitoring and improving reproductive management in extensive systems.

Session 54

Theatre 8

Characterisation of calving site selection by free-ranging cows using GNSS tracking data

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Calving is a critical event in the production cycle of cows. Real-time monitoring of calving to assist with dystocia and/or to prevent peripartum mortality can be challenging in extensive systems due to the size of grazing paddocks. GNSS collars have been used to monitor ethological changes associated with calving, allowing it to be detected automatically. However, collaring all cows in a herd is not always possible. A complementary approach to calving monitoring is to identify patterns of calving site selection, which would allow the identification of the areas of the farm where calving cows are most likely to be found, even if they are not wearing a GNSS collar. The aim of this study was to characterise calving site selection by beef cows under free-grazing conditions. Sixty-five cows from six different farms were monitored with GNSS collars during the peripartum period. Calving dates recorded by farmers and GNSS coordinates of cows isolated from the rest of the herd for several hours were used to identify calving locations. To characterise the farms, maps of distance to water troughs, distance to fences or walls (< 25 m, 25-100 m and >100 m), geomorphological units (peak, slope, valley, etc.) and tree cover (0%, 0-10%, 10-20%, 20-40% and over 40%) were produced using orthophotos and digital terrain models. For each of these categories, the Jacobs Selection Index (JSI) was calculated from the GNSS positions of the cow during calving. Data processing and calculations were performed using the open-source software R. A preference for areas further from water (>100 m) and areas with more tree cover (20-40%) was observed on all the farms. Regarding the distance to fences, cows preferentially selected areas close to the walls (0-100 m), except in large paddocks, probably due to the distance they had to travel to reach the fences. In terms of geomorphology, cows were more likely to select gentle slopes (between 5 and 10%). Despite the existence of common patterns, some variability was observed between individual cows. These results suggest that there are several drivers for calving site selection, the characterisation of which may facilitate the location of calving cows on pastures.

Drone monitoring for grazing animals: Not yet fully PLF, but already a major time-saver

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Drones are now easily accessible, with 9% of ruminant farmers in France already equipped with them. However, this enthusiasm reflects a somewhat “wild” adoption, often disregarding regulations and leading to suboptimal equipment choices and inefficient usage frameworks. After defining the scope of drone use in livestock farming and establishing guidelines for their implementation, the European ICAERUS project focused on evaluating the impact of these technologies on working conditions. Following a six-month familiarization period on a beef cattle farm (100 dams on 200 hectares of natural grasslands), an experiment was conducted. The primary use of the drone was to facilitate animal observation rounds, as livestock were scattered in different paddocks. A “traditional approach”, where the farmer visited the animals in person by car and on foot, was compared to a “drone-assisted management” approach. In the latter, the drone was used in relevant paddocks to observe the animals remotely, reducing time-consuming movements on foot or by vehicle. The drone-assisted approach was introduced at most one day on two to maintain human-animal interaction and allow for operations that could not be replaced by the drone (e.g., paddock changes). Rainy weather conditions limited drone use. A total of 69 recordings were made—48 under traditional management and 21 under drone-assisted management—smoothed over a homogeneous grazing period (autumn and spring, representing different grazing conditions). Utilizing drones for grazing animal monitoring reduced the farmer’s daily observation time by 12% in autumn and 38% in spring, compared to the respective baseline average of 81 and 133 minutes per day. According to a qualitative survey, the perceived benefits of this technology exceed its purely time-saving advantage. Substantial variations in time spent or saved with drones were observed throughout the season, mainly due to grazing conditions variations (intensity of rotational grazing, number of animal batches). These findings help define the farming systems where drones provide the greatest benefit and guide farmers toward a rational and efficient adoption of this technology.

Session 54

Theatre 10

Estimating Cattle Body Weight in Grazing Livestock Using UAV-Based Monitoring Systems

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Accurate body weight (BW) assessment is essential for precision livestock monitoring and optimizing grazing management. However, in extensive grazing systems, frequent BW measurements remain challenging due to labor-intensive handling. This study explores the potential of unmanned aerial vehicle (UAV)-based computer vision systems (CVS) to estimate BW in dairy heifers using 2D imaging. A total of 153 top-down RGB images from 71 heifers were collected using a DJI Mavic 3M UAV at 20 meters altitude. Key anatomical landmarks were manually annotated to extract Euclidean distances, along with additional geometric features (pixel area, perimeter, circularity, and eccentricity). A Support Vector Machine regression was developed using a leave-one-out cross-validation (LOOCV) approach, optimizing hyperparameters based on root mean square error percentage (RMSEP). The model achieved an R^2 of 0.88, a concordance correlation coefficient (CCC) of 0.93, and an RMSEP of 47.5 kg (12.1% of observed BW). These results indicate that UAV-based CVS is a promising, non-invasive tool for BW estimation in grazing systems, outperforming previous UAV-based 3D imaging methods. This approach has the potential to improve livestock management by enabling real-time BW monitoring, optimizing nutrition, and integrating pasture quality assessments for sustainable grazing systems.

Adaptation of heifers trained to virtual fencing in a rotational grazing system

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Virtual fencing (VF) offers an innovative and promising alternative to conventional fencing for managing grazing systems. This study, part of the DaVaSus project (Data and value-based decision-making for a sustainable land use), aimed to evaluate the adaptation of heifers previously trained to VF in a rotational grazing system. The study was conducted from July to September 2024 at the regenerative and ecological farm Gut & Bösel from Finck Foundation in eastern Brandenburg, Germany. Twenty-seven non-pregnant Angus heifers (39 ± 0.9 months, 335 ± 74 kg) were included in the trial in rotational grazing with perennial forage. All animals were trained with the eShepherd™ VF system (Gallagher Group Limited, Hamilton, New Zealand) three months before the study. Each heifer wore a solar-powered neckband equipped with GPS and an accelerometer. When an animal approached within five meters of the VF, the neckband emitted an audio tone, followed by an electrical pulse if the animal continued forward, ignoring the audio cue. The trial area of 6.4 ha consisted of six paddocks, each enclosed with electric wire fencing and at least one side controlled by VF only. All paddocks provided shade and water troughs. Paddock sizes were determined based on estimated dry matter availability per ha, and animals were rotated about every seven days. The neckbands recorded each animal's location, walking distances, audio warnings, and electrical pulses per minute. The audio warnings and electrical pulses showed animal individual differences ($p < 0.001$). Over time, audio warnings decreased both within and across paddocks ($p < 0.001$). Despite prior VF training, our present study indicated that an adaptation period was necessary for the animals to adjust to the system. With repeated exposure to new paddock, heifers reduced their interactions with the VF system, resulting in fewer audio warnings and electrical pulses. This research was funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE).

Session 54

Poster 12

Evaluation of spectroscopy-based milk analysis tools within precision dairy sheep farming systems

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Milk quality assessment is a key component of precision livestock farming across the dairy sheep value chain. It facilitates the improvement of animal husbandry systems, enhances animal productivity, health, and welfare, and improves the overall management and sustainability of the farms. The objective of the study was to evaluate the use of spectroscopy-based tools as rapid and non-destructive solutions for milk quality assessment at the point-of-service. Milk samples from 200 dairy ewes were collected and analyzed using Ultraviolet/Visible Spectroscopy (UV/Vis), and Near-Infrared Spectroscopy (NIRS) tools with spectral range between 340–780 and 500–1700 nm, respectively. To determine the functionality and diagnostic performance, spectra of these tools were analyzed and results were validated against the Fourier-Transform Infrared (FTIR) which was used as “gold standard” to estimate milk fat, protein, and lactose contents. Herein, we present preliminary results of the development of analytical protocols and milk quality prediction models. We also present results on their diagnostic performance when applied in raw untreated ovine milk derived from individual animals. Despite the initially promising results, trials continue and relevant modifications of the protocols are tested to improve the performance and applicability of the tools for the real-time, in situ, milk composition monitoring. In any case, the demand of efficient calibration and further validation of the exploited tools has been evidenced. For this reason, various real-world scenarios need to be considered to justify the applicability of the tools and their integration in quality assessment and data-driven decision support systems at the farm level.

Sensor based ENvironmental Surveillance and Observation with Real-time data in Pig and Poultry houses

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The objective of the Sensor-PP project is to create a complete toolbox for monitoring the climate in pig and poultry houses. This includes optimization of the hardware part (climate sensors, cameras) as well as the development of new software. The final tool should support farmers, advisors and veterinarians to make data driven decisions in order to improve and adjust the barn climate. An optimized climate results in an improved health (and less medication), a higher productivity and animal welfare. Decisions can be based on large amounts of real-time data rather than on short-term or subjective observations as is often the case today. Unique to the system is that in addition to sensor data, digitized production data (mortality, feed- and water intake, treatments) and external weather data will be incorporated to make correlation analyses. Through the user friendly web-interface farm-specific parameters such as geolocation, animal species, breed, type of ventilation and age category of the animals can be selected so that interpretation of the obtained measurements can be done fully automatically but case-dependent. In addition to sensors data, the toolbox also included cameras (RGB, infrared). By applying artificial intelligence (AI) to the generated images, animal behaviour and any abnormalities will be quantified in an objective way because often, in addition to animal health problems, incorrect barn climate is also accompanied by abnormal behaviour (uneven distribution of animals, aggression, etc.). An overview will be given of the current status of the toolbox. The performance of the tool will improve as the database is growing and the deep learning technologies are kept being trained.

Evaluating Sensor-Derived Rumination and Activity Data for Monitoring Pre-Weaning Dairy Calf: A Correlation Study with Traditional Metrics

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Sensor technologies have gained traction in dairy farming, but remain underutilized during the calf pre-weaning phase. The objective of this study was to evaluate the correlation between sensor-derived variables (rumination time and activity index) and traditional performance metrics (average daily gain, morphometric measurements height at withers (HW), Chest Circumference (CC), Rump Length (RL), and Rump Height (RH) in pre-weaning dairy calves. Thirty-four randomly selected Holstein calves were housed in individual pens and fitted with SCR HR-Tag Heatime collars one day after birth. The morphometric variables (HW, CC, RL, and RH), body weight (BW) and average daily gain (ADG) were measured weekly. Pearson correlation analysis was performed to explore relationships between variables. The correlation coefficients were low between activity and ADG ($r = 0.03$), BW ($r = 0.03$), HW ($r = 0.1$), CC ($r = 0.08$), RL ($r = 0.1$), and RH ($r = 0.11$). The correlation coefficient between rumination and ADG ($r = 0.11$) was weak as well. HW ($r = 0.54$), CC ($r = 0.57$), RL ($r = 0.55$), and RH ($r = 0.54$) mirrored the variation patterns in conventional measurements, suggesting that increased rumination time corresponds to greater physical growth. These findings show promise for rumination time to be used as a complementary variable for calf development evaluation and highlight the potential of sensor-derived rumination data to enhance the development of automated systems for monitoring pre-weaning dairy calves. Keywords: dairy calves, pre-weaning, sensors, rumination, activity index, performance monitoring.

Using precision livestock farming to reduce the environmental impacts of cow milk production

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This study investigates the environmental sustainability of a dairy farm in Emilia Romagna, Italy, equipped with Precision Livestock Farming (PLF) technologies. The research compares the environmental impact of milk production where different technologies were installed one by one to a base configuration: first a mechanical ventilation system (MV) and second an automatic milking system (AMS). The evaluation considers various environmental indicators to assess the sustainability performance of the dairy operation and the latter was quantified via Life Cycle Assessment (LCA) method. The base farm configuration was compared with the two scenarios in which the technology was implemented. Fat and protein corrected milk (FPCM) was used as a functional unit, and a cradle to farm gate system boundary and biophysical allocation method were selected. The reduction in labour requirements and optimized milking schedules contribute to reduce energy consumption and greenhouse gas emissions per litre of milk. The biggest benefit can be observed with the installation of mechanical ventilation, to which correspond benefits in terms of animal health, welfare and productivity. However, the installation and maintenance of robotic systems introduce additional energy and material inputs, which must be carefully evaluated to guarantee overall sustainability advantages. The results of this study are expected to inform dairy farmers about the potential environmental benefits and challenges of integrating PLF technologies into dairy farming operations. Reducing environmental impacts and guaranteeing the long-term viability of the agricultural sector firmly rely on supporting sustainable practices in dairy production.

Session 55

Theatre 1

Colostrum intake and piglet survival: The imbalance between genetic selection and neonatal nutrition

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Colostrum, the first milk produced by a sow after farrowing, is vital for the survival and growth of neonatal piglets. It is rich in immunoglobulins, essential nutrients, and bioactive compounds, all of which are vital to support the early physiological functions of the piglets. One of its primary benefits is the transfer of maternal antibodies, which protect piglets from infections before their immune system matures. Since piglets are born without placental antibody transfer, colostrum is their only source of early immune defense. Therefore, timely and sufficient intake of colostrum is crucial to reduce pre-weaning mortality, which remains a significant challenge in pig production. Additionally, colostrum contains high-energy compounds such as fat, lactose, and protein, which help maintain body temperature and prevent hypoglycemia, a common cause of neonatal mortality during the first three days postpartum. Beyond immunity and energy, colostrum promotes gut development and enhances nutrient absorption. Bioactive components like growth factors, including insulin-like growth factors, stimulate intestinal maturation and improve digestive efficiency and long-term health. Colostrum also positively influences microbiota colonization, fostering beneficial bacterial populations that support gut health and disease resistance. However, colostrum intake varies within and between litters because genetic selection in the swine industry has prioritized large litter sizes, which creates challenges in ensuring adequate colostrum intake of the individual piglet. This has vast implications for neonatal nutrition and piglet survival. Insufficient colostrum intake can increase susceptibility to infections, slow growth rate, and elevate pre-weaning mortality. Management strategies like split suckling, cross-fostering, and sow nutrition could optimize colostrum intake, thus improving piglet survival rates. In conclusion, colostrum intake is crucial for piglet survival, offering immune protection, energy, and gut development, but selection for large litter sizes compromise these traits. Effective management practices to ensure adequate colostrum intake can significantly enhance piglet health, growth, and overall productivity in swine production.

Effect of a simulated udder on survival and weight gain of neonatal piglets in a practical farm setting

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Hyperprolificity in sows presents challenges for piglet nutrition and survival in modern pig production, leading to an elevated piglet mortality rate and increased vulnerability. Simulated udders have emerged as an innovative solution, offering consistent and accessible nutrition for piglets. This study investigates the potential to ensure the survival of one additional piglet by using a designed simulated udder (test group, T) compared to the control group (C). The hypothesis further holds that in general, there will be no negative effect on piglet weight gain. The experiment involved 53 litters from 26 C-sows and 27 T-sows, conducted over seven weeks on a Danish pig farm. In the T-group, litters were divided into sets of six based on birth order, rotating between nursing by the sow and the simulated udder, while the C-group piglets nursed continuously by the sow. The data were modelled by linear mixed-effects models incorporating both fixed and random effects. The survival and weight development analyses were performed at litter level. The treatment did not have a significant effect on survival ($P = 0.7$). In this study, 69.8% of the sows successfully nursed 17 piglets, with 74.1% of these sows belonging to the T-group. Among the piglets, 96.5% maintained or exceeded their birth weight; 86.8% experienced a weight gain of $\leq 10\%$ within 76 hours post partum. The litter weight gain was 5.8 ± 1.3 kg (T-group) and 4.9 ± 1.6 kg (C-group). The utilization of simulated udders in combination with sow nursing as a supplemental feeding method did not result in significant changes in piglet survival, though it did have an impact on weight gain compared to sow nursing. The sample size in this study was too low to detect smaller effects – numerically, 0.6 piglets more survived in the T-group. Importantly, no negative consequences were observed from the use of simulated udders, indicating that this approach has potential to improve piglet survival rates and welfare.

Session 55

Theatre 3

Repeatability and heritability of maternal behaviour traits in sows

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In sows, maternal behaviour is a commercially important trait which affects sow and piglet welfare and the quality and safety of human-animal interactions. The aim of this study was to estimate the quantitative genetic parameters of maternal behaviour in Swiss Landrace (SLR) pigs. The lactating sows (parities 1 to 11) at 4 nucleus farms of the breeding company SUISAG underwent tests and observations during two seasons. The behaviour towards a human when handling one of their piglets was scored in 569 sows. The reaction to the playback of squealing piglets from a different litter was tested for 521 sows. Vocalisations to the piglets before lying down and time to lying down were recorded for 451 sows. Number of samples per measure varied between 1 and 6. Variance components were estimated based on Bayesian generalised linear mixed models, including as random effects the animal genetic effects and the permanent environmental sow effects. The additive genetic relationships were either computed from the pedigree (1418 animals, 6 generations), the genomic data (376 sows genotyped with FBF SNP-Chip 60K), or their combination. All traits were found to be repeatable when modelling the relationship between individuals using pedigree information, with means of the posterior distributions ranging from 0.23 to 0.66. When genomic data alone were considered, only the repeatability of response to a human and probability of vocalising to piglets before lying down had means higher than 0.20 (response to a human: 95% CI = [0.47, 0.71], vocalising to piglets: 95% CI = [0.03, 0.69]). When using pedigree data only, response to a human was found to be heritable (h^2 : mean = 0.44, 95% CI = [0.22, 0.69]). Results suggest that the measured traits show temporally stable variation (repeatability) between sows within and across parities, and that their response to a human is consistently genetically determined. This suggests selection for calmer behaviour of sows towards humans in breeding programmes would be feasible, improving stockpersons' safety and animal welfare.

Review of validated tools and methods to measure piglet milk and feed intake and weights

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Proper milk intake is crucial for the survival and performance of newborn piglets and sets them up for a good start in life. Main causes of death in the pre-weaning phase are crushing, starvation and hypothermia. These causes could be linked to insufficient intake and energy reserves. However, directly measuring piglet milk intake is very challenging. For the H2020 project PIGWEB a literature review is done to find validated methods to measure milk and feed supplement intake, suckling and feeding behaviour of piglets, and piglet weight; and to identify future opportunities for automation. The most common methods to estimate milk yield in sows or individual piglet milk intake are: the weigh-suckle-weigh method, the deuterium oxide (D2O) dilution technique and regular measurements of piglet weight gains. These are all manual methods with varying degrees of invasiveness, accuracy and precision. For milk and feed supplement intake, chemical markers techniques can estimate intake by comparing indigestible markers in feed and feces. These techniques face challenges in accuracy, sampling and environmental impact. Electronic feeding and milking stations also exist and are being developed further. These systems are precise, but verification and recalibration are essential. With advancements in image analysis, individual piglet feeding and drinking behaviors can also be monitored, sometimes combined with recording the weight of the feed removed. Progress is being made to be able to accurately classify different behaviors and track individual piglets on camera. To measure piglet weights, direct weighing on a scale, morphometric measurements, and indirect approaches using image analysis can be used. Direct weighing is accurate but labor-intensive. Camera systems can be used both to track piglet movements and weight. These methods aim to provide accurate, non-invasive, and efficient ways to monitor pig growth and health, but also still have limitations. By further developing novel tools, such as feeding and drinking stations and computer vision, researchers and farmers can ensure that piglets receive adequate nutrition and care, leading to improved welfare and productivity in pig farming.

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Theatre 5

Colostrum intake and early-life nutrition: implications for growth, immune development, and antibiotic reduction strategies

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Insufficient colostrum intake (CI) in neonatal piglets can increase susceptibility to disease, necessitating antibiotic treatments. This study examined the relationships between individual CI, passive immunity transfer, growth performance, nutritional status, and specific immune responses in suckling piglets raised either solely on sow milk or provided with a liquid nutritional supplementation (LNS). Newborns from 24 (Landrace×Yorkshire) sows were studied for individual CI, using piglet birth weight, weight gain and duration of colostrum suckling in the prediction model. After the colostrum period, litters were randomly allocated to experimental treatments: 12 litters (n=180 piglets) received LNS (milk replacer from day 2, transitioning to wet feed at day 12), and 12 litters (n=180 piglets) were raised on sow milk (RSM). Plasma samples were harvested at 3, 11, and 24 days of age for determination of immunoglobulins (Ig) G, A, and M, using a commercial ELISA kit. Linear mixed models were used for statistical analyses. High CI was associated with greater weaning weight ($P = 0.0004$) and ADG ($P = 0.001$), whereas nutrition had no significant effect ($P > 0.05$). A positive correlation between CI and plasma IgG ($P = 0.02$), IgA ($P = 0.0007$), and IgM ($P = 0.04$) concentrations reflected effective passive immunity transfer for piglets with high CI. However, specific antibody titers (*E. coli* O138 and O149 serotypes) at weaning indicated no effect of CI ($P = 0.08$) and nutrition ($P > 0.05$) on the acquired immunity. Plasma triglyceride concentrations were negatively associated with CI on day 3 ($P = 0.05$) and day 11 ($P = 0.02$), indicating increased body fat mobilization among piglets with low CI. Provision of LNS improved the electrolyte balance, as higher ($P = 0.04$) plasma albumin concentration (dehydration) was measured in RSM piglets. These findings emphasize the importance of optimizing piglets' CI and implementing nutritional interventions during suckling to enhance neonatal outcomes and reduce the risk of enteric pathogen-associated diseases. Overall, such strategies may help minimize antimicrobial use in pig production and prevent the development of antibiotic resistance among pathogens by enhancing piglet robustness.

Assessing sow body condition and body weight dynamics in individual and multi-suckling primiparous sows during lactation

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This study aimed to understand the dynamics in body composition changes in individual (IS) and multi-suckling (MS) sows. This was part of a bigger project studying feeding management strategies in gilts. Body weight (BW) and body condition (BC) were recorded for 690 PIC X54 primiparous sows (N=329 IS and 361 MS) at d90 of gestation, pre-farrowing, and at weaning. Post-farrowing 48h BW was predicted. Differences in BW and BC d90 vs weaning (BW90-W; BC90-W), BW post-farrow vs weaning (BWPF-W), and BC pre-farrow vs weaning (BCPF-W) were calculated. Lactation feed intake was collected and sow average daily feed intake (ADFI) was calculated. Lactation piglet average daily gain, piglet BW at weaning, and number of weaned piglets were also collected. Data for IS and MS were analyzed separately using linear models, including feeding frequency and feeding level during the peripartum period as fixed effects, and litter information was included as covariates. For the MS sows, BW, BC, ADFI from the adjacent sow (Sow2) were included as covariates in the model. Average BW change for IS was $11.7 \pm 1.31\%$ and $7.9 \pm 1.11\%$ for BW90-W and BWPF-W, respectively. Average BC change was $22.8 \pm 1.96\%$ and $22.9 \pm 1.54\%$ for BC90-W and BCPF-W, respectively. For the MS, BW change was $11.9 \pm 1.07\%$ and $8.3 \pm 0.77\%$ for BW90-W and BWPF-W, respectively. Average BC change was $24.4 \pm 1.23\%$ and $23.2 \pm 0.99\%$ for BC90-W and BCPF-W, respectively. Results showed a positive association between Sow2 BW changes and BW90-W and BWPF-W, as well as between Sow2 BC changes and BCPF-W ($P < 0.05$). Results suggest that MS may not create higher demand on sows than IS. However, a limitation of the study was that IS and MS were not comparable side by side, so further research is needed. Nevertheless, MS affects sow BW and BC dynamics, which is slightly influenced by Sow2. The latter could be explained because both sows share the same environment, management, and lactation demands, but their individual responses (genetics, nutrient requirements or maternal traits) still allow for some independent variation. This balance between shared factors and individual differences leads to a low, but a positive association in the present study.

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The Colostrum Chronicles: Identifying Porcine Milk Oligosaccharides in Colostrum and Investigating Their Role in Litter Performance

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This study aimed to identify porcine milk oligosaccharides (PMOs) in colostrum of high performing sows and identify associations between PMO concentrations and litter performance. Colostrum was opportunistically sampled during parturition from 45 Large White x Landrace sows (parity 2-8) and analysed by HPLC-UV. Oligosaccharides were identified by using reference materials as well as HPLC-MS measurements. Where reference materials were not available, constituent monosaccharides were estimated from the mass. The Spearman's rank correlation was used to identify correlations between PMO concentrations, parity, and litter performance parameters. A total of 11 PMOs were identified; 2'-FL; 3'-GL; 3'-SL; 6'-SL; LNNH; LNNt; LSTc and 4 others based on mass alone: galactosyllactose 1 and 2 (GSL 1 and GSL 2), sialyl-lacto-N-triose (S-LN-Tri), and sialyl-lacto-N-neohexaose (SLNNH). Between-sow variation was large, with total oligosaccharide concentrations ranging from 0.83g/L to 4.32g/L and a median value of 1.90g/L. Significant ($p < 0.05$) and moderate ($0.5 > \rho > 0.25$) positive correlation of sow parity with PMO concentration was identified in GSL 1, 3'-SL, 6'-SL, and total concentration of PMOs. Significant ($p < 0.05$) negative correlation of a moderate magnitude ($-0.25 > \rho > -0.5$) was shown between LNNt and parity as well as between GSL 2, 3'-SL, 6'-SL, SLNNH, total concentration of PMOs ($p \leq 0.0001$) and weight of piglets at 1 day of age. Porcine milk oligosaccharide (PMO) concentrations are highly individual to each sow, despite controlled breeding and environment. Concentrations of some PMOs appear to increase with parity, although survivorship bias must be considered. Concentrations of some PMOs were negatively correlated with piglet weight at 1 day of age, but not correlated with any other litter performance parameter measured. Avenues to explore further include the potential phenomenon that PMO concentration is enhanced when birthing smaller piglets.

Piglets benefit from supplementation of a complementary milk replacer, even in moderately sized litters or when kept 5 weeks with the sow

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In moderately-sized litters (< 15 piglets at day 4) and systems where piglets are weaned late, the practice of providing complementary piglet milk replacer (cPMR) is under debate. Our aim was to investigate the effect of providing cPMR to piglets via an automated cup system in moderately sized litters (trial 1) and in 5 weeks weaning systems (trial 2). In trial 1, 480 TN70 sows were divided over 2 treatments: Control (CTRL3) (n=233) and cPMR3 (n=247). In trial 2, 110 TN70 sows were divided over two treatments: CTRL5 (n=62) and cPMR5 (n=48). All piglets received dry feed from day 6 on. Additionally, cPMR3 piglets received PMR from day 4-12 and a follow-up cPMR from day 13-19 via an automatic cup system. cPMR5 piglets received cPMR from day 4-14 and a follow-up cPMR from day 15-28. All piglets were tagged with an RFID eartag at day 4 and were individually weighed at day 4 and at weaning (day 21 in trial 1, day 35 trial 2). At the end of the nursery and at slaughter, pen weights were recorded per 13 and 10 pigs, respectively. The effect of cPMR supplementation was tested using the GLM procedure (SAS 9.4). cPMR3 and cPMR5 piglets were significantly heavier than CTRL3 and CTRL5 piglets at weaning, respectively + 0.28 kg ($p < 0.01$) and + 0.98 kg ($p < 0.001$) and at the end of the nursery, respectively + 0.85 kg ($p < 0.01$) and + 1.64 kg ($p < 0.001$). There were fewer small piglets in both cPMR groups compared to the CTRL groups ($p < 0.01$ and $p < 0.01$). CTRL3 and cPMR3 pigs had the same body weight at slaughter (126.2 kg vs 126.2 kg; $p > 0.1$), but cPMR3 pigs were younger than CTRL3 pigs (169.6 days vs 166.3 days; $p < 0.001$). In trial 2, 144 CTRL5 pigs and 120 cPMR5 pigs were followed in the fattening phase. The final weights (corrected to 171.5 days) of CTRL5 and cPMR5 pigs were respectively 135.1 kg and 142.7 kg. Contrary to common believe, provision of cPMR via an automated cup system to both piglets in moderately sized litters and to piglets weaned at 5 weeks, did result in significantly improved results from weaning up until slaughter. Automated provision of cPMR to piglets is thus an interesting strategy to improve piglet performance and reduce pressure on the sow without the need of additional labour and with a high economical return.

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Comparison of individual milk intake of piglets fed by an automatic feeding system with total milk intake measured by the D2O dilution method

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In piglet production, large litters are supplied with milk supplement (MS) during the suckling period to provide piglets with sufficient energy and protein. This is usually done by group feeding without measuring of individual MS intake. Together with industry partners, we have developed an automatic piglet feeding system that measures individual MS intake. Piglets were recognized by RFID and MS intake was measured via the weight difference at the drinking cup. We compared the individual MS intake by the automatic feeder with the total milk/MS intake as determined by the deuterated water (D2O) dilution method. In a pilot study, a total of 16 piglets from 4 litters (3 wk old; with sow) and 12 piglets from 3 litters (5 wk old; without sow) were investigated. The piglets were injected ip. with 1 g D2O/kg BW (20 atom %) and blood was taken 1 h and 48 h after the injection to determine the enrichment of plasma deuterium. Total milk/MS intake was calculated from D2O data and compared with consumed amounts of MS via the automatic feeder using linear regression models. Time differences (wk 3 vs wk 5) were calculated by Student's t test. The mean ($\pm$ SE) BW of piglets was 5.3 ± 0.2 kg at wk 3 and 8.0 ± 0.3 kg at wk 5. Total intake measured by D2O increased ($P < 0.05$) from 1.6 ± 0.1 kg/d in wk 3 (MS plus sow's milk) to 3.3 ± 0.3 kg/d (no sow's milk; only MS intake) in wk 5. The MS intake measured by the feeder increased ($P < 0.05$) from 14 ± 4.6 g/d in wk 3 to 390.3 ± 102 g/d in wk 5. There was a weak linear regression between MS intake measured with D2O and the MS intake measured with the feeder of $R^2 = 0.16$ for wk 5. The low MS intake at the feeder in wk 3 was probably due to the fact that the piglets could still drink enough milk from the sow. The low intake measured in wk 5 by the automate was likely due to missing measurements at the feeder, as the feeder could not identify the piglets when several piglets were standing close to the feeding cup at the same time. Feed intake measurement calculated by the D2O method in wk 5 was probably too high because of additional water intake by the piglets. Funding: EU H2020-INFRAIA "PIGWEB" no.101004770

Effect of supplementation of Iberian lactating sows with betaine or amino acids on the nutritional composition of milk and its redox status under heat stress conditions

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Lactating sows are particularly vulnerable to heat stress due to the high metabolic rate associated with milk production. Heat stress during lactation could affect sow's metabolic state, the quantity and quality of the milk and consequently the proper development of the litter. The effects of supplementing lactating Iberian sows from day 70th of pregnancy onwards with betaine (BET; n=15) or amino acids (AA; n=13; arginine + valine) as nutritional strategies to mitigate heat stress was evaluated against a non-supplemented group (C; n=14). The effect was evaluated under heat stress (HS; n=18) and thermoneutral (NO-HS; n=24) conditions, in primiparous (P; n=21) and multiparous (M; n=21) sows. The average maximum temperature was 32.3°C for HS and 23.7°C for NO-HS. At 15 days postpartum, milk samples were collected and analysed for nutritional composition: total solids, protein, fat, ash, lactose, and energy content, as well as milk antioxidant capacity (FRAP) and lipid peroxidation (TBARs). The intake of lactating sows was measured daily and the average daily gain (ADG) of each piglets was recorded (n=407). The Statgraphics Centurion XVI package was used, and a multifactorial ANOVA was performed. The results showed that HS reduces the intake of the lactating sows ($P<0.001$) and decreased total solids, fat, and energy content and increased protein, ash, and lactose in milk ($P<0.001$). Furthermore, piglets under HS showed lower ADG ($P<0.001$). Milk in BET group had a higher percentage of fat, total solids, and energy content than C group ($P<0.05$). A treatment $\times$ parity interaction was observed, such that piglets from M sows supplemented with betaine had the highest ADG ($P<0.05$). Moreover, P sows showed higher antioxidant capacity than M sows, regardless of temperature or treatment ($P<0.05$). In conclusion, heat stress had a negative effect on milk quality and reduced litter average daily gain. Supplementation with BET could improve milk composition increasing energy and fat content. Funded by Spanish Ministry of Science and Innovation.

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Effects of Pine-Derived Polyphenol Supplementation on Sow Performance, Milk Quality, and Piglet Growth
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Botanical extracts are increasingly being explored for their potential benefits in the search for sustainable and natural solutions in livestock nutrition. In a three-run experiment, OXILEM, a polyphenol-rich commercial pine extract, was supplemented at 1% from day 106 of gestation through lactation in 10 sows (OX), while another 10 received a control diet (CON). At weaning, 229 piglets were assigned to one of four dietary groups based on maternal and post-weaning diets (CON/CON, CON/OX, OX/CON, OX/OX), with OX post-weaning diets containing 1% polyphenols. The study evaluated sow body weight and backfat changes during lactation, feed intake, milk composition (at day 3 and 17), piglet growth and feed intake from birth through early post-weaning. Sow performance was analyzed via ANOVA (R-studio software), considering the sow as the experimental unit. A linear mixed model assessed the effects of diet and parity (fixed factors) and run (random factor). Milk composition was analyzed using a model that incorporated diet, test day (day 3 vs. day 17), and their interaction as fixed effects, with run as a random factor. Post-weaning piglet performance was evaluated using a linear mixed model, with sow and piglet diets and their interaction as fixed effects, while run and box were random factors, with piglets nested within their foster mother. OXILEM supplementation had no significant impact on sow performance but increased total milk protein content at day 3 post-partum (5.58% in CON vs. 5.84% in OX, $P = 0.04$) with no differences observed at day 17. The sow diet significantly influenced piglet post-weaning growth, with higher body weight in piglets nursed by CON-fed sows and those fed the CON diet post-weaning ($P < 0.05$). However, the sow $\times$ piglet diet interaction was not significant. These findings indicate that while OXILEM supplementation may enhance total milk protein content, its direct application to piglets needs further investigations to optimize the supplementation strategy to balance sow benefits with optimal piglet development.

Supplementation of a unique fatty acid blend improves sow productivity and subsequent piglet lifetime performance

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Colostrum is critical for neonates, providing nutrients and driving immunological and physiological development. Effects of colostrum on lifetime performance are hypothesised, although published evidence is scarce. The aim of this study was to supplement sows with ColfaPig, a unique fatty acid blend, and monitor colostrum quality and subsequent progeny performance. Sow and progeny performance was recorded, on a commercial unit, for 10 baseline batches, followed by 32 supplemented batches. Colostrum brix values, a proxy of IgG content, were obtained from 184 actively farrowing sows using a digital refractometer. During supplementation, 1kg/t of the fatty acid blend (ColfaPig, Devenish Ltd, Belfast, UK) was incorporated into the gestating and lactating sow diets. Grower, finisher and sow diets were consistent throughout the recording period. Data was analysed univariately. The wean to slaughter period was corrected to 140 days prior to analysis using a sigmoidal growth curve. Fatty acid supplementation increased brix ($p=0.042$) from $22.1\pm 1.18\%$ to $25.4\pm 1.42\%$, with a notable increase ($p<0.001$) in samples classified as sufficient, from $31\pm 2.0\%$ to $62\pm 6.6\%$. Pigs born alive per sow increased ($p<0.001$) from 15.9 ± 0.20 to 16.9 ± 0.17 , and subsequent pigs weaned per sow increased ($p=0.019$) from 13.1 ± 0.36 to 14.1 ± 0.13 , with no reduction ($p>0.05$) in weaning weight. Litter weaning weight increased ($p=0.043$) from $85.6\pm 2.49\text{kg}$ to $91.3\pm 1.19\text{kg}$. Weaning pen weights were also less variable, with the Coefficient of Variation reducing from $27\pm 1.1\%$ to $23\pm 0.7\%$. In the wean-finish period the average slaughter weight of the progeny from fatty acid supplemented sows increased ($p=0.014$) by 3.2kg from $117.3\pm 0.91\text{kg}$ to $120.5\pm 0.81\text{kg}$, and wean to finish growth rate increased ($p=0.016$) by 23g/day from $789\pm 7.4\text{g/day}$ to $812\pm 5.3\text{g/day}$. This study demonstrates that improvements in colostrum quality can be achieved through dietary supplementation, with long term benefits on sow productivity, a major determinant of sustainability and profitability. Furthermore, a reduction in weaning weight variability was recorded from the resulting progeny, which also benefited lifelong performance through improved wean to finish growth rates and concomitantly higher slaughter weights.

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Poster 13

Glutamine and glucose oxidation and intestinal absorption capacity in low and normal birthweight suckling piglets supplemented orally with glutamine at 2–15 days of age

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Glutamine (Gln) followed by Glucose (Glc) are important fuels for rapidly dividing cells. Their metabolic pathways share significant crosstalk. We have previously reported that Gln supplementation supports weight gain in low (L) birthweight suckling piglets, however, information on the metabolism of Gln and Glc is limited, especially in suckling low birthweight piglets. Male normal (N) and L birthweight German Landrace littermates were supplemented with 1 g/kg BW/day (d) Gln (L-Gln; N-Gln, $n = 12$), dissolved in water, or water (W) as a control (L-W; N-W, $n = 11$) until age d 15. The oxidation and plasma rate of appearance (Ra) of Gln (d 14; $^{13}\text{C5-Gln}$) and Glc (d 16; $^{13}\text{C6-Glc}$) were determined with tracers. Small intestine absorptive capacity was measured using mannitol (Man; d 15) and xylose (Xyl; d 16). Tracer ^{13}C enrichments and plasma Xyl and Man levels were analysed in plasma and red blood cell- CO_2 using mass spectrometry and HPLC, respectively. Statistical analysis was performed with the Generalized Linear Mixed Model (SAS). Glutamine supplementation did not enhance piglet BW, and N piglets were always heavier than L throughout the study. Oxidation of Gln and Glc were always higher in L than in N piglets ($P<0.01$), whereas Glc oxidation was lower than that of Gln (43.4 vs. 52.8% ; $P=0.005$). Plasma $^{13}\text{C6-Glc}$ Ra was higher than that of $^{13}\text{C5-Gln}$ Ra ($P<0.01$). One hour after administration, L-Gln piglets showed higher plasma Xyl and Man concentrations suggesting that the absorptive capacity and area is higher in L-Gln piglets compared to their N-Gln littermates. We conclude that L piglets are more reliant on the utilisation of Gln and Glc as energy sources, whereas N-piglets may transition more rapidly to the utilization of a lipid-based energy supply. The lower oxidation of Glc compared to Gln could indicate that Glc is spared for organs with an absolute glucose requirement, which is consistent with the higher Glc Ra. Funding: European Union's Horizon 2020 – MSC Innovative Training Network grant No 955374; *metges@fbn-dummerstorf.de.

More eaters in 21 day weaned piglets by providing a complementary piglet milk replacer during lactation
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Piglets that consume dry feed during lactation start eating faster after weaning. However, up to 50% of the piglets do not consume dry feed at 21 days of age (DOA), which puts early-weaned piglets at greater risk of post-weaning health issues. Our aim was to investigate the effect of providing sow-reared piglets with a complex complementary piglet milk replacer (cPMR) via an automated cup system on the number of eaters at weaning. Piglets of 59 primi and multiparous TN70 sows were used. Control piglets (n=396) received only sow milk and dry feed before weaning, while cPMR piglets (n=404) were also supplemented with cPMR from 4 till 19 DOA by an automated cup system. Dry feed was provided twice daily from 6 DOA till weaning and was recorded per litter. Piglets were weighed individually at 4 DOA and at weaning. Rectal swabs were taken at weaning day. Piglets were classified as eater when the swab was covered with light to dark brown manure and contained coarse textured particles. Non-eaters had yellowish manure on the swabs without textured particles. When no clear distinction could be made, piglets were labelled as undefined. The effect of cPMR supplementation on body weight and dry feed consumption was tested using a GLM test, while the prevalence of eaters and non-eaters between groups was compared with a Chi² test (SAS 9.4). More cPMR piglets were eaters compared to control piglets (67.3% vs 50.5%; $p < 0.0001$). Most importantly, provision of cPMR reduced the number of non-eaters (20.5% vs 6.4%; $p < 0.0001$). cPMR piglets were also heavier (5.37 kg vs 6.25 kg; $p < 0.0001$) at weaning and consumed more dry feed per piglet (119 g vs 152 g; $p < 0.0001$). Next to the dry feed, cPMR piglets consumed 206 g cPMR per piglet (on dry matter basis, single measurement on group level), resulting in a total dry matter intake (DMI) of 358 g per piglet. Our results show that supplementation of cPMR during lactation increases the number of eaters, DMI of dry feed, total DMI and body weight of piglets at weaning. An improved preweaning DMI enhances adaptation of the piglets to solid feed, supporting gut development and facilitating a smoother weaning transition.

Session 55

Poster 15

Easy and fast prediction of sow milk immunoglobulins and main milk components using mid-infrared analysis of milk and colostrum

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Quality of sow colostrum and milk is an essential element regarding piglets immunity and growth. Among others, the content in immunoglobulins in colostrum is directly impacting piglets immunity in the first weeks of life, thereby impacting their intestinal health at weaning. In addition, the global composition of milk, through its fat, protein and lactose content is the main source of energy transfer from the sow to the piglets. Therefore, even if milk sampling is laborious, in research frame there is an interest in measuring colostrum and milk composition, but this usually implies reference chemical methods which are tedious and expensive. The objective of this study is to derive a key method in dairy animals to be used with sow milk: the mid-infrared (MIR) analysis of milk. Milk analysis of milk requires only a few seconds to run, is cost-effective and non-destructive. For this, 37 sows from CRA-W experimental farm were milked from week 0 to 3 after farrowing, and milk was analyzed both in chemical reference methods and in MIR spectrometry. First, the existing models predicting fat and protein for dairy cows were evaluated and corrected with sow milk main components. Second, new MIR models were developed to attempt predicting the immunoglobulins A, G and M contents in colostrum, in milk or in a common model using both colostrum and milk. Results show that cow MIR models were perfectly linear when predicting sow milk fat and protein, with an R^2 of 0.99, but were biased and needed to be adjusted for slope and bias. Then MIR models to predict immunoglobulins provided very heterogeneous results, with R^2 ranging from 0.16 to 0.97 depending of the model (colostrum, milk or both) and the molecules (immunoglobulins A, G, M or total). These results are only preliminary findings, as a proof of concept with a limited dataset. However, they show that an accurate estimation of fat and protein, but also potentially immunoglobulin, is possible with MIR analysis of sow milk and could be developed to go deeper in sow milk phenotyping.

The importance of paternal influence on the survivability and vitality of piglets

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Piglet mortality has a negative impact on both animal welfare and public acceptance of piglet production. The aim of this study was to investigate the influence of artificial insemination (AI)-boars on piglet vitality and survival. In total 115k piglet litters from 52 piglet producer herds were analyzed. These herds are part of the progeny testing scheme of the AI station GFS. Comparisons were made between a) the boar breeds Duroc (Du) and Pietrain (Pi) and b) different sire lines within Pi and Du. In addition, within the Pi-boars, the interaction between sire-line and dam-line was tested. For a) only herds using both AI breeds (12k litters) were included; b) and c) were performed within the Du (2k) and Pi (112k) boar breeds. Appropriate linear contrasts of the herd-year-season×breed included in trait-specific linear models were used to answer questions a-c. Finally, variances and genetic parameters were estimated for piglets born alive, born dead and weaned (NBA, NBD, NW), pre-weaning mortality (PWM) and the subjectively scored litter homogeneity, weight and vitality (EXH,W,V). The results revealed significant higher NBA (+1.3 piglets), NW (+0.2), PWM (+1.4), EXH (+0.4 points) and lower NBD (-0.3) of the Du AI-boars, compared to Pi. The more pronounced PWM can be explained by the higher NBA with an estimated related regression coefficient of 0.2. In contrast to the Du, clear differences between the lines were found in Pi. Further, relevant sire×sow-line interactions were observed. Heritability (h²) estimates within Pi-lines showed similar values between 4% and 14%. Slightly higher h² up to 26% were found for EXV. Genetic (rg) correlations between PWM and NBA and were high (>0.6), highlighting the negative influence of litter size on PWM. In most Pi-lines the EX scoring traits showed moderate favourable rg to PWM between 0.2-0.4, so that these traits can be used as informative genetic indicators for PWM. This study provides initial evidence that sire breed/line has a relevant influence on piglet vitality and survival.

Session 55

Poster 17

Review of technologies to measure pig body temperature

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Monitoring physiological parameters offers insights in animal health and functioning. Core body temperature is particularly valuable as it can indicate the presence of diseases, the time of ovulation in sows or the adaptation to thermal stress. Traditionally, core body temperature is measured manually using a rectal thermometer, however automated technologies would allow to measure pig body temperature in a (semi-)continuous way for a longer term follow-up. As part of the H2020 project PIGWEB a systematic literature identified 1773 records on sensors to monitor pig body temperature. After removing duplicates, filtering based on year and language, 836 records remained, of which 129 were focused on measurement of temperature in livestock. Information on the used sensor technology was collected including measurement location (e.g. rectal, skin at ear base), development stage, invasiveness (e.g. remote sensing, wearable, implanted), cost, and automation level. The found temperature sensors can be divided into three main categories. Implanted temperature sensors provide the most accurate core body temperature readings in pigs but are invasive and impractical for large numbers of pigs like on a farm. Contact temperature sensors, e.g. ear tags or wearable bands, have been explored for on-farm use but limited published results hinder the assessment of their practical applicability. Thermal imaging enables non-invasive remote monitoring of pig body temperature, yet challenges remain in correlating surface and core body temperature prompting the use of various modelling techniques. Other factors like thermoregulation, dirt, and calibration requirements contribute to measurement inaccuracies. While temperature sensors exist for all pig life stages, costs, manual data retrieval and the need for a controlled environment or frequent calibration limit on-farm adoption. Future efforts should therefore focus on developing cost-effective, automated and barn-adapted pig body temperature monitoring solutions.

Production diseases reflect production intensity - consideration of the OneWelfare implications

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In spite of decades of research on animal health and welfare and significant on-going investment by the EU (e.g. EUPAHW), there is little progress on some of the most intractable welfare problems for farm animals. These are largely associated with what are commonly known as production diseases and include lameness and poor longevity in breeding females and peri and neonatal mortality. These issues have grave One Welfare implications as they cause enormous suffering for the animals involved, high economic losses to the farmer and represent a significant waste of valuable environmental resources. Production diseases reflect the intensity of production we place farm animals under. This is to the extent that some are reaching their biological limit (e.g. hyper prolific sows). These limits are analogous to the limits being placed on farmers and farming systems in general, examples of which will be presented during this talk. My central thesis is that employing the One Welfare framework is crucial to identifying the commonality of the drivers of production intensity and thereby in addressing solutions to these 'wicked problems'.

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Theatre 2

Sustainability in Meat Production: A Preliminary Analysis of Beef Cattle Farms in Veneto (Italy)

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Cattle farming is a key sector in the Italian agri-food supply chain. According to 2023 data, there are about 2.33 million cattle in Italy for meat production: the Veneto region alone is home to 459,839 cattle, making it a primary production area. These figures highlight the structural evolution of the sector and its impact on economic and environmental sustainability. The Farmer Association in Veneto is developing a specification for sustainable meat production and, in collaboration with the University, has carried out a validation test to assess the ability of farms to meet the requirements and the challenges they face in implementing the specification. The questionnaire, consisting of 52 binary questions ("applied" and "not applied") regarding feeding plans, drug use, manure management, biosecurity and animal welfare, was administered by technicians through direct interviews with farmers from February to December 2024. Seventy-five interviews were conducted, excluding nine questionnaires due to missing identifying data. Preliminary results indicate that 21.2% of the requirements surveyed are less than 30% implemented, suggesting that beef producers face challenges in adopting practices related to farm management, particularly in manure management, nutrition, biosecurity and environmental sustainability. These findings highlight the difficulties in investing in sustainability and animal welfare and highlight the need for technical support and training policies. In addition, farms without employees (n=39) have to comply with mandatory occupational health and safety legislation, but only 12.8% of them fully meet all the requirements. Targeted interventions are essential to improve environmental management, animal welfare, and safety, particularly on farms without employees, and they should include increased training and incentives to support the adoption of good practices.

Biosecurity in dairy buffalo from 2018 to 2023 assessed by ClassyFarm check list in Campania Region
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Biosecurity in ruminants is often an overlooked area, but it is a key aspect in a One Health approach. The principles relating to the biosecurity provisions in Reg. (EU) 2016/429 require reflection on the critical issues and possible mitigation measures that can be planned and possibly implemented in this sector. ClassyFarm processes biosecurity data by a checklist of 15 items. Each item can be scored as 'inadequate', 'acceptable' or 'optimal'. For each item, a weight was determined using an expert opinion elicitation process. Score is expressed as percentages on a scale from 0 to 100%. In Caserta province, the tool has been used to assess 216 and 475 buffalo farms in 2018 and 2023 respectively, in order to investigate the level and the main critical issues. In this period biosecurity value increased from 44.6% to 65.4% level. In particular, the frequency of unsatisfactory responses decreased from 38.72% to 11.14%. Conversely, acceptable ratings increased from 48.84% to 65.46% and optimal ratings increased from 12.43% to 23.4%. The items with the greatest reduction in the frequency of negative thresholds were: lack of quarantine area; possible contact with other species due to lack of fencing; loading and unloading areas for live animals close to the housing areas; lack of disinfection facilities for foreign vehicles; possibility of contact between foreign vehicles and farmed animals. The data obtained show that public and private awareness-raising activities, thanks to the receptiveness of the sector, have allowed a significant improvement in the overall level of biosecurity. It should be emphasized that the main improvements have been in structural aspects, which, although necessary, are not sufficient to be effective if they are not accompanied by management improvements that allow effective awareness and monitoring of the biosecurity status of the farm.

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Theatre 4

Towards an effective farm evaluation: Methods, barriers and best practices of record-keeping in Indonesian smallholder dairy farmers

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Appropriate record-keeping method improves the accuracy of data collected by farmers, thus optimising farm analysis, which leads to long-term growth in the livestock sector. This study assesses the record-keeping methods by smallholder dairy farmers in Indonesia, emphasising the challenges and potential enhancements in their effectiveness. The study was conducted in Bandung, Bogor, Boyolali, and Malang districts from June 2023 to July 2024. Survey questionnaires were employed to interview 100 farmers, observations were carried out on 12 farms, and in-depth interviews were undertaken with five dairy cooperatives. Our findings indicated that manual record-keeping remains the predominant method, particularly among small cooperatives with restricted access to technology and support facilities. Conversely, digital record-keeping is common among farmers affiliated with large cooperatives. Farmers frequently utilise storage media for manual record-keeping, including sheets of paper, books, whiteboards, or barn walls. Farmers indicated that manual record-keeping requires additional time to summarise and identify data. At the same time, digital record-keeping, especially data collected using mobile phone applications, encounters issues, including unreliable internet connectivity and device malfunctions. Furthermore, the discussion with farmers pointed out that their inadequate knowledge and data evaluation skills hamper the efficiency of their record-keeping practices, both using manual and digital methods. Though digitisation will inevitably occur, manual record-keeping is still needed. Therefore, both methods need to be harmonised to suit the farmer's requirements. In addition, given the strategic role of dairy cooperatives for farmers, it is necessary to strengthen the capacity of cooperatives to provide regular support to farmers to encourage farmers' motivation, awareness, and knowledge regarding the importance of record-keeping. Regular feedback on the results of recording is crucial to ensure that data evaluation is compelling so that farm management decisions are made correctly and accurately.

Self-reported dry-off procedure in 21 commercial Irish dairy herds

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The period around dry-off (including the drying-off) represents a high-risk period for new intramammary infections. After drying-off, the teat ends can remain open for several weeks, which facilitates bacteria entering the gland. When applying the dry-off treatments it is essential to reduce the exposure of teats and teat canal to bacteria to reduce the risk of infections. As non-antibiotic dry cow therapy (selective DCT) is implemented on dairy farms, there is a greater need to focus on the procedure because antibiotics could have masked the impact of a poor procedure in the past. Therefore, this study aimed to identify the dry-off procedure in 21 commercial dairy farms conducting selective DCT. This qualitative study used survey data from 21 commercial dairy farms in Ireland to understand farmers self-reported dry-off procedure. The survey included questions about the method of drying-off the cows (abruptly or gradually) and the steps and order of tasks when carrying out this procedure. Data was compiled and frequencies were calculated using Microsoft Excel. Prior to dry-off, 15 farmers restricted the cows diet to reduce milk yield. Ten farmers dried-off the cows gradually (reducing milking frequency prior to dry-off). Twenty farmers dried-off the cows immediately after the last milking. Thirteen farmers reported drying-off the cows following these steps: 1- using a different pair of gloves than the ones used for the preceding milking, 2- cleaning and disinfecting teats with alcohol or methylated spirits swabs, 3- massage the antibiotic after infusion, 4- pinching the base of the teats for infusion of the teat sealant and 5- applying teat disinfectant after drying-off. Only 5 farmers applied teat disinfectant before the alcohol swabbing step. Seventeen farmers cleaned front quarters followed by rear quarters and 11 applied treatments to rear quarters first then front quarters. This study contributes to identify self-reported steps taken by farmers for drying-off the cows. This highlights areas that would increase the risk of infections being introduced while carrying-out the procedure, such as the lack of disinfection or cleaning steps prior to disinfecting the teats with alcohol spirits. It also highlights the need for further research to empirically assess the cleanliness and quality of the procedure on Irish farms.

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Theatre 6

SCC dynamics over the dry period in 21 Irish dairy herds

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Replacing antibiotic use at dry-off for an internal teat sealant (ITS) alone in low SCC cows (selective DCT) is now compulsory for most herds in Ireland. Commercial farmers are implementing this practice, with approximately 88% of cows receiving antibiotic at dry-off in 2023, a decrease from almost 100% in 2019. Irish research has shown that using ITS alone could result in higher SCC in the following lactation. The aim of this study was to describe the somatic cell count (SCC) dynamics from the last milk recording of the previous lactation to the first milk recording of the following lactation, in herds that were conducting selective DCT. This study used SCC data from October 2020 to April 2021 from 21 commercial spring-calving grazing dairy herds. These herds were conducting selective DCT for > 1 season. Cows were classified based on their last milk recording SCC of 2020 (Dry-off) and first milk recording SCC of 2021 (calving). Categories were: 1- Healthy: a dry-off and calving SCC <200,000 cells/mL; 2- New SCC: a dry-off SCC <200,000 cells/mL and a calving SCC ≥200,000 cells/mL; 3- Cured SCC: a dry-off SCC ≥200,000 cells/mL and a calving SCC <200,000 cells/mL and; 4- Persistent SCC: a dry-off and calving SCC ≥200,000 cells/mL. We evaluated the percentages within each classification accounting for the treatment at dry-off (i.e. antibiotic [AB] + ITS or ITS alone) in SAS (Proc Freq). We evaluated the impact of treatment and farm on the odds of a Healthy cow (versus a New SCC) using Proc Genmod. Average SCC prior to dry-off was 240,000 and 54,500 cells/mL for cows treated with AB+ITS and ITS alone, respectively. For cows treated with AB+ITS 71%, 19%, 6% and 4% were classified as Healthy, Cured SCC, New SCC and Persistent SCC, respectively. For cows treated with ITS alone 88%, 0.7%, 11% and 0.3% were classified as Healthy, Cured SCC, New SCC and Persistent SCC, respectively. Farm was significantly associated with the odds of a Healthy cow ($p < 0.01$). Cows treated with AB+ITS had a non-significant 1.4 higher odds of a Healthy status compared to ITS alone ($p = 0.06$). The majority of cows treated with ITS alone remained with a low SCC in the following lactation, however a greater proportion of ITS cows had new high SCC after calving. This study helps identify potential areas of risk for cows treated with ITS alone and target prevention strategies.

Effects of lactoferrin on the growth and biofilm formation activities of mastitis-associated Enterobacteriaceae pathogens

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Escherichia coli and *Klebsiella pneumoniae* are major mastitis-causing pathogens, and their antimicrobial resistance of these bacteria is a major concern. Therefore, new therapeutics of mastitis other than antimicrobial drugs are urgently needed for mastitis. We focused on bovine lactoferrin (LF), an endogenous antimicrobial peptide, as an alternative to antimicrobial drugs. LF exhibits antimicrobial and antibiofilm activities. In this study, we aimed to investigate the effects of LF on the growth and biofilm (BF) formation activities of *E. coli* and *K. pneumoniae*. In total, 24 *E. coli* and 20 *K. pneumoniae* strains were isolated from cows with mastitis and their dairy farm environment. Relationship between bacterial growth and LF concentration in bovine milk was assessed via milk growth experiments and ELISA assay. Additionally, minimum inhibitory concentration (MIC) via broth microdilution method and BF formation via crystal violet method were assessed to determine the antimicrobial and antibiofilm effects of LF. Notably, LF concentration in bovine milk was not correlated with *E. coli* growth but was negatively correlated with *K. pneumoniae* growth. MIC values for over half of all strains were higher than 4,000 µg/mL, exceeding the concentrations in the bovine udder. LF inhibited the growth of *E. coli* and *K. pneumoniae*, and BF formation activity of *K. pneumoniae*. However, BF formation activity of *E. coli* was increased in the presence of LF. Collectively, our findings suggest that bovine milk LF partly regulates the growth of *E. coli* and *K. pneumoniae*. However, further investigation of its specific effects on each bacterial species is necessary to facilitate its application to control mastitis.

Antimicrobial component concentrations in milk at dry-off and postpartum and their association with new high somatic cell counts at the quarter level in dairy cows

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Antimicrobials are commonly administered intramammarily at dry-off to treat intramammary infections during the dry period. Due to the concern of antimicrobial resistance, there is a need to establish antimicrobial-independent udder health management strategies for the dry period. It was reported that high concentrations of lactoferrin in milk at dry-off are important to control the new high (NH) somatic cell counts (SCCs) at subsequent lactations. Lactoferrin is an endogenous antimicrobial protein secreted in milk, whereas bovine milk contains many other types of antimicrobial components; however, their specific roles during the dry-off period remain unclear. Therefore, in this study, we aimed to determine the concentrations of various milk antimicrobial components, including immunoglobulin (IgG), IgA, lactoferrin, lingual antimicrobial peptide (LAP), and S100A7, during the dry-off and postpartum periods and evaluate their roles in preventing high SCCs at the quarter level in dairy cows. In this study, Quarters with SCC < 300 000 cells/ml at both dry off and postpartum were classified as persistent low SCCs (PL) whereas those that rose above that same threshold postpartum were classified as NH SCCs. Notably, IgG, IgA, and lactoferrin concentrations were significantly higher at dry-off than at postpartum. In contrast, LAP concentrations were significantly lower at dry-off than at postpartum. Moreover, LAP concentrations at dry-off and postpartum showed a significant positive correlation. Comparison of the concentrations of antimicrobial components at dry-off with PL and NH SCCs in postpartum quarter milk revealed that LAP and IgG concentrations in PL SCCs milk were significantly higher than those in NH SCC milk. These results suggest that the IgG and LAP concentrations at dry off influence the postpartum SCCs. Specifically, the maintenance of LAP concentration in the postpartum period may explain NH SCC prevention, requiring further research.

Modifications of the Dutch functional hoof trimming method may increase the risk of hoof lesions in cattle
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Regular hoof care is essential for effective lameness control in cattle. To accommodate modern management conditions, several modifications of the widely used five-step Dutch hoof trimming method have been emerged. One common modification involves significantly increasing the modelling of the sole area (axial groove slope) to relieve pressure on vulnerable parts of the sole corium. However, published findings on the impact of this adaptation on hoof health remain inconsistent. Using the state-of-the-art biomechanical ex-vivo model of a bovine hoof, we evaluated different scenarios of extensive sole modelling by measuring internal pressure distribution. The results indicated that while larger modelling can reduce harmful pressure on the sole corium in healthy hooves, it may conversely increase the pressure when the weight-bearing function of the hoof bulbs is compromised (e.g., due to heel horn erosion), associated with the sole slope shifted cranially. When extensive modelling was applied to hooves with reduced bulb support, sole corium pressure increased significantly ($P < 0.05$) compared to hooves with minimal or no modelling. Field studies analysing trimming profiles from 19 certified hoof trimmers and hoof health data from over 52,000 dairy cows further confirmed that more cranially positioned modelling of the sole was presumably associated with a higher prevalence of severe hoof horn lesions. These findings emphasise the importance of adapting hoof-trimming techniques to individual properties of the hoof and the ability to restore proper weight bearing to prevent hoof lesions and lameness. This information is particularly relevant for professional hoof trimmers, veterinarians, and other experts involved in cattle lameness management.

Session 56

Theatre 10

Transcriptomic characterisation of cattle lameness (CHDL) susceptibility in blood and foot biopsies
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Lameness in dairy cows has significant welfare, economic and environmental effects through reduced reproduction and milk production and increased culling rates. Claw horn disruption lesions (CHDL) are non-infectious foot lesions that can cause lameness, reported to affect >40% of dairy cattle globally. CHDL is comprised of three lesions, sole haemorrhage (SH), sole ulcers (SU) and white line disease (WL). Genetic variation is related with lesions susceptibility and severity, therefore, genetic improvement, with farm management interventions could reduce their incidence. From 2,352 Holstein dairy cows with phenotypic and genetic data on CHDL lesions, 24 phenotypically and genetically (based on GEBVs) extreme animals were selected and peripheral blood lymphocytes (PBLs) and foot tissue (FT) biopsies taken to study systematic and local responses. We performed whole genome sequencing (WGS) and RNA-seq to identify differentially expressed genes, potential candidate variants and performed eQTL analysis for each lesion type. We identified cis eQTLs in FT and PBLs in ~200-320 genes for SH, SU and WL. Enriched functions of these included immune response and inflammation and the complement cascade. Differentially expressed genes were identified for all lesion types in FT samples (17-40 by lesion) and SU and SH in PBLs (6-117). Data was combined with RRBS differential methylation data from CHDL and healthy cows and existing GWAS data to understand the interplay between regulation, genomics and transcriptomic profiles with susceptibility to CHDL lesions. These advances contribute to informing the genetic improvement of dairy cattle resistant to all types of non-infectious foot lesion, reducing the burden of lameness on the dairy cattle industry.

Investigating cattle response to digital dermatitis using single cell RNA sequencing and in vitro data

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Bovine Digital Dermatitis (BDD) is a painful, polybacterial disease causing severe lameness in cattle, with significant welfare and economic impacts. Spirochetes from the *Treponema* genus are key causative agents in BDD. Resistance to BDD has been shown as a complex heritable trait by previous studies. To explore genetic predisposition and immune response mechanisms, we developed an in vitro model and generated single-cell RNA sequencing data from foot biopsies. Using the UK Agriculture and Horticulture Development Board's Digital Dermatitis Index, 361 heifers were classified into high (HG), medium (MG), and low (LG) genetic merit groups, and three key phenotypes were studied: HG that were healthy (HGH), LG that were chronically infected (LGC), and LG that were infected and then healed (LGH). Foot skin biopsies (n=5 per phenotype) were used to culture fibroblasts. Granulocytes from blood samples (n=3 per phenotype) were co-cultured with treponeme-exposed fibroblasts in a transwell migration system. After 16 hours of incubation, HGH heifers exhibited significantly reduced granulocyte migration compared to LGH and LGC, indicating a potential genetic basis for immune response differences. Additionally, we performed single-cell and single-nuclei RNA sequencing (scRNAseq/snRNAseq) on foot skin biopsies (n=4 per phenotype) to profile immune and stromal cell populations. Using 10X Genomics system, we profiled transcriptomic data from over 25,000 cells, identifying keratinocytes, fibroblasts, and multiple immune cell types. Notably, snRNAseq yielded higher cell recovery rates than scRNAseq, due to the challenges of skin tissue dissociation. Ongoing differential gene expression analysis aims to pinpoint molecular signatures driving disease susceptibility and immune response variation within each cell types. By linking in vitro and in vivo immune responses, our study provides further insights in the genetic and cellular mechanisms underlying BDD susceptibility.

Session 56

Poster 12

Comparing immunogenicity of lipopolysaccharide (LPS) from the rumen and from *E. coli* LPS

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This study compared the immunogenicity of LPS from rumen digesta and *E. coli* O111:B4 using an in vitro inflammation model with rumen epithelial cells (REC) from dairy cows and intestinal epithelial cells (IPEC-J2) from swine. LPS exposure reduced cell viability in a dose-dependent manner, measured by the WST-1 assay. At 200,000 EU/mL, both rumen and *E. coli* LPS reduced REC viability to 85%. In IPEC-J2 cells, rumen LPS reduced viability to 80%, while *E. coli* LPS reduced it to 74% ($P < 0.001$). At 75,000 EU/mL, rumen LPS decreased TEER and increased FITC-Dextran permeability by 52.8% and 106.9%, respectively. *E. coli* LPS increased permeability by 109.9%, but did not affect TEER. Gene expression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-8, and IL-10) increased in a dose-dependent manner in both REC and IPEC-J2 cells ($P < 0.001$). However, increases were more pronounced in REC cells exposed to rumen LPS. Exposure to 75,000 EU/mL rumen LPS upregulated IL-1 β , IL-6, and IL-8 expression by 44.5, 12.3, and 17.0-fold, respectively, compared to 10.0, 4.0, and 5.9-fold with *E. coli* LPS. In IPEC-J2 cells, 75,000 EU/mL *E. coli* LPS induced a higher TNF- α expression than rumen LPS (10.5 vs. 2.5-fold, $P < 0.001$), but rumen LPS caused larger increases in IL-6 (2.2 vs. 1.6-fold, $P < 0.05$), IL-8 (3.1 vs. 1.5-fold, $P < 0.05$), and IL-10 (3.5 vs. 1.9-fold, $P < 0.05$). Exposure to 75,000 EU/mL rumen LPS downregulated ZO-1 expression to 0.7-fold in REC cells, while upregulating OCLN, CLDN1, and CLDN3 by 1.6, 1.6, and 1.8-fold, respectively. *E. coli* LPS increased these tight junction proteins mRNA expression by 1.4, 1.6, 1.7, and 2.8-fold. In IPEC-J2 cells, rumen LPS increased the tight junction proteins expression by 1.4, 1.2, 2.0, and 1.4-fold, while *E. coli* LPS downregulated ZO-1 and CLDN1 by 0.7 and 0.8-fold, respectively, and increased OCLN and CLDN3 by 1.1 and 1.7-fold. Both rumen and *E. coli* LPS reduced cell viability, TEER, and increased permeability, along with upregulating pro-inflammatory cytokines and tight junction proteins in REC and IPEC-J2 cells. However, rumen LPS had a more significant effect. Keywords: lipopolysaccharide, cell viability, cytokines, tight junctions

Stabilized extracellular vesicles from bovine milk as anti-inflammatory and immunomodulatory agent on human macrophage-like cells

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Milk is a rich source of bioactive components, including extracellular vesicles (EVs), which play a key role in cell communication. Bovine milk EVs (mEVs) have therapeutic potential as anti-inflammatory agents for inflammatory bowel disease (IBD) and as antimicrobial alternatives to antibiotics in livestock. In a 2D IBD model, mEVs reduced proinflammatory cytokine production and improved enterocyte homeostasis increasing gene expression of tight junctions and mucins. Additionally, in a neonatal calf diarrhea model caused by *Escherichia coli*, mEVs reduced bacterial adherence, modulating host-pathogen interactions. Clinical application enhances the milk value as a livestock product, benefiting farmers and stakeholders. However, EV storage is a pivotal limitation, as low-temperature preservation (-80°C) is the most effective method but unfeasible for clinical use. This study aimed to develop a prototype formulation by converting mEV suspensions into microparticle powders, ensuring stability at room temperature and facilitating clinical application. mEVs were isolated using differential centrifugation and ultracentrifugation, incorporating an EDTA washing step before the stabilization. Morphology was analyzed via scanning electron microscopy and nanoparticle tracking analysis, while western blot confirmed the presence of EV markers CD81 and TSG101. Both fresh and stabilized mEVs were tested on human macrophage THP-1 cells to assess bioactivity retention. Gene expression was analyzed at two treatment points (6h and overnight), including powder shelf-life evaluation (18 months). When applied to pre-inflamed cells, stabilized mEVs showed comparable effects to fresh ones, demonstrating their remarkable stability. This study allowed the production of a stabilized mEV product to be used for clinical application although these results have to be confirmed in vivo in wider studies.

Session 56

Poster 14

Impact of Besnoitia besnoiti Seropositivity on Seminal Quality in Beef Bulls Under Extensive Management

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Besnoitiosis, caused by *Besnoitia besnoiti* (Bb), is an emerging parasitic disease affecting cattle, particularly in southern Europe. The infection leads to economic losses due to reduced fertility, poor body condition, and, in severe cases, irreversible testicular degeneration. Despite these known impacts, its potential relationship with seminal quality in beef bulls remains unclear. Progressive sperm motility is a key parameter of reproductive efficiency, influencing conception rates and herd productivity. This study examines whether Bb antibody titres correlate with progressive sperm motility in bulls raised extensively in Castilla y León. A total of 20 beef bulls (Limousine and Charolais breeds), aged 15–18 months, were included. Semen samples were collected using electroejaculation, and blood samples were obtained from the coccygeal vein using the Vacutainer system. Seminal analysis was conducted within two hours of collection using the Computer-Assisted Sperm Analysis (CASA) system. Serum samples were tested for Bb antibodies using an indirect ELISA commercial kit (Innovative Diagnostics, Grabels, France). Data analysis was performed using RStudio software, with statistical significance set at $p < 0.05$. The median progressive motility value was used as the threshold to classify samples as suitable or unsuitable. No significant association ($P > 0.05$) was found between Bb antibody titres and progressive sperm motility. However, bulls classified as having unsuitable semen based on motility exhibited higher antibody titres than those with suitable semen. Bulls with unsuitable semen showed higher antibody titres, suggesting a possible link, though statistical significance was not reached. The findings suggest that Bb seropositivity alone may not be a reliable predictor of seminal quality in beef bulls. However, the trend of higher antibody titres in bulls with lower motility suggests potential subclinical effects on reproductive performance. Given the study's limited sample size, further large-scale research is necessary to confirm these findings and explore physiological mechanisms linking Bb infection to reproductive health in cattle.

Sub-acute acidosis in dairy cows: towards a metabolite-based diagnosis

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With the objective of identifying metabolites suitable for diagnosing sub-acute acidosis (SARA) in lactating dairy cows, we conducted a study using cows fed either a control or a SARA inducing diet. Throughout the experimental period, a total of 23 dairy cows in mid-lactation were housed in a free-stall barn equipped with an automatic milking system. The study lasted 63 d, comprising 3 periods (P) of 21 d each. During P1, cows were fed a control diet (52:48 forage to concentrate ratio; CONT1), followed by P2, during which cows were fed a SARA inducing diet (35:65 forage to concentrate ratio; SAR2); and finally, during P3, cows were fed the same control diet as in CONT1 (CONT3). During the last 2 days of each experimental period, cows were sampled for milk, blood, ruminal fluid, urine, faeces and saliva. In this report, we present preliminary results of metabolomics analysis performed in samples of milk and blood. Samples were analysed using non-targeted metabolomics with UPLC-QTOF-MS (in the positive and negative ionization mode). The pairwise comparisons between the SARA and control groups highlighted distinct overall metabolite profiles. In total, 54 annotated metabolites exhibited significant differences in both milk and plasma samples between the groups. These metabolites were primarily classified as amino acids and derivatives, glycerophospholipids, phosphosphingolipids, and nucleotides and their derivatives. A refined MUVR analysis identified four metabolites that distinctly segregated the CONT and SARA groups: 1-methyladenosine, isovaleryl glycine, PS P-35:0, and PC P-31:0. In addition, in the serum samples the highest fold change (FC > 2) was observed in C₁₈H₃₂NO₂, C₁₈H₃₄NO₂ and N₂O while in milk samples Cholic acid and Cholic acid adduct were dominant.

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Poster 16

Host Responses to *Treponema* spp. in Bovine Digital Dermatitis: Transcriptional Insights from a Murine ModelH. Espiritu¹, E. J. Valette¹, M. H. Jung², S. S. Lee¹, Y. I. Cho¹¹ Suncheon National University, Department of Animal Science and Technology, 255 Jungang-ro, 57922 Suncheon, South Korea, ² Gyeongsang National University, Department of Microbiology, 501 Jinju-daero, 52828 Jinju, South Korea

Bovine digital dermatitis (BDD) is a complex, polymicrobial hoof disease in cattle, with *Treponema* spp. as primary pathogens driving inflammation, immune dysregulation, and tissue damage. This study employed a murine model to investigate the host transcriptional responses to infections with *Treponema pedis*, *Treponema phagedenis*, and *Treponema pescar*, both individually and in combination, using RNA sequencing (RNA-seq) analysis. The results revealed species-specific pathogenic mechanisms and distinct host responses. *T. pedis* induced a robust inflammatory response, upregulating cytokine signaling (Cxcl2, Saa3, Fpr1, S100a8) and neutrophil recruitment, leading to extensive tissue disruption and epidermal barrier breakdown. In contrast, *T. phagedenis* promoted extracellular matrix remodeling and metabolic reprogramming (Nos2, Mmp8), facilitating chronic lesion persistence. *T. pescar* triggered a localized immune response, characterized by immune cell trafficking (Fpr1, Clec4e) with minimal systemic effects. Notably, combined infections resulted in reduced lesion formation but triggered compensatory tissue repair pathways (Dsg4, Krt83, Padi3), suggesting bacterial interactions that alter disease severity. Gene ontology and KEGG pathway analysis highlighted cytokine-cytokine receptor interactions, immune cell chemotaxis, and keratinization disruption as key drivers of *Treponema*-mediated pathology. Calcium signaling suppression emerged as a common feature across all infections, impairing muscle function, tissue repair, and epidermal homeostasis, contributing to systemic dysfunction and lameness. These findings underscore the distinct yet interrelated roles of *Treponema* spp. in BDD pathogenesis, emphasizing the interplay between inflammation, tissue remodeling, and barrier integrity loss. By elucidating host-pathogen interactions at the transcriptional level, this study provides a foundation for targeted therapeutic strategies aimed at modulating immune responses, restoring epidermal integrity, and mitigating lesion severity in affected cattle.

Utilization of milk components to predict the risk of negative energy balance in Holstein cows using receiver operating characteristic analysis

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Most dairy cows experience a certain degree of negative energy balance (NEB) after calving, which may result in various health problems. The aim of the study was to predict the incidence of NEB in Holstein cows using milk components collected during milk performance tests conducted in the period from day 5 to day 35 of lactation. A total of 732 cows from 5 herds were used in the experiment. Depending on the blood concentration of non-esterified fatty acids (threshold 0.6 mmol/l) they were considered healthy ($n = 621$) or in NEB ($n = 111$). A binary logistic regression model was developed to predict NEB incidence from milk yield and milk components including milk fatty acids as explanatory variables. The results of the regression analysis applied to the data from individual herds and from all herds combined showed that milk fat content and milk C18:1 and C18:0 concentrations were the main significant factors ($P < 0.05$) in the diagnosis of NEB. Receiver operating characteristic (ROC) curves were then constructed to evaluate the performance of the model and to visualize its use as a diagnostic test. The area under curve (AUC) was used as a measure of the diagnostic accuracy of the model outcome, with an AUC of 1 meaning perfect diagnosis and an AUC of 0.5 indicating no discriminative ability. When milk fat content and milk C18:1 and C18:0 concentrations were included in the model applied to the dataset containing the cows from all herds, the resulting AUC value was 0.82, indicating quite high diagnostic ability. The optimal prediction cut-point was determined by Index of Union method. The resulting sensitivity and specificity values were 0.73 and 0.73, respectively. This means that the test detected 73% of real positive NEB cases, while the false positive rate was 27%, which makes it reasonably useful for on-farm diagnosis of energy-imbalanced cows. It was concluded that milk fat, C18:1 and C18:0 could be used as biomarkers potentially applicable for screening the incidence of NEB in early lactation Holstein cows. However, the results need to be validated by further research and large-scale studies.

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Poster 18

Prediction of metabolic and reproductive disorders in Holstein cows using milk composition and logistic regression

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Prevention and early detection of metabolic and reproductive disorders can significantly reduce veterinary costs and loss of performance. The objective of the study was to predict these disorders in dairy cows using the models that include milk characteristics. A total of 1783 Holstein cows from three herds were involved in the study. Data from monthly milk performance tests (milk yield, milk composition including milk fatty acids) were recorded from September 2023 to December 2024. In addition, the health of the cows was monitored and diagnosed metabolic and reproductive disorders were documented. In total, metabolic disorders were detected in 33 primiparous (PP) and 77 multiparous (MP) cows, while reproductive problems were detected in 93 PP and 253 MP cows. Binary logistic regression models were developed to predict the incidence of disorders in PP and MP cows using milk yield and milk composition data as explanatory variables. Receiver operating characteristic (ROC) curves, which graphically represent the relationship between true positive and false positive rates, and area under the curve (AUC), which quantifies the accuracy of the model, were used to assess the predictive ability of the models. The Index of Union method, which minimizes the absolute differences between the AUC and the diagnostic measures (sensitivity and specificity), was used to determine the optimal cut-off values. For the prediction of metabolic disorders in PP cows, the resulting model included milk fat, C18:0, and C18:1 (AUC 0.81) and correctly identified 72.7% of affected animals when the optimal cut-off value was applied. In MP cows, a model based on milk protein, C16:0, C18:1 and C18:0 resulted in an AUC of 0.72 and correctly predicted 64.3% of metabolic disorders. With regard to reproductive disorders in PP cows, a model with milk fat, C18:0 and C18:1 (AUC 0.85) was able to detect 78.5% of the affected animals. Milk composition, particularly fatty acid content, appears to be a suitable indicator for predicting metabolic and reproductive disorders in Holstein dairy cows, and binary logistic regression has proven to be an effective method for developing prediction models.

Examining factors affecting *Treponema phagedenis* abundance in foot swabs taken from dairy heifers

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Digital dermatitis (DD) is a disease affecting the feet of dairy cattle, negatively impacting animal welfare and causing significant economic losses. Bacteria of the genus *Treponema* have a key role in disease pathogenesis. We investigated how *Treponema phagedenis* abundance changes in the weeks leading up to visible lesion presence, and how abundance changes with a heifers Digital Dermatitis Index (DDI), an established breeding value for resistance to DD. Additionally, this research contributes further knowledge as to how *T. phagedenis* abundance changes between active and inactive lesions and between DD asymptomatic cattle and DD symptomatic cattle. Thirty-six heifers were swabbed on both hind feet on four consecutive months and the extracted DNA was used to calculate absolute *Treponema phagedenis* abundance using qPCR. Cycle threshold values were then used to estimate absolute genome copy number of the test DNA. Using Mann-Whitney U, Wilcoxon signed rank and Chi-Squared tests, the presence of any type of DD lesion was significantly associated with higher *T. phagedenis* abundance than heifers with no lesions, however there appeared to be no significant difference between active and inactive lesions. Additionally, heifers which were affected with DD were found to have a significantly higher abundance of *T. phagedenis* one month before a lesion was present compared to heifers that were not affected. The presence of *T. phagedenis* on a foot before a lesion is observed highlights the need for early detection of lesions as well as the usefulness of routine preventative treatments. Finally, this research does not find that DDI has any significant effect on *T. phagedenis* abundance.

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Poster 20

Autoantibodies in colostrum and plasma of cows and calves and its relation with dry period length

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Natural antibodies (NAbs) are polyreactive low affinity antibodies that bind to exogenous antigens but also to modified self-antigens. NAb reacting towards self-antigens or danger signals are also known as Natural Autoantibodies (NAAbs). The aim of this study was to evaluate the relation between dry period (DP) length and anti-PC-BSA autoantibodies in colostrum and plasma of cows and their calves. Holstein Friesian dairy cows (N=93) were assigned to 3 DP lengths (0, 30 or 60 d) and 2 diets (glucogenic or lipogenic). Weekly blood samples were taken from week -3 till 9 relative to calving and colostrum was sampled at calving. Weekly blood samples from the heifer calves (N=59) were taken from day 2 till 12 weeks of age. Cow and calf plasma NAAbs anti-PC-BSA IgG and IgM isotype data were measured by Elisa and analysed using a repeated measurements mixed model including fixed effects of DP, diet, parity, week and interactions. Values presented are LSMEANS $\pm$ SEM. NAAbs of the IgG and IgM isotype in plasma of cows did not differ among DP lengths (IgG: 8.8, 8.5, 8.6 $\pm$ 0.2 ; and IgM: 9.1, 9.3 or 9.1 $\pm$ 0.1; for cows with 0, 30 or 60 d DP). Colostrum of cows and plasma of their calves at day 2 were analysed using a linear regression model including fixed effects of type of sample (colostrum mother, plasma calf), DP, diet, parity and interactions. NAAbs in colostrum of cows and plasma of calves was equal for IgG, but lower for calves for the IgM isotype. Lower levels were present for colostrum of cows with a 0 d DP (IgG: 5.4, 8.4, or 8.5 $\pm$ 0.5; and IgM: 6.2, 8.3 or 8.0 $\pm$ 0.4; for cows with 0, 30 or 60 d DP). Also calves' NAAbs anti-PC-BSA of the IgG isotype were lower for calves from cows with a 0 d DP (IgG: 5.6, 8.4, or 8.5 $\pm$ 0.5; and IgM: 4.7, 7.3 or 7.2 $\pm$ 0.4; for 0, 30 or 60 d DP). Diet did not affect NAAbs levels, but NAAbs anti-PC-BSA in plasma of multiparous cows was higher than for primiparous cows. In conclusion, autoantibodies were present in colostrum and plasma of cows and calves with lower levels for colostrum of cows with a 0 d DP and in plasma of their calves.

Behavioral and production differences in Holstein cows with known severity of rumen acidosis transitioned from low to high-grain diets

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The main objective of this study was to evaluate behavioral and production differences in Holstein cows, with known severity of subacute rumen acidosis (SARA), when transitioned from low to high-grain diets during their second lactation. Eighteen Holstein cows, previously classified according to SARA-severity (SARA-resistant $n = 9$; SARA-susceptible $n = 9$) were fed 40% and 65 % concentrate for two and four weeks respectively, in a longitudinal experimental design. The effect of diet change and severity of SARA were evaluated based on behavior (chewing, lying, eating, feed sorting), salivary characteristics and milk production in second lactation. The results showed that cows that were susceptible to SARA in the first lactation had impaired rumination behavior ($P < 0.05$), compared to the cows resistant to SARA. But this effect was not reflected in ruminal pH variables, which did not differ between the SARA groups. Variables linked with behavior (chewing, lying, eating and feed sorting), salivary characteristics and milk production were not different between the SARA groups ($P > 0.05$). Feeding the high-grain diet increased sorting for physically effective fiber, meal frequency, total lying bouts, milk yield and salivary osmolality ($P < 0.01$). However, rumination variables, meal duration, average bout time lying on left side, salivary mucin, milk fat concentration, fat to protein ratio were decreased ($P < 0.01$). Interaction effect of the SARA-severity by diet was found for variables such as rumination chews per bolus, ruminating time per kg DMI and meal frequency, which were higher ($P < 0.05$) in SARA-resistant cows during high-grain feeding. Overall, previously identified SARA-severity in first lactating cows was reflected in lower rumination during second lactation, but not in other variables related to animal behavior or milk production, regardless of dietary concentrate level.

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Detection of Treponemes in popliteal lymph nodes of dairy cattle with and without Digital Dermatitis lesions

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To gain further understanding into the complex pathogenesis of Digital Dermatitis (DD), an infectious skin disease causing lameness in cattle, this project aimed to uncover if Treponema bacteria can invade and exist within the lymphatic system. Popliteal lymph nodes were collected at post-mortem from ten dairy cattle with and without presence of a DD lesion, and DNA was extracted from three different locations: the medulla, cortex and capsule. A 234 base-pair sequence within the Recombinase A gene of *T. phagedenis* was targeted by qPCR assay to identify treponemal DNA within the lymphoid tissue. Positive controls were generated by inoculating bovine mammary tissue with cultured treponemes. Presence of *T. phagedenis* DNA was confirmed in at least one biopsy location from all lymph nodes, and all medullary biopsies were positive. Eleven of these lymph nodes were fixed in formalin and examined using Immunohistochemistry (IHC) to detect DD associated treponemal antigen using a rabbit polyclonal antibody. One lymph node showed an activated cortex and visible germinal centres on histology and IHC results indicated a large amount of strong and specific labelling in a subscapular sinus and in the medullary sinus. Interestingly, this was a cow that was recorded as not being currently symptomatic for DD. The significance of this novel finding for disease aetiopathogenesis warrants further investigation.

Uses of DNA methylation data to understand variation: from studies in humans to applications in livestock
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Livestock farming has seen substantial advances in the last decades, largely due to genetic selection improving production of species like cattle, pigs, chickens, and salmon. Genomic technologies have sped up genetic gains, but genetics alone does not explain all variability in livestock traits. DNA methylation, an epigenetic marker influenced by genetics and environment, can explain additional trait variability and enhance breeding programs, especially for difficult-to-measure traits like infectious diseases. DNA methylation is affected by genetic makeup and environmental factors such as nutrition, and regulates gene expression, offering insight into trait variability. Assessing DNA methylation's role in health, production, and adaptation traits in livestock populations is crucial for breeding improvements, but requires large-scale studies to ensure robust statistical evidence. Initial research in livestock shows DNA methylation differences related to production, size, fertility, and disease resistance. There are also documented differences linked to environmental exposures. Although current evidence in livestock mainly derives from small studies, larger human studies using affordable high-throughput DNA methylation arrays have uncovered environmental exposure signatures. These findings indicate DNA methylation's potential in measuring environmental impacts and gene-environment interactions in livestock breeding, improving selection and management. Currently, cost-effective, array-based, methods for studying DNA methylation at scale in livestock are still few (an example is the RUMIGEN Epichip). In this talk, I will describe work in humans using the Illumina Infinium EPIC BeadChip that illustrates the potential uses of DNA methylation arrays in livestock, to understand biology of key traits and enhance breeding programmes and management practices.

The RUMIGEN Epichip: a versatile, medium density DNA methylation BeadChip for large population studies in cattle

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Responding to the objective of assessing the potential of epigenetics in cattle breeding within the European RUMIGEN project, a DNA methylation BeadChip, called "RUMIGEN Epichip," has been developed. It targets 44K CpGs at an affordable cost, enabling the analysis of thousands of individuals, which will generate robust conclusions about the impact of the methylome on phenotypic variations in cattle. This is the first DNA methylation BeadChip specifically dedicated to the bovine species. We therefore propose to describe the strategy used for the design, as well as some results on the technical validation. The selection of informative CpGs was achieved by combining two different and complementary strategies: (i) selecting CpGs showing variations in relation to fertility, age, breed, nutrition, physiological and inflammatory status, somatic cell count in milk, heat stress and metabolic stress, mainly in blood and sperm; (ii) targeting CpGs linked to relevant functional elements of the genome. The Epichip has been manufactured by Illumina. The technical validation demonstrates a good technical repeatability (Pearson $R^2 > 0.99$); a good correlation with gold standard methods (Pearson $R^2 > 0.9$) and a good accuracy (Pearson $R^2 > 0.98$); which demonstrates a reliable DNA methylation estimation by the Epichip. Funding: H2020 101000226 RUMIGEN, APIS-GENE POLYPHEME AP-2024-004

Harnessing RUMIGEN EpiChip data to predict dairy cow performance

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The DNA methylation BeadChip (EpiChip), developed in the H2020 RUMIGEN project, offers a powerful approach to understanding the epigenetic factors influencing dairy cow performance. This study used the RUMIGEN EpiChip on blood samples from 3,087 Holstein cows to evaluate the extent to which epigenetic biomarkers may help predict cow phenotypes. Key performance indicators such as milk production, fertility, and udder health were analyzed using a mixed model that included the random effects of genetic and/or epigenetic values. Genetic covariance structure was computed from SNP genotypes obtained from the EuroGMD chip, while epigenome covariance structure was derived from methylation similarity between individuals estimated from EpiChip data, after adjusting data from leucocyte composition. Three distinct models were applied to estimate genetic and/or epigenetic variances, all including environmental effects along with i) a genetic component, ii) an epigenetic component, or iii) both genetic and epigenetic components. The population of cows was divided into a training and validation set to estimate prediction accuracy across the three models. Results presented in detail include the proportion of epigenetic variance relative to the total variance for all traits considered in this study. Specifically, we compare the performance of the three models in terms of accuracy and bias in predicting cow performance. These findings pave the way for EpiChip data to improve predictive capacity beyond conventional genetic and environmental factors. This provides a more comprehensive framework for animal performance management, supporting more informed breeding and management decisions to advance efficiency and sustainability in the dairy industry. This project has received funding from the European Union's Horizon 2020 Programme for Research & Innovation under grant agreement n°101000226 (RUMIGEN) and from APIS-GENE (PolyPHEME).

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Theatre 4

Applying the RUMIGEN Methylation Array to Identify Epigenetic Markers Associated to fetal development during maternal lactation in Cattle

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This work was supported by European Union's Horizon 2020 Research and Innovation Program, 101000226. Genotyping arrays have become an essential tool for genetic evaluations. Positions of interest are selected to estimate individuals' genetic merit and identify the best parents to enhance the rate of genetic gain. Alongside improvements in phenotype recording and computation, the genetic rate of gain has doubled over the past decade. However, genomic information and genetic biomarkers such as SNPs explain only part of the phenotypic variance and the heritability of economically relevant traits in livestock. In this context, epigenetic biomarkers, particularly DNA methylation marks, are being explored for their potential role in livestock breeding programmes. These marks provide insights into the heritability of traits and the environmental influences on gene expression, ultimately improving selection strategies. To assess the impact of methylation marks, we epi-genotyped 470 samples from new-born calves from lactating or non-lactating cows using the H2020 RUMIGEN methylation array designed within the RUMIGEN consortium and manufactured by Illumina Inc., which includes approximately 44,000 CpGs, of which 37,337 passed quality control filters. We combined this epigenetic data with genomic information from a 60k SNP chip to identify CpGs associated with maternal lactation status and estimate the genetic control of DNA methylation at different genomic regions using the Gibbsf90+ software. We identified 155 CpGs significantly associated with maternal lactation status (Bonferroni-adjusted p-value < 0.05). These CpGs were linked to genes such as RCAN3 and NOBOX, which are involved in nervous system development, and pre-weaning survival; ALDH1A3, associated with growth traits; WNT7A, which plays a role in embryo development; and several HOX family genes, which are key regulators of embryonic development and body plan organisation. The heritability of DNA methylation at these CpGs ranged from 0.30 to 0.95. Given the nature of DNA methylation marks, this tool could enhance our knowledge of the epigenetic importance on dairy cattle production and may serve as a tool to personalized breeding strategies.

Epigenetic Signatures of Prenatal Heat Stress: Persistent DNA Methylation Changes in Blood of Dairy Calves A. Boucher¹, H. Martin¹, M. Oudihat¹, J. Laporta², M. A. Sirard¹, V. Ouellet¹

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Suboptimal intrauterine environments, such as prenatal heat stress, can have lasting postnatal effects on production and longevity, some of which may not be reversible. DNA methylation changes have been associated with these outcomes, but it remains unclear whether these epigenetic marks persist over time or how they evolve postnatally. Blood cells offer an opportunity to address this question, providing a minimally invasive tool to study the long-term effects of prenatal stress, gain insights into DNA programming, and identify potential early-life biomarkers. This epigenome-wide study examined DNA methylation in blood cells at birth (D0) and weaning (D63) in Holstein calves whose dams were exposed to either cooling (n=5 at D0, n=8 at D63) or heat stress (n=3 at D0, n=8 at D63) during late gestation (~54 days pre-calving). Whole-Genome Enzymatic Methyl Sequencing identified Differentially Methylated CpGs (DMCs) between treatments at D0 and D63 (q-value<0.05; methylation difference>|25%|). At D0, principal component analysis of genes expressed in immune cells revealed 21,124 DMCs that separated calves based on prenatal treatment, with 250 DMCs located in promoter regions. By D63, clustering based on all 22,727 DMCs (including 229 in promoters) no longer clustered prenatal treatments. However, using the top 400 most significant DMCs restored this separation. Pathway analysis identified enrichment in genes related to immune function (TLR4, TXK, CYLD), growth and development (CDH20, PHF3, BTG1, VPS37B), metabolism (IGFBP7, ITPR2, RPLP1, DUSP6), and apoptosis (BCL2), all of which were consistently differentially methylated at both D0 and D63 and detected across all genomic regions. These findings suggest that prenatal heat stress induces lasting epigenetic reprogramming across multiple interconnected biological pathways, reinforcing its broad systemic impact. The persistence of these epigenetic marks highlights their potential as blood-based biomarkers for detection of prenatal heat stress susceptibility within the first two months of life, supporting selection strategies to enhance resilience in dairy herds.

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Theatre 6

Adaptation of concepts and frameworks from human epigenetics to livestock epigenetics

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Biomarkers for livestock breeding and monitoring represent a considerable unmet need in veterinary medicine. Epigenetic modifications, like DNA methylation, provide important information about cellular states and environments, which establishes them as highly attractive biomarker candidates. However, methodological limitations and the lack of suitable computational frameworks have so far precluded their systematic evaluation and development. I will discuss key aspects that have shaped the analysis of human DNA methylation datasets and their adaptation to livestock epigenetics. Examples include the focus on dynamically methylated regions, the avoidance of known confounding factors and the development of composite DNA methylation biomarkers.

Towards a comprehensive bovine lncRNA Atlas: Multi-tissue transcriptomic profiling in cattle

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Long non-coding RNAs (lncRNAs) constitute a significant portion of the non-coding transcriptome and are implicated in the regulation of gene expression through post-transcriptional modification, chromatin remodelling, and interactions with regulatory elements. While their roles in governing gene expression variation and complex traits are well-documented in humans, lncRNAs in bovine genomes remain poorly characterized. To address this, we are generating an extensive catalogue of bovine lncRNAs by analyzing 12,506 publicly available RNA-seq data of *Bos taurus* spanning multiple tissues and both sexes. We retained only paired-end data with a minimum read length of 75 bp and at least 10 million reads per run to ensure data quality. Using a batch-wise computational workflow for efficient and reproducible lncRNA identification and annotation, we perform quality control with FastQC, trim adapters and low-quality sequences using Fastp, and align reads to the ARS-UCD2.0 bovine reference genome using STAR. We assemble and quantify transcripts with StringTie and Kallisto, then filter transcripts by removing those shorter than 200 bp, overlapping protein-coding genes, or classified as rRNA, tRNA, snoRNA, or snRNA. We assess coding potential using PLEK, CPC2, and FEELnc, integrating multiple prediction tools to compute a combined confidence score. We cross-reference the final lncRNA set with existing lncRNA databases, including LncBook, and NONCODE. Furthermore, the final integrated lncRNA atlas, merged with existing annotations, will be used to quantify expression per sample, enabling the identification of tissue-specific and sex-based expression patterns. Our large-scale analysis generates a high-confidence catalogue of bovine lncRNAs, providing novel insights into their regulatory roles in *Bos taurus*. We will present the initial findings from the Bovine lncRNA Atlas, including expression dynamics and functional characterization. This study establishes a foundational resource for bovine functional genomics and enhances our understanding of the role of lncRNA in mammalian biology.

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Theatre 8

Bovine sperm sncRNAs vary according to bull fertility

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Semen fertility remains a key concern in the breeding sector. As conventional sperm quality assessments fail to accurately predict fertility, sperm small non coding RNAs (sncRNAs) content is emerging as a promising approach for fertility issues. Ejaculates from 100 Montbéliarde bulls (43 subfertiles vs 57 fertiles according to their adjusted non-return rate at 56 days) were analysed to identify potential sperm sncRNAs fertility biomarkers. These ejaculates were collected between 17 and 19 months of age from bulls born from 2011 to 2015. Extracted RNAs have been used for UMI sequencing (Illumina HiSeq2000, 20 million reads, 75 bases single-end). MiRDeep2 was used for annotation and quantification, while DeSeq2 identified differentially expressed sncRNAs between fertility groups. Expression differences were considered significant at Benjamini-Hochberg adjusted p-value <0.05 and differential at <0.1. TargetScan was used to predict the genes potentially targeted by the identified microRNAs (miRNAs). Among the 413 196 analysed sncRNAs, 94% are expressed under 10 copies per sample, nearly 5% between 5 to 100 copies, and less than 1% above 100 copies. The twenty most expressed sncRNAs, mainly miRNAs and transfer RNA fragments (tsRNAs), represent 33% of total reads expression. A total of 552 differential sncRNAs was considered by DeSeq2, with 151 showing a significant differential expression. Interestingly, 49.6% of these sequences were acquired in the epididymis cauda. Several differentially expressed miRNAs, such as miR-6526 or miR-30d, have already been associated with sperm motility or abnormalities. Enriched biological pathways among predicted target genes include anatomical structure morphogenesis, system development and regulation of transcription. Some of these genes, such as STAT3 or SMAD1, play established roles in embryo development. These interesting findings indicate that specific sperm sncRNAs are associated with fertility status. Further studies are necessary to confirm their potential as biomarkers for bull fertility.

Breed-specific small RNA profiles in pig sperm

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In selective breeding, economically beneficial traits are selected to shape the next generation of livestock. Beyond genetic selection, paternal epigenetic inheritance includes factors such as small non-coding RNA (sRNA, <200 nucleotides), which are involved in regulating gene expression and safeguard genomic integrity by silencing transposable elements (TEs), also known as jumping genes. However, some insertions of TEs within genes with economically important traits have shown to be beneficial in livestock production. Environmental factors such as diet and stress alter the composition of sRNA in sperm, which in turn leads to an altered phenotype in the offspring. With this background, our aim was to investigate differences in sperm sRNA profiles between sire (s) and dam (d) lines, which are selectively bred for production and reproduction purposes, respectively. We extracted and sequenced sRNA from 24 commercially acquired sperm samples (six individuals per breed) from four breeds; Duroc (s), Hampshire (s), Landrace (d), Yorkshire (d). Data analysis was mainly performed by an in-house developed R package for comprehensive sRNA analysis. We found that mitochondrial sRNA drives the differences between sire and dam lines, followed closely by miRNA. tRNA-derived sRNA (tsRNA) accounted for the largest sire-to-dam variance. The second largest mitochondrial sRNA driver was rRNA-derived sRNA (rsRNA), which was generally more abundant in sire lines than in dam lines. Both tsRNA and rsRNA were also the most differentially expressed in sire-to-dam comparisons, followed by piRNA, which primarily function in TE silencing. Differentially expressed piRNAs from sire and dam lines primarily originate from LINE and SINE elements, respectively. Additionally, piRNA drives a distinct separation among dam line breeds, a pattern not observed in sire line breeds. These findings highlight distinct sRNA profiles between sire and dam lines, with potential implications for selective breeding strategies and the role of environmental exposure in livestock epigenetics.

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Using the sperm methylome to predict Holstein bull fertility : creation and comparaison of machine learning-based models

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Bull fertility is a key parameter in the dairy cattle industry, with significant economic implications. Previous attempts, primarily focusing on genetics and spermatogenic parameters, have shown limited predictive power. Meanwhile, DNA methylation has been related to male fertility in various species, and has emerged as a potential source of biomarkers for male fertility. The RUMIGEN Epichip, a DNA methylation array covering 48,351 CpG sites could be used for large-scale and routine methylation measurements in cattle. We leveraged methylation data obtained with Reduced Representation Bisulfite Sequencing (RRBS), targeting CpGs found on the Epichip, to evaluate the potential of DNA methylation for predicting bull fertility. Our study was conducted on a cohort of 351 Holstein individuals, classified into subfertile and fertile based on a fertility index (NRR56). Using semen RRBS data, we developed classification models to discriminate Subfertile and Fertile individuals. Four machine learning algorithms were tested: Least Absolute Shrinkage and Selection Operator (LASSO), Random Forest, Gradient Boosting and Neural Networks. To optimize performances of each algorithm, we used a three-fold cross validation, repeated five times, before final testing on an independent test set. We managed to reach a moderate predictive accuracy of 64% on this independent set, with an Area Under Curve (AUC) of 69%. These results suggest that sperm DNA methylation holds potential as a relevant source of marker in bull fertility, even though current predictive accuracy remains insufficient for field applications. We provide insights for potential future improvements, highlighting key methodological aspects that could enhance predictive performance. Funding: POLYPHEME ANR-21-CE20-0021 and APIS-GENE, ABIES (Paris-Saclay University)

DNA Methylation Dynamics during Goat Spermatogenesis

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The epigenetic regulation of spermatogenesis has not been explored in goats and remains poorly understood in other species. This study provides important insights into the epigenomic landscape of spermatogenesis in goats. Different populations of spermatogenic cells were sorted from the testes of three male goats using Fluorescence-Activated Cell Sorting (FACS). We obtained near-pure populations of primary spermatocytes and spermatids. In addition, spermatozoa were collected in the cauda epididymis. Each population of cells was then used to prepare Reduced Representation Bisulfite Sequencing (RRBS) libraries, which were subsequently sequenced. Using a custom-designed bioinformatics pipeline, we analyzed DNA methylation differences between primary spermatocytes, spermatids, and spermatozoa across 2.08 million CpG sites. Differentially methylated cytosines (DMCs) were identified based on a minimum of 20% methylation difference between two spermatogenesis stages and a q-value threshold of <0.01. We detected 765 DMCs between primary spermatocytes and spermatids, 1057 DMCs between spermatids and spermatozoa, and 7417 DMCs between primary spermatocytes and spermatozoa. Genome feature analysis revealed methylation changes in open sea and CpG islands, with Type I transposons and tandem repeats enriched. Intergenic and intron regions were most affected by methylation. Pathway analysis revealed key roles of GTPase regulation in spermatogenesis. Comparisons between spermatozoa and spermatids showed enrichments in cytoskeleton components. This study offers insights into epigenomic regulation in goat spermatogenesis and highlights potential vulnerability to environmental factors affecting reproductive health. Funding: APIS-GENE GERMIMALE and BullNet (Horizon MSCA Doctoral Network 101120104)

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Theatre 1

Effects of pesticide residues in substrates on reared insects

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Reared insects, such as black soldier fly larvae (BSFL, *Hermetia illucens* (L.); Diptera: Stratiomyidae) and yellow mealworm (YMW, *Tenebrio molitor* (L.); Coleoptera: Tenebrionidae), can contribute to a more sustainable food and feed system. However, their rearing substrates may contain pesticide residues from agricultural use. Legal limits have been established for pesticides in food and feed products in the European Union (EU), but these do not account for their effects on reared insects. The potential issues related to the presence of pesticide residues in insect-rearing substrates are threefold. Firstly, the presence of pesticide residues, even at or below legal limits, may adversely affect the insect survival and yield. These effects have been observed to be compounded by the presence of synergists as well as other insecticidal substances in compound feed ('cocktails'). This means that even lower concentrations of active substances, if jointly present, could have negative effects leading to economic implications for the insect farmers, as well as welfare implications for the reared insects. Secondly, pesticidal substances may be transferred to or even accumulated in the larval biomass, thereby raising a food/feed safety or compliance concerns. Existing safety limits for insects as a food or feed product are set at very low levels compared to the limits for feed commodities such as wheat, meaning that even limited transfer from substrate to reared insects could result in non-compliance. Thirdly, dietary exposure to pesticide residues may cause changes in the insects' macro- and micro-nutrient composition, affecting the nutritional quality of the insect products. Limited research on these three issues is available, making it difficult for stakeholders to make informed decisions for their rearing substrates (i.e., accept / reject). Most recent experimental findings in this area of research will be presented.

Insect rearing on livestock residues: How to combine sustainability and safety?

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The EU Green Deal aims for climate neutrality by 2050, requiring a shift to sustainable agriculture. One crucial aspect is reducing the use of human-edible crops, such as cereals and soy, for animal feed and shifting towards sustainable alternatives. For about a decade, insects have been promoted as an alternative protein source, converting waste into high-value products while lowering reliance on imports. However, financial challenges threaten the sector's future. Without proper support, this promising alternative may fail before reaching its full potential. A possible solution is using locally sourced, low-value feedstocks, such as agricultural by-products or food waste, but EU regulations prohibit their use in insect farming. Previous research revealed residue persistence from rearing substrates in insect meal, raising concerns: Can insects be reared on these materials without leaving dietary residues in the final product? Given the cost advantages of such substrates and regulatory differences, do more flexible regulations outside Europe increase the risk of fraud in imported products? To address these issues, a collaborative research project between Canada and Belgium was launched to assess if current analytical methods can ensure compliance. Black soldier fly larvae were reared on fermented diets containing category 2 and 3 animal by-products. Diets were optimized for nutrient content and texture, while growth performance (weight, length, mortality) and bioconversion (feed intake, waste reduction) were monitored. At ~15 days, larvae were sieved, rinsed, oven-dried, and ground. Additional processing methods (including fasting, boiling, and microwave drying) were tested for their effect on contamination. Insect meals were analyzed using official EU methods (light microscopy, PCR) and complementary techniques (MS-proteomics, NIRM) for animal residues detection. This research will provide analytical tools for quality monitoring and assess whether cleaning methods can eliminate substrate residues, potentially enabling the production of safe and sustainable alternative proteins for livestock feed.

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Theatre 3

Research as a foundation for legislative decision-making: utilizing canteen waste as a substrate for black soldier fly

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Canteen waste (CW) is not authorized as a substrate for insects, but research could inform future legislative decisions. CW may pose challenges for larvae production, including safety risks and variability in insect products due to waste inconsistencies. A protocol for collection, processing and supplementing CW with a core diet is needed for nutritional stability. The study evaluated processed CW treated with vinegar (until pH<4.2), combined with a core diet (Gainesville diet, GA) at inclusion levels of 0%, 10%, 20%, 40%, 60%, 80%, and 100% (GA, W10, W20, W40, W60, W80, and W100, respectively). A total of 12500 black soldier fly larvae (5-day-old) were inoculated in each replicate (0.8 g of feed/larva; 4 replicates/treatment). Growth was monitored every 2 days until the weight difference between two samples dropped below 50%. At the trial end, survival rate, growth, and bioconversion indices (BER, RR, WRI) were calculated. Larvae were sampled for chemical composition analysis (dry matter [DM], crude protein [CP], ether extract [EE]). Protein rate (PR; grams of protein obtained/rearing box) was calculated based on growth performance and chemical composition. Data were analysed by One-Way ANOVA ($p<0.05$). Results showed that W100 larvae experienced high mortality and were unable to complete the trial. The W40 group had the highest larval weight and BER ($p<0.001$). GA had the highest WRI ($p<0.001$), indicating faster growth, while W60 and W80 had the highest RR ($p<0.001$) due to high substrates moisture content. DM content increased with higher waste inclusion ($p<0.001$). Compared to inclusion levels above 10% waste, GA larvae had higher CP and lower EE content ($p<0.001$). PR was higher in W40 than GA, W20, W60, and W80 ($p<0.001$). In short, W40 appears the most effective, but further studies are needed to assess larval composition with different CW batches (Supported by the project HiTech (MUR 20229T94FL) funded by the EU – NextGenerationEU, NRRP Missione 4 “Istruzione e Ricerca” – Componente C2, Investimento 1.1, “Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN).

Comprehensive screening of different mixtures of agricultural by-products for black soldier fly larval performance, body composition and microbial landscapes across production scales

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Valorisation of agricultural by-products via black soldier fly larvae (BSFL) processing into high value insect products has potential, provided that ecological and economic efficiency can be demonstrated, and that feed safety can be ensured. This study investigated the effects of distinct mixtures of common by-products on BSFL performance, survival, conversion efficiency and body composition, including amino acid profiles. Further, complementary comprehensive genetic profiling of temporal microbiota dynamics associated with different matrices, i.e. feed, larvae and frass, were performed based on 16S sequencing, as well as plate counts of possibly critical pathogens according to standardised microbiological procedures. A basic laboratory scale trial assessed seven dietary treatments in four replicates where 1'200 inoculated neonates were fattened until first prepupae appeared. In a separate setup, the influence of production upscaling on relevant larval performance traits and microbial communities relative to diet was evaluated. In this trial, three highly similar experimental diets as in the laboratory experiment were subject to large-scale BSFL rearing at quasi-industrial production scale (3 replicates starting with 75'000 BSFL each). The results provide interesting insights into how dietary input and particularly production parameters influence growth efficiency as well as microbial species richness and community composition, and ultimately product safety. We conclude that key production variables need to be extrapolated across scales with caution due to complex interactions.

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Theatre 5

Authentication of insect-derived products by real-time PCR and High-Throughput Sequencing

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Eight insect species are currently authorized by regulation in the European Union as feed for aquaculture, pigs, and poultry. Authenticating insect species is essential to protect producers and users. At the end of the FARMY-NG project, methods were developed using real-time PCR to detect the six most commonly reared insect species: *Tenebrio molitor*, *Hermetia illucens*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllus assimilis*, and *Bombyx mori*. The methods were evaluated against performance criteria recommended in international guidelines. However, real-time PCR only allows detection of the targeted species. For a more complete and untargeted approach, we used the metabarcoding approach, which relies on high-throughput sequencing. We compared 34 PCR targets (also known as barcodes) found in scientific literature or developed at CRA-W. The barcodes were tested on mock communities composed of 10 insect species, including the 8 authorized in feed and 2 species commonly found as contaminants of this kind of product. Three types of DNA were used: DNA extracted from whole insects, DNA extracted from the legs of insects, and artificial DNA. Significant differences were observed in the results obtained with the different barcodes. This highlights the importance of taking precautions when interpreting results in studies that rely on a single barcode. In our approach, we selected a set of appropriate barcodes and created databases for the targeted genomic regions. We also developed the TDmerger (Taxonomic Data merger) bioinformatics pipeline, a script to study sample composition with several PCR targets and generate consensus taxonomy. Using real-time PCR and sequencing, it was also observed that some commercial samples from outside the EU were not correctly labeled.

Authentication of insect-based products : use of Near Infrared Microscopy to highlight inconsistent labelling
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With the authorisation of insects in feed and the need to find alternative and more environmentally friendly sources of protein, new insect-based products have emerged on the feed market. These new products, with their advantageous nutritional values, represent a major economic challenge. However, insect-based products are subject to very few quality controls, leaving the door open to potential fraudsters. One of the most common types of fraud is mislabelling. In the feed sector, and more specifically for animal proteins, feed authentication is generally carried out using light microscopy and Real Time PCR. Both techniques require the use of harmful products, as well as sample preparation, which can be time-consuming. In this context, Near Infrared Microscopy (NIRM) was used to authenticate the contents of several commercially available feeds described as containing one or more insect species. These same feeds were also analysed by Real Time PCR to best assess the performance of NIRM. By combining the results obtained using NIRM with chemometric analyses, it was possible to detect the presence of different insects and authenticate the species in the insect-based products. These results were broadly consistent with those obtained using PCR. The different results obtained were compared with the composition labels of the products analysed and revealed labelling errors, with insect species sometimes differing from those indicated, or even a total absence of certain species despite being notified. Besides highlighting the ability of the Near Infrared Microscopy technique to detect and authenticate insect species in feeds, these analyses emphasise the importance of better control of insect-based products to avoid possible fraud.

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Theatre 7

Rice by-products as rearing substrates for black soldier fly

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Rice processing generates several by-products that can be valorized by *Hermetia illucens* (HI) larvae. Therefore, this study aimed to evaluate the effects of rice by-products (RBP)-based diets on growth performance, bioconversion efficiency, nutritional profile and microbiological traits of HI larvae. Four isonitrogenous, isolipidic and isoenergetic diets were tested (5 replicates/diet, 14286 6-days-old larvae/replicate, 0.7g/larva): C (control diet, containing different agri-food by-products), CR1 (11.5% white RBP), CR2 (13.5% combined white and par-boiled RBP) and CR3 (15% parboiled RBP). Larval weight was recorded at the beginning of trial and every two days, and survival rate, growth rate (GR), specific growth rate (SGR), bioconversion efficiency corrected for residue (BER), reduction rate (RR) and waste reduction index (WRI) were calculated at the end of larval growth (growth %≤50%). Larvae were also submitted to proximate composition and microbiological analyses. Data were analysed using SPSS software ($P \leq 0.05$). The CR1 showed the best average larval weight, GR, BER, RR and WRI among diets ($P < 0.001$). In contrast, C displayed the lowest larval weight, GR and WRI among treatments ($P < 0.001$), but similar BER and RR when compared to CR2 and CR3 ($P > 0.05$). The CR1-fed larvae showed the lowest crude protein content, but the highest protein yield among the diets ($P < 0.001$). Furthermore, their dry matter, ether extract and gross energy, and ash contents were higher and lower, respectively, when compared to C-fed larvae ($P < 0.05$). *Listeria* spp., *Salmonella* spp. and *Campylobacter* spp. were not detected for all the diets, while total bacterial count (TBC) and enterococci were lower for CR1 than C and CR2 (TBC, $P < 0.001$) and other diets (enterococci, $P < 0.001$). In conclusion, the use of 11.5% white rice by-products led to the best outcomes in terms of larval performance, nutritional profile and microbiological safety. Research supported by Fondazione Cariplo (newRIFF project).

Assessment of the interest price of three products from black soldier fly larvae used to feed fast-growing broilers

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Black soldier fly (BSF) larvae are a promising protein source for poultry, but their high cost still greatly limits their use. The aim of this study was to evaluate the interest price of three products derived from BSF larvae. In a previous study, four feeding strategies were tested in fast-growing broilers (D0-D35): a control without BSF (C), the use of 10% defatted BSF larvae meal (LM) in feed, or the use of live (LL) or dried (DL) larvae, provided to represent 15% of daily dry matter intake in addition to an ad libitum access to a deconcentrated feed (-150 kcal and -0.8 to -1.0 g digestible lysine per kg, compared to C). Compared to C strategy, no effect of LM was observed. In contrast, final body weight (BW) and breast meat (BM) yield were reduced with DL (-9.6% and -2.7% points), while BM yield was increased by 1.2% points with LL. Using individual animal performance, the average feeding costs (avFC) per bird, kg BW and kg BM were calculated in six different feedstuffs price contexts, for a price of BSF product (PriceBSF) varying between 200 and 2500 €/t. A linear model avFC vs. PriceBSF was then fitted for each BSF product and unit (i.e., bird, kg BW, or kg BM). Finally, for each economic context, the average interest price of each was estimated as the PriceBSF for which avFC was equal to avFC in the C strategy. For avFC expressed in €/kg BW, interest price ranges were 639-1125, 216-414, and 600-1150 €/t for LM, LL, and DL, respectively. For avFC expressed in €/kg BM, due to the large LL amount provided to birds (1.4 kg/bird) and the higher BM yield, LL had a very low interest price compared to LM (206-397 vs. 876-1516 €/t). On the opposite, despite lower amounts used (0.44 vs. 1.4 kg/bird), the decrease in BW and BM yield led to a higher interest price of DL compared to LL (310-672 vs. 206-397 €/t). These results confirm that the cost of BSF is still too high to compete with soybean meal. Substantial reduction in production costs is thus required to increase the use of BSF in poultry. Moreover, the economic “value” of other benefits, such as animal welfare, should also be evaluated.

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Theatre 9

StayPrime: Revolutionizing Black Soldier Fly Larvae (BSFL) Juvenile Logistics for Decentralized Production

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The efficient and cost-effective shipment of Black Soldier Fly Larvae (BSFL) juveniles has been a significant bottleneck in decentralized BSFL production, particularly for operations like FarmInsect farms. Challenges associated with high mortality during transport, short shelf life, and the need for costly express shipping with controlled climate conditions have hindered scalability. This presentation introduces StayPrime, a technology designed to address these challenges by enabling the storage and shipment of highly viable juveniles under regular conditions, significantly reducing costs and mortality while extending shelf life. We present data from 19 production-scale trials conducted between 13.09.2024 and 02.12.2024, where a total of 6.3 tonnes of BSFL biomass was produced using StayPrime-arrested juveniles. Approximately 80,000 juveniles (1-2mg) were individually packaged with StayPrime and shipped to FarmInsect fattening sites for storage up to 7 days. These juveniles, along with a control group (non-arrested), were then placed in individual production crates and reared for 7 days under FarmInsect farm conditions. Results show that StayPrime-treated juveniles exhibited high survival (92-94%), average larval weight (128-136 mg/larvae) and Feed Conversion Ratios (FCR, 1.35-1.39 DM/FW), comparable to the control. Crucially, StayPrime dramatically reduced shipping costs by 50%, by eliminating the need for express shipping and controlled climate conditions. The technology allows bulk shipments under standard conditions and on-site storage at FarmInsect farms, streamlining production schedules. StayPrime is now the primary method for juvenile shipment and storage across FarmInsect farms, contributing to the successful production of 26 tonnes of BSFL biomass and scaling rapidly. Ongoing optimization efforts focus on extending the arrestment period and further reducing shipping costs, making juvenile delivery more accessible and efficient than ever before for FarmInsect's decentralized production network. This presentation will showcase the practical application and significant impact of StayPrime in revolutionizing BSFL juvenile logistics for sustainable and scalable production at FarmInsect.

Restoring Ruminant Herds After Disaster Events

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Small ruminants are a vital part of global food security, especially in rural and marginalized areas. Their role extends beyond just being a source of food; they provide economic stability, environmental benefits, and cultural significance. They are adaptable animals that support agricultural diversity and can be crucial to sustainable development efforts in many parts of the world. Natural disasters and pandemics, especially those that affect sheep and goats can have devastating impacts on food security, livelihoods, and social stability. Restoring small animal populations following these events will take planning, coordination, resources, and an understanding of local production systems and cultures. Consideration must be given to several of the principles of disaster response. Response must be timely, appropriate, culturally and socially acceptable, economically feasible, equitable, and sustainable. Examples of appropriate response would be restoring dairy goats with locally utilized and familiar breeds rather introducing meat goats into the population. Reintroduced animals should be adapted to climate and disease resistant. Timeliness is important in that animals can be reintroduced too soon to survive the current conditions such as in severe drought or too late to provide the food security and economic benefit for recovery. In this presentation we will provide numerous examples of both appropriate and inappropriate responses. Finally, it is important to consider the prevention aspect of small ruminant health. One key project to promote this is the UN Food and Agriculture (FAO) and World Organisation for Animal Health (WOAH) Global Control and Eradication Strategy of Peste des Petites Ruminants (PPR) with a goal of eradicating the disease by 2030.

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Theatre 2

Navigating Epidemics Amidst Climate and Livestock Restoration Challenges: Lessons from the recent PPR and Capripox Outbreaks in Greece

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Over the past few years, the impact of extreme climatic events and transboundary animal disease outbreaks resulted in important losses for small ruminant populations and production systems. Greece recently dealt with two epidemics of great importance—Peste des Petits Ruminants (PPR) and Sheep and Goat pox, that led to massive animal losses but also seriously impacted production systems which were already in a fragile equilibrium due to natural disasters, like floods. This presentation traces the history and key factors shaping the emergence and spread of the PPR/Capripox viruses in Greece in 2024-2025. It also assesses the regional initiatives and controls implemented to limit the spread e.g. active sampling/clinical surveillance campaigns, and movement restrictions/biosecurity measures. Additionally, the challenge of restoring livestock after crises like natural disasters and disease outbreaks is discussed. The importance of synergistic tactics including disease prevention programs, climate adaptive farm practices, and farmer support when rebuilding small ruminant herds is demonstrated. Institutional roles, policy frameworks and stakeholder cooperation are considered as pivotal elements in restoration efforts. With the expected rise in frequency of these negative events like climatic disasters and disease outbreaks, this recent Greek paradigm will provide useful information and insight to be considered from other countries and their experts when dealing with similar situations. During this presentation experiences will be shared fostering new discussions on how to preserve small ruminant systems against future challenges as well as maintain rural livelihoods sustainable.

Balancing biosafety and free-range farming: Contribution of an action research on biosafety for pig farming in Corsica

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Biosafety refers to measures designed to minimize the risk of pathogen introduction into farms by preventing contact with wild or domestic compartments. While primarily intended for conventional systems, biosafety can lead to the standardization of farming practices, which threatens outdoor systems despite their environmental and animal welfare benefits. In 2018, after the outbreak of African Swine Fever in Belgium, the French agricultural ministry elaborated a prevention plan, focusing on conventional and outdoor pig farms, requiring measures like farm zoning, record-keeping and animal confinement. In Corsica, where pig farming relies on extensive systems, these biosafety measures were difficult to implement due to grazing on large open pastures. We carried on an action-research to support a technical committee, mandated by farmers, to develop a health management plan tailored to this context. The goal was to find a compromise between biosafety and the preservation of local pastoral practices, to be validated by farmers representatives and negotiated with public authorities. The compromise focused on three areas. First, the grazing area was recognized as essential. Traditional Corsican farming system base rearing pigs on natural resources like acorns, mandatory for protecting the origin of delicacies. However, maintaining grazing access requires accepting certain risks, mainly during autumn. To limit disease transmission, breeding animals must be confined with double fencing, and only non-reproductive pigs can graze. Fattening pigs are neutered, and leading sows must be pregnant before grazing. The second part of the compromise involved establishing a quarantine zone in case of an outbreak. Finally, the third aspect focused on developing monitoring tools to help farmers manage their systems including a biosafety grid, a farm audit, and a customized timeline for transition. This approach integrated farming system knowledge with biosafety challenges, providing a practical solution avoiding conflicts between farmers and institutions. It highlights how systemic and participatory approach can adapt farming systems to biosafety constraints respecting the core of the production model.

Improving Food Security and Pandemic Preparedness in livestock

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Food security and pandemic preparedness are global challenges that are intertwined. Any disruption in livestock systems as a result of zoonoses or natural disasters and other hazards has a direct effect on food supply and thus local and global economies. In this presentation we will summarize findings from a recent seminar at the Veterinary Health Innovation Engine (vHive; <https://vhive.buzz>). Themes include government action to protect food security, preparedness for disease outbreaks from a governmental and industry perspective, and risk assessment in international disease monitoring. Our objective is to foster discussion thereby to improve policy on pandemic preparedness in livestock by engaging with livestock managers, researchers, public agencies and industry.

An Entrepreneurial Ecosystem Approach for Climate-Smart Small Ruminant Production in Pastoral Communities

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Improving the productivity and profitability of sheep and goats is critical for pastoral communities whose livelihoods depend on livestock. However, many communities prioritize herd growth and milk production over sales, limiting the number of animals sold. Ensuring the few animals pastoralists are willing to sell are of the highest quality is essential to maximize income. Improvement is hindered by uncontrolled breeding, limited fodder and lack of fattening expertise and grading systems. Market-based services offer a promising path to commercialization but gaps in financial literacy, business experience, and operational capacity threaten sustainable engagements. We propose an approach based on the principles of an entrepreneurial ecosystem—a network of actors and factors enabling productive entrepreneurship. This model fosters trust, collaboration, and value creation across interconnected actors, enabling sustained value chain engagement. Entrepreneurs are active participants in shaping and sustaining the ecosystem. Our strategy emphasizes identifying community-level innovations and integrating them where feasible with productivity-enhancing and environmentally sustainable technologies. These can be scaled to offer practical tools for managing livestock, water, and pasture. We focus on three critical value chain nodes: producers, input/service providers, and market agents. Producers are supported to engage in organized community groups to improve livestock productivity, access technical and business training in animal husbandry, feed resources and health management, and improved market linkages. Input and service providers receive training to deliver integrated solutions tailored to producers' needs. Market agents benefit from branding, marketing, and use of technologies to ensure quality and safe livestock product delivery. Within this framework, service providers and market agents help de-risk producers, transforming them into viable business entities. This approach strengthens financial literacy, operational capacity, and inclusivity, promoting sustainability and resilience in pastoral systems.

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Community-Based Breeding Programs Enhance Farmer Adaptation to Climate Change in Ethiopia

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Climate change poses significant challenges to agricultural systems, particularly in livestock-dependent communities. This study examines farmer perceptions of climate change, its impacts and adaptation strategies in two sheep Community-Based Breeding Program (CBBP) sites in Ethiopia: Bonga and Menz. Using a mixed-methods approach, meteorological data from 1993–2023 were analyzed alongside household surveys of 407 farmers. Findings revealed notable differences in farmer perceptions and adaptation practices between regions, largely shaped by environmental conditions and resource availability. Farmers in Menz reported a higher frequency of droughts and water scarcity, while Bonga farmers highlighted deforestation and grazing land loss as critical challenges. The analysis of adaptation practices identified key determinants such as geographical location, membership in cooperatives, training attendance, and access to climate-related information. Participation in cooperatives, particularly the CBBP, was a strong enabler of climate-smart adaptation, with CBBP members adopting nearly twice as many adaptation measures as compared to non-members. However, barriers such as limited access to feed resources, veterinary services, and microfinance were prevalent across all groups. The study underscores the importance of farmer cooperatives and organizations, and tailored policy interventions to enhance resilience and foster climate-smart agricultural practices in Ethiopia.

Improved competitiveness of dairy producers through systematic crossbreeding – a holistic view

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Systematic crossbreeding has for decades been used for improving the performance of production animals in other species like pigs and poultry. In dairy cattle there has been an increase in systematic crossbreeding, but at a much lower rate. A newly finished research project in Denmark (DairyCross – Optimization across breeds) focused on how to improve competitiveness for both Danish milk producers and VikingGenetics as breeding company by improving the resource efficiency from crossbreeding. Also, the obstacles for systematic crossbreeding at a larger scale were investigated. The project focused on solutions to overcome these challenges and developing tools to utilize the full potential of crossbreeding in dairy cattle. In this presentation we will focus on the main areas for improvement of crossbreeding: 1) developing genomic breeding values for crossbred dairy cattle for all main traits in the Nordic total merit index, 2) Incorporating the value of heterozygosity in mating plans for crossbred animals, 3) development of a Simherd CrossFlex App that supports farmers to choose the optimal crossbreeding strategy, 4) a sector analysis of an alternative plan for use of systematic crossbreeding in which purebred lines in nucleus herds are selected to maximize crossbred performance at production units, and 5) a survey with interviews of farmers clarifying the barriers for starting a crossbreeding program. Overall, the results from the project showed that crossbreeding is a competitive strategy towards a more resource efficient milk production.

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Accurate determination of breed origin of alleles in a simulated smallholder crossbred dairy cattle population

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Accurate assignment of breed origin of alleles at a heterozygote locus may help to introduce a resilient or adaptive haplotype in crossbreeding. In this study, we developed and tested a method to assign breed of origin for individual alleles in crossbred dairy cattle. After generations of mating within and between local breeds as well as the importation of exotic bulls, five rounds of selected crossbred cows were simulated to mimic a dairy breeding programme in the low- and middle-income countries (LMICs). In each round of selection, the alleles of those crossbred animals were phased and assigned to their breed of origin (being either local or exotic). Across all core lengths and modes of phasing (with offset – move 50% of the core length forward or no-offset), the average percentage of alleles correctly assigned to a breed origin was 95.76%, with only 1.39% incorrectly assigned and 2.85% missing or unassigned. On consensus, the average percentage of alleles correctly assigned to a breed origin was 93.21%, with only 0.46% incorrectly assigned and 6.33% missing or unassigned. This high proportion of alleles correctly assigned to a breed origin resulted in a high core-based mean accuracy of 0.99 and a very high consensus-based (most frequently observed assignment across all the scenarios) mean accuracy of 1.00. The algorithm's assignment yield and accuracy were affected by the choice of threshold levels for the best match of assignments. The threshold level had the opposite effect on assignment yield and assignment accuracy. A less stringent threshold generated higher assignment yields and lower assignment accuracy. The developed algorithm is straightforward in its application and does not require prior knowledge of pedigree, which makes it more relevant and applicable in LMICs breeding programmes.

Crossbreeding strategies for improved adaptability and productivity in small-scale cattle systems

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This study investigated effective structured crossbreeding strategies for small-scale beef cattle systems. Phenotypic and genetic gains were assessed via simulation for productive and adaptive traits over 20 generations in F1, rotational and synthetic crosses. The trait variation was driven by additive and dominance genetic effects and environment. Four synthetic breeding schemes (farm bull (FB), intra-village bull (IVB), exchanged-village bull (EVB) and population-wide bull (PWB)) evaluated the impacts of different bull selection methods on animal performance and inbreeding. The productive trait (body weight) and the adaptive trait (tick count incidence) were modelled as polygenic traits in a local taurine breed (BWL and TCL) and in an exotic indicine breed (BWE and TCE). We varied the genetic correlation between the traits ($rg = -0.4, 0, 0.4$), genotype-by-environment effects between local and exotic environment ($rgxe = 0.4, 0.6, 0.8$), and the relative emphasis of TCL in a selection index (SI_TCL10%, SI_TCL30%, SI_TCL50%), a total of 27 scenarios. The highest phenotypic and genetic gains were obtained in EVB crosses, irrespective of rg and $rgxe$. Synthetic crosses displayed higher additive genetic variances compared to F1 crosses, consistent with the higher responses to selection and genetic gains in EVB. However, synthetic crosses showed lower phenotypic values than F1 crosses in the first seven generations, due to the loss of heterosis. Extensive exchange of semen between villages prevented rapid buildup of inbreeding in EVB. Moreover, synthetic breeding offers the opportunity of combining both adaptive and productive traits in selection indexes, while minimizing antagonist responses to selection. Low emphasis on TCL (SI_TCL30%, SI_TCL10%) implied negative phenotypic gain for TCL in synthetic crosses when $rg = -0.4$. Taken together, the EVB scheme is a feasible option for genetic improvement programs in smallholder cattle farms. The developed simulation pipeline in this study is freely available to evaluate additional scenarios and traits.

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Evaluating genotype-by-environment Interactions in Ethiopian Dairy Systems: Implications for Crossbreeding Strategies

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Nationwide studies on crossbreeding in dairy cattle often classify production systems but rarely integrate it with agro-climate and weather conditions, to define crossbreeding strategies and breeding goals in East Africa, particularly Ethiopia. Genetic variation in environmental sensitivity among cattle breeds highlights the need to consider genotype-by-environment interactions (GxE) in breeding programs. This study assessed these interactions by examining the effects of agro-climate, market proximity, farm size, and weather conditions on types of crossbred dairy cattle in Oromia and Sidama. Weekly performance data from 320 dairy farms (June 2022–April 2023) were combined with meteorological data, household surveys, and breed composition reconstructed from genotypic data (100K SNPs) from 848 crossbred animals across 295 farms. Factor analysis of mixed data, linear mixed models, and estimated means were used to integrate farm characteristics, agro-climates, and genotypes to evaluate performance across environments. Among other findings, the Arsi-Bale breed showed strong positive GxE in highlands, while Borana cattle performed better in lowlands. Crossbreeds with over 80% exotic ancestry had reduced milk yield in highlands and higher numbers of services per conception. We conclude that optimal crossbreeding strategies in these regions in Ethiopia depend on farming system and agro-climatic zone.

The importance of trait stability in crossbreeding: uncovering the impacts of genotype-by-environment interaction

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Intensive dairy farming has evolved to maximise milk production in optimised conditions. However, in tropical systems, optimal conditions are often unavailable due to challenges like heat stress, resource variability, and climate instability. These factors limit response to selection, delaying genetic progress. Crossbreeding is a strategy designed to overcome the challenges of tropical systems. The goal is to produce crossbred animals that, on average, outperform the mean of the parents. In practice, response to crossbreeding is highly inconsistent, which can be attributed to a primary focus on mean performance while overlooking genotype-by-environment interaction (GEI). Despite evidence of the role of GEI on performance, few solutions address this issue, reflecting a gap in understanding how to optimise crossbreeding effectively. Given the increasing unpredictability of tropical climates driven by climate change, understanding how GEI affects dairy production in these areas is crucial. Our research aims to uncover the impacts of GEI on tropical dairy production and demonstrate the advantages of pursuing trait stability — the consistent performance of a genotype across environments — for the success of crossbreeding strategies. Using stochastic simulations, we compare three breeding programmes: a purebred intensive programme (e.g., Holstein), a purebred tropical programme (e.g., locally adapted indicine breeds), and a crossbreeding programme. The intensive programme is tested in a single favourable environment, while the tropical purebred and crossbred programmes are evaluated in five environments representing varying tropical conditions. Environments are correlated, with stronger correlations between neighbours. We evaluate response to selection for the six environments, examining the impacts of GEI. Our results demonstrate the trade-off between performance and stability, with loss in fitness across environments reflected by an increase in genetic variance over time. This work offers a framework for understanding the impacts of environmental variability on dairy production and provides insights for designing breeding strategies better suited to these conditions, supporting the sustainable future of tropical dairy farming.

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Consequences of including CO2 taxes in the selection index of the Danish Blue line used for beef on dairy crossbreeding

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From 2030, Danish cattle farms will be taxed based on their greenhouse gas emissions. This study aims at evaluating how incorporation of CO2 taxes in the total merit index for beef bulls used on dairy cows (BxD) impact expected genetic gain for selected traits. The breeding scheme of the Danish Blue population used for BxD was simulated over 25 years. The selection index comprised eleven traits: Carcass growth, carcass conformation, fat score, slaughter percentage, calving ease and survival, early and late youngstock survival, health, feed efficiency (FE) and methane production (CH4). There were four economic values of CH4, respectively zero, 0.14, 0.35 and 0.87 EUR/index units – corresponding to 0.1 genetic standard deviation unit (SDU) respectively for three taxes of CO2 (120, 300 and 750 DKK pr ton CO2e). Eight scenarios were simulated: four with varying CH4 values based on the taxes and four where the economic value of FE was halved. Genetic parameters from current EBV evaluations were applied, and each scenario was replicated 30 times. With no CO2 tax, the annual trend for CH4 was 0.03 SDU. Introducing an economic value of 0.14 EUR/index unit on CH4 increased the gain to 0.05 SDU, while an economic value of 0.87 EUR/index unit further increased genetic gain to 0.11 SDU. Having the economic value of FE reduced its gain, while increasing gain for the other traits. The results indicated a favorable trend for CH4 when the economic value was increased for the trait. Increased economic values of CH4 and FE increased growth, carcass conformation, and slaughter percentage, due to positive breeding value correlations, while survival and health decreased by approximately 0.05 SDU. This study found that including economic value for CH4 in a BxD breeding scheme led to favorable genetic trends for CH4, FE, growth, and slaughter percentage but had negative effects on survival, calving ease, and health. In conclusion, the CO2 taxation scheme will reduce CH4 but will have small negative effects on functional traits.

Genetic correlations between feed efficiency, methane emission and the traits in the Danish BeefxDairy evaluation

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Genetic improvement of new traits, including feed efficiency and methane emission, is essential for sustainable production of BeefxDairy crossbreds (BonD). Before implementing these traits in the breeding program, it is important to know their genetic correlations with the current traits used for genetic evaluations of beef bulls used for BonD. The traits in the Danish BonD breeding program are calving ease, calf survival, young stock survival in the early period (1-30 days) and the late period (31-200 days), health, daily carcass gain, carcass conformation score, fat score and slaughter percentage, which are all recorded routinely for all BonD calves. The aim of this study is to estimate genetic correlations between feed efficiency, methane emission and the current traits in BonD genetic evaluation. In this study, feed efficiency was defined in two ways, feed conversion rate (FCR), defined as average daily gain (ADG)/dry matter intake (DMI), and residual feed intake (RFI). Methane emission was expressed as the ratio of CH₄ to CO₂. In Danish BonD breeding program, the three beef breeds: Danish Blue (DBL), Angus (AAN) and Charolais (CHA) have been heavily used. Methane emissions were recorded for 9774 animals, of which 2079 were AAN x Holstein (HOL) crossbreds, 6429 were DBL x HOL and 1266 were CHA x HOL crossbreds. DMI was on average measured for a period of 30 days, and ADG for a period of 33 days for each animal, and the phenotypes used are full-test means of these periods. As all other traits are recorded routinely on all BonD calves, the number of animals with data are much higher than for methane emission and feed efficiency. For BonD with HOL dams and one of the three beef breeds as sires, more than 348,000 animals have birth trait data, 133,000 have slaughter %, 191,000 have all the other carcass traits, 284,000 have young stock survival and 257,000 have health data. Preliminary results indicate genetic variation for feed efficiency, supporting its potential inclusion in the BonD breeding program. Further analyses are ongoing to assess the other genetic parameters relevant to the study objectives.

Dynamic breed definition for admixture Nordic Red dairy cattle – An ad-hoc approach for modelling heterosis in genomic evaluation for production traits

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In 2006, Denmark, Finland, and Sweden introduced joint genetic evaluations for yield traits in Holstein, Red Dairy Cattle (RDC), and Jersey populations using breed-specific random regression test-day models. Initially, the RDC population exhibited an admixture structure, requiring complex modeling to account for breed differences and non-additive effects. After nearly two decades of joint Nordic breeding efforts, the RDC population has become more homogeneous, and studying genotype information shows that Finnish, Swedish, and Danish RDC cows have become increasingly similar. Thus, a new approach was required to model heterosis and recombination effects. In the updated breed definition, each animal's individual breed composition is compared to the average breed composition of all animals born in the same year. A Mean Squared Deviation (MSD) value is assigned to each animal to quantify how much an individual deviates from the average breed composition. If MSD is below a threshold, the animal is classified as 100% Nordic Red Cattle (NRC) with no heterosis or recombination effects. For others, only positive deviations contribute to heterozygosity. We tested MSD thresholds (0.001, 0.002, 0.003, and 0.004) to assess their impact on estimated breeding values (EBVs) compared to the current model. The new definition reduces total heterozygosity and recombination. For instance, under the original approach, the average total heterozygosity for cows born between 2005 and 2010 was 65%. With the new method applying a 0.002 threshold, it dropped to 50%. For cows born between 2015 and 2020, the average total heterozygosity was 65% under the original approach and 22% with the new method. The model estimate of total heterosis for protein yield in lactations 1-3 varied between 4.7-5.3% and 2.9-3.6% of the mean for the original and new definition applying the 0.002 threshold, respectively. Genetic trends and standard deviations in bulls and cows remain stable, with correlations >0.999 between GEBVs from the current and new models.

Deep Learning for Crossbred Genomic Prediction: Integrating Transfer Learning and Heterosis Effects

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In recent years, crossbreeding in dairy cattle production has gained interest given the potential of crossbred animals to outperform their purebred parents, mostly due to heterosis. Since in dairy cattle production crossbred animals remain in the system as parents to be considered for future generations, accurately predicting their breeding values (BV) is crucial. However, despite various approaches that account for breed composition or breed of origin of the allele (BOA) in genomic evaluations, BV prediction for crossbred animals often shows lower accuracy than for purebreds. This is due to the relatively small crossbred population size, varying breed compositions, genetic differences between purebred populations, and the effect of heterosis. Most methods do not account for heterosis at each individual SNP effect, being the heterosis thus confounded with the additive effects, therefore reducing prediction accuracy. To address this issue, we developed a Deep Learning model that incorporates BOA and heterosis at each SNP to predict genomic BVs in crossbred animals, utilizing Transfer Learning from purebred parents. Transfer Learning in Deep Learning enables a model trained on one dataset (purebreds) to improve performance on a related dataset (crossbreds), allowing better recognition of genetic patterns despite limited crossbred data. We validated our model on a simulated population resembling a rotational crossbreeding strategy, for which genotypes and phenotypes were simulated, for traits with varying heritability and levels of heterosis. Using the simulated genotypes (QTL excluded) and phenotypes from purebred and crossbred animals, the predicted BVs of young candidates obtained with our model presented an accuracy up to 0.15 greater than BVs from a Bayes A accounting for the BOA, when heterosis represented 30% of the total genetic variance.

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Theatre 10

Harnessing Genetic Selection to Combat PRRS: Evidence from a Multiyear, Multiline Study

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Porcine reproductive and respiratory syndrome (PRRS) is a major global disease affecting pigs at all stages of production. Breeding for increased natural resilience to PRRS virus (PRRSv)-infection is a promising control strategy. Since 2018, Topigs Norsvin has collected data for crossbred animals from multiple genetic lines exposed to various disease challenge conditions. Our previous analyses validate the existence of substantial, naturally occurring genetic variation in host response to PRRSv challenge. This specific study evaluated the impact of selecting boars for reduced mortality under PRRS challenge (using these trials data as reference population) on the performance of their offspring post-PRRSv-infection. Data was collected on 2,711 three-way commercial crossbred Topigs Norsvin pigs. A total of 93 boars from two sire lines were selected based on extreme breeding values (BV) for mortality under challenge, derived using data collected from previous PRRS challenge trials. Boars with low vs. high BVs were selected to sire the High Robust and Low Robust progeny groups, respectively. Pigs were inoculated with a highly pathogenic North American PRRSv isolate at 52 days of age and followed until marketing. Data was recorded at the individual level, including body weight at multiple time points and mortality events. Mortality rate and average daily gain (ADG) were calculated using data collected from 0 to 28 days post-inoculation. Results from this study demonstrated that pigs sired by High Robust boars had a significantly ($P = 0.05$) lower mortality rate (18.6%) than those sired by Low Robust boars (24.2%). Additionally, progeny of High Robust sires had significantly ($P = 0.02$) greater ADG under challenge (360 g/day) than the progeny of Low Robust sires (320 g/day). In conclusion, this study shows that a multiline, multipathogen training population can be used to select for reduced mortality under PRRS challenge in an independent population. The sizeable and significant impact of selection on mortality, even for a binary trait, demonstrates that genetic selection can be used as an effective strategy to mitigate the impact of PRRS on health and production.

Improving efficiency and robustness of introgression by the use of haplotype-based founder detection
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The transfer of mendelian traits, e.g. from local populations into large commercial breeds, is a relevant task for breeding organizations. The goal hereby is to introgress only the causal variant, while the overall genome of the recipient population shall remain original. With genetic editing being more efficient but still lacking political acceptance, backcrossing schemes will stay relevant for the next years. They however come with the drawback of also introgressing non-target genome together with the actual target, in case of two backcross generations 12.5% (87.5% recipient genome) on average. Selection against donor genome in non-target regions was demonstrated in the EU project IMAGE by the introgression of blue egg shell color from Araucana chickens into a White-Layer (WL) line, using oppositely fixed SNPs as markers for the founders. After retrospectively reviewing the experiment, however, we did not see significant deviations from expectation, likely being caused by a) a few separated SNPs, and b) a batch effect causing a larger share of markers falsely appearing separated. We therefore performed simulations based on the data of the original founder animals, proposing haplotype-based methods as a solution. Simulations showed that the strict selection on the shell color decreased WL genome in the final intercross to 86.6%. SNP-based selection on WL genome peaked at 90.7% WL genome when using $\geq 1k$ separated SNPs. Fewer markers or erroneous genotypes decreased performance of SNP-based selection. However, haplotype-based selection reached 90.8% WL genome in all scenarios, even when simulating 20k/46k markers as being erroneous, proving the efficiency and robustness of haplotype-based founder detection methods in backcross schemes.

Session 60

Theatre 12

The role of dominance in laying hen breeding schemes including crossbreeding information

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Crossbreeding improves laying hen breeding by maximizing heterosis and performance. Traditional selection relies on purebred populations, assuming a strong correlation with crossbred performance, but this correlation may differ from 1 due to non-additive effects like dominance. This study evaluates the impact of incorporating dominance and crossbred information into genetic evaluations to optimize selection response in laying hens. We modeled a breeding scheme using stochastic simulations with two pure lines and their crossbred population. Phenotypes were generated under two models: (M1) additive effects only and (M2) additive plus dominance effects. Breeding values were estimated using single-step genomic BLUP (ssGBLUP), integrating purebred data with six crossbreeding information strategies: NC (no crossbred data), PI (individual phenotypes), PP (pooled phenotypes), IP_IG (individual phenotypes and genotypes), PP_IG (pooled phenotypes with individual genotypes), and PP_PG (pooled phenotypes and genotypes). Results showed a steady decline in additive variance across generations, confirming selection effectiveness. Strategies integrating more crossbred data (IP_IG, PP_IG) exhibited greater variance reductions, improving selection efficiency. The selection response analysis found that IP_IG strategy achieved the highest genetic gains, followed by PP_IG, with 85% response in M1 and 77% in M2. Strategies using pooled phenotypic data (PP, PP_PG) and only individual phenotypic data (IP) showed lower but notable gains. Comparing models, M2 showed a sharper decline in additive variance, as dominance effects captured more genetic variation. However, when dominance was present in phenotypes but omitted from evaluations, selection response dropped by 5–20%, highlighting the need to model dominance in genetic assessments. In conclusion, integrating crossbred data improves selection response. Additionally, dominance effects significantly influence variance reduction and selection outcomes, emphasizing the need to include both in poultry breeding programs.

Farmers' views on production systems, management and breeding strategies for beef on dairy cattle crosses in Sweden

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The interest for using beef breed semen on dairy cows not aimed for producing recruitment heifers is increasing worldwide. It provides an opportunity for a climate efficient way to increase the beef production from calves born in dairy herds. Production systems where these beef on dairy (BxD) crossbred calves are born and raised for slaughter vary within Sweden. Hence, further investigations are needed for validation and continued development of breeding values for bulls aimed for BxD crossing. This interview study aimed to investigate Swedish dairy and beef farmers' perspectives on BxD crossbreeding, focusing on calf value, management and breeding strategies, market demand, and production system differences. By using strategic snowballing, 23 farmers located all over Sweden with either dairy, beef or both beef and dairy production where identified based on certain characteristics and selected to be included in the study. Interview guides with checklists were used when conducting the digital semi-structured interviews. The interviews were recorded and transcribed for further analysis. The qualitative data analysis focused on uncovering meanings within the participants' responses, and the preliminary results show both similarities and variability between farmers. Examples of themes that emerged in the preliminary analysis were "traits of value in BxD calves", "calf sexes and their different value in production", "opinions on bull breeds", "economic value of BxD calves". A common view among beef producers was that BxD crosses were preferred over purebred dairy calves. Dairy producers highly valued the beef sires effect on calving ease when choosing which breed or bull to cross with. The emerging themes this far provide insight into the variability of Swedish production of BxD calves and what expectations, obstacles and future prospects these farmers experience with BxD crosses.

Session 60

Poster 14

Fast and accurate estimation of breed origin of alleles in crossbred populations

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Estimating the breed origin of alleles (or "chromosome painting") is of great interest in crossbred individuals to determine the exact genetic composition of an individual, to estimate the genotype at genes of interest, or to predict breeding values with SNP effects depending on their origin. By extension, this approach can provide information on the contributions of individual ancestors in purebreds or crossbreds. Several algorithms already exist but many of them are computationally intensive. For large-scale routine evaluation of crossbreds, this algorithm needs to be run frequently and to provide fast results without compromising accuracy. In many situations, the genomic evaluation pipelines include an imputation and phasing step. This phasing information can be efficiently used to estimate allele origins. The proposed algorithm considers haplotypes of 16 SNPs and estimates their frequency in each potential breed of origin from a reference population of purebred individuals. The breed of these individuals is assumed to be known with certainty. The haplotype of a crossbred is then assigned to the breed with the highest frequency of the same haplotype. A breed likelihood ratio of at least 10 is required to ensure accuracy. The whole genome is scanned with a sliding window of 16 SNPs with a step size of 1. If a small indeterminate segment is surrounded by two segments of the same origin, it is assigned to that origin. The process is iterated with haplotypes of decreasing size (down to single SNPs) to fill the remaining gaps. This algorithm has been shown to be very accurate (frequently >99%) and has been found to be at least 10 times faster than tested alternatives. While it is particularly suitable to medium-density marker panels, it can also be applied to higher densities up to the sequence level but prior selection of informative markers is recommended to ensure haplotype informativeness. This work was part of the European GenTore project (No 727213) and of the Evagenoc project funded by APIS-GENE.

Influence of an active form of Vitamin D combined with triterpenes on lactating sows productive performance, plasma calcitriol status, immunity markers and serum proteome

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Lactating sows are prone to calcium and vitamin D deficiency, therefore, offsprings health can be deeply affected. We hypothesized that an active form of Vitamin D (Active D, Phytobiotics GmbH) constituted by glycosylated calcitriol combined with triterpenes, could enhance lactating sows productivity and health. One week prior to farrowing, twenty-four multiparous sows (320.08 ± 26.21 kg) were randomly allocated to 3 homogenous groups. Control (CTR) was fed a basal diet (1800 IU of cholecalciferol), Treatment 1 (ACTD1) and Treatment 2 (ACTD2) were fed the basal diet + 0.64 µg/kg and 0.96 µg/kg of glycosylated calcitriol, respectively. Litters growth performances were monitored during the trial. Blood samples were collected from each sow at trial start, farrowing and at lactation end. Plasma pro-inflammatory cytokines (TNF-α, IL1-β and IL1-α), calcitriol, parathormone (PTH) and calcitonin levels were analyzed through ELISA tests. Differential shotgun proteomics analysis was performed on serum samples. Data were evaluated with a general linearized model (GLM) and Tukey's test. ACTD1 and ACTD2 litters revealed better mean BW at weaning compared to CTR (7.28 ± 1.06 kg and 7.43 ± 1.07 kg vs 6.58 ± 1.30 kg; $p < 0.05$). In comparison to CTR, higher calcitriol levels were detected in ACTD1 and ACTD2 after farrowing (15.83 ± 4.63 pg/ml and 17.49 ± 4.48 pg/ml vs 7.12 ± 3.45 pg/ml; $p < 0.01$) and after lactation (16.79 ± 5.00 pg/ml and 18.61 ± 4.39 pg/ml vs 10.58 ± 3.01 pg/ml; $p < 0.05$). At trial end, PTH levels were reduced in ACTD1 and ACTD2 groups in comparison to CTR (21.46 ± 2.86 pg/ml and 24.04 ± 4.55 pg/ml vs 31.50 ± 4.67 pg/ml; $p < 0.05$). TNF-α, IL1-α and IL1-β were markedly lower at the end of the trial in ACTD1 and ACTD2 sows ($p < 0.05$). Calcitonin was not affected by treatments. Proteomics analysis suggested that glycosylated calcitriol influenced metabolic and immune-related pathways. In conclusion, the enhanced bioavailability of glycosylated calcitriol and triterpenes combination improved lactating sows productivity by modulating circulating calcitriol and immune status.

Session 61

Theatre 2

Tributyrin: a solution to enhance growth performance in low mature pigs

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Dietary tributyrin supported intra-uterine-growth restricted pigs. It was hypothesized that it could benefit pigs that have low maturity at weaning. The study aimed to determine the effect of tributyrin on growth performance of low vs high mature nursery pigs. A maturity score was developed based on gestational age (score 1 <114, score 2 114-115, or score 3 >115 days), weaning age (score 1 <23, score 2 23-24, or score 3 >24 days of age), prewean body weight gain (score 2 <25% quartile, score 4 25-75% quartile, or score 6 >75% quartile of pig batch), colostrum intake (score 2 <25% quartile, score 4 25-75% quartile, or score 6 >75% quartile of pig batch), and birth weight (score 1 <25% quartile, score 2 25-75% quartile, or score 3 >75% quartile of pig batch). Body weight gain and colostrum intake were given a higher weighting in the scoring due to their relevance for health status. Scores for each parameter were summed per pig and a total score of 7 (minimum) to 14 was considered LOW and 15 to 21 (maximum) was considered HIGH maturity. A 2×3 factorial design was used with maturity HIGH/LOW and three experimental diets as main factors. There were 20 pens and 3 pigs/pen per treatment. The three diets were formulated similarly (18% CP; 1.32% SID Lys; 2500 kCal NE) but with 0% (CONTROL), 0.75% (0.75TRI), or 1.5% (1.5TRI) tributyrin both in the first (d0-14) and second phase (d14-35). Growth performance data were analyzed with the main effects and the interaction between maturity score and diet as fixed effects (MIXED model). Compared to HIGH mature pigs, LOW mature pigs had lower average daily gain during d0-14 when fed CONTROL (253 vs 215 g/pig/d) or 1.5TRI (253 vs 207 g/pig/d) while gain was similarly high when fed 0.75TRI (246 g/pig/d for both; Pinteraction=0.039). This was driven by a feed intake effect (Pinteraction=0.022). The interaction disappeared in the second phase with only a main effect of maturity score on gain ($P < 0.001$) and feed intake ($P < 0.001$). Separating pigs based on maturity score seems key when evaluating feed additives like tributyrin. In conclusion, the effectiveness of tributyrin was limited to low mature pigs and at 0.75% inclusion.

The SID Thr and total dietary fibre (TDF) ratio in piglet diet affects intestinal development and mucin secretion

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Improved intestinal development can be achieved by increasing mucus secretion by feeding dietary fibre (DF) and supplying an adequate SID Thr. In growing pigs, the benefits of high DF are achieved when SID Thr increases. The present study investigated the impact of increasing total DF (TDF) while compensating for the SID Thr on intestinal development, mucin secretion, and piglet growth. A total of 270 piglets weaned at ~25 d of age (6.4 ± 0.3 kg) were group-housed (3 pigs/pen) and pens were assigned to 1 of 5 treatments ($n=18$ pens) for 28-d. The diets were formulated with TDF (145, 165 and 175, g/kg) and SID Thr (0.63, 0.75, and 0.81), achieving five (5) diets, T1; TDF145/Thr0.63, T2; TDF165/Thr0.63, T3; TDF165/Thr0.75, T4; TDF175/Thr0.75 and T5; TDF175/Thr0.81. Growth performance and feed intake were measured weekly. At the end of the experimental period, a total of 10 pigs/treatment were selected and euthanized for intestinal tissue samples for morphological, goblet cells, and organ weight measurements. Fecal samples were collected for fecal mucin analysis. All data were analyzed as a one-way ANOVA with treatment as a fixed effect and room as a random effect. The initial body weight was used as a covariate in the statistical model. No differences were observed for growth performance ($P>0.05$). Ileal goblet cell size was greater in T3 and T4 compared to the rest of the treatments ($P<0.05$). Goblet cell numbers were lower in T3 and T4 (i.e., higher SID Thr levels) compared to the other treatments ($P<0.05$). A higher fecal mucin for T3 than for T2 (i.e., higher SID Thr with similar TDF; $P<0.05$; 0.86 vs 0.27 $\mu\text{g/g}$) was observed, and T3 was not different from T1 and T4 ($P>0.05$; 0.86 vs. 0.48 and 0.60 $\mu\text{g/g}$ respectively). In summary, TDF and SID Thr supply did not affect growth performance, however, with fecal mucin, a higher SID Thr was required to support a high mucin production even at a similar TDF level (T3 vs T2). Similarly, comparing T3 vs. T5 indicates that, a much higher TDF (175 g/kg) and a further increase in SID Thr (0.81) did not enhance mucus secretion or growth; possibly, other limitations impacted the piglet's metabolism. In conclusion, feeding a high DF compensating for the dietary SID Thr supply may improve intestinal development in piglets, but perhaps, the TDF levels should not exceed 175 g/kg, as this may negatively impact intestinal development, even when dietary SID Thr supply is further increased.

Session 61

Theatre 4

The effect of dietary micelle quercetin supplementation on growth performance, nutrient digestibility, gas emission, fecal score, and blood profile in growing-finishing pigs

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Antioxidant polyphenols from plants are potential dietary supplementation to alleviate early intestinal disorders in piglets. Recent evidence showed that polyphenol quercetin could reshape the gut microbiota when it functioned as anti-inflammation or antioxidation agents in rodent models. To date, the effect of micelle form of quercetin supplementation in grow-finishing pigs' performance study is very limited. Therefore, we aimed to investigate the effects of dietary micelle quercetin (MQ) on growth performance, nutrient digestibility, gas emission, fecal score, and blood profile in growing-finishing pigs. A total of 105 growing pigs (with an average body weight of 24.3 ± 3.51 kg) were allocated to three groups and fed diets containing different levels of MQ for 15 weeks. The treatments included CON (basal diet), TRT1 (basal diet + 0.025% MQ), and TRT2 (basal diet + 0.05% MQ), each with seven replicates and five pigs per replicate. Experimental data were analyzed in a complete randomized block design using the GLM procedures (USA). Linear and Quadratic polynomial contrasts were used to examine the graded levels (0.05% and 0.1%) of MQ supplementation. The results indicated that dietary supplementation with MQ had no significant effect on growth performance, nutrient digestibility, fecal score, and gas emission. However, there was quadratic change on bile acids of blood parameters ($P<0.05$), with no significant changes in aspartate aminotransferase (AST), and alanine aminotransferase (ALT) ($P>0.05$). Also, fecal scores remained unchanged ($P>0.05$). In summary, dietary MQ supplementation reveal no adverse effect on growth performance, nutrient digestibility, fecal scores, and gas emissions, but influenced bile acid levels without impacting key liver enzymes (AST, and ALT), suggesting its safety and potential for targeted metabolic benefits.

Nitrogen and Phosphorus reduced feeding strategies and its influence on bone related traits in Landrace boars

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The reduction of nitrogen (N) and phosphorus (P) in animal feed aims to minimise environmental pollution such as excess nutrients in water bodies and the impairment of soil quality without impairing the growth and health of the animals. The aim of this work was to investigate the effects of N/P-restricted diets on the morphological bone structure, chemical bone composition, bone density as well as fundament conformation in pigs. In total 1,082 purebred landrace (LR) boars were split into 2 feeding groups. The diet of the control group contained, depending on the live weight, crude protein and P in a range of 187 to 171g/ kg DM (CP) and 6 to 5.5 g/kg DM (P), respectively. The N/P -reduced diet contained ~10% less CP and ~7% less P following the recommendations by DLG. Before slaughtering leg and body conformation of 835 boars was evaluated subjectively by a routinely applied linear scoring system. After slaughtering the right front foot from all animals was collected and the metacarpal bones 3 (Mc3) and 4 (Mc4) were extracted. Length, weight and diameter of Mc3 and Mc4 were measured and bone density of Mc3 were determined by computer tomography. Afterwards the content of ash, calcium, phosphorus, magnesium and sodium of Mc3 was analysed. There was no significant impact of the diet group on bone density. The chemical analyses showed significant differences for the magnesium content only. Four conformation traits were significantly affected by the diet group. Variance components were estimated using a sire model. The highest heritability (h^2) was observed for bone mass traits ranging from 0.14 to 0.56, whereas lower h^2 from 0.05 to 0.23 were estimated for all other traits. The sire $\times$ feed interaction was not significant. We will further investigate the genetic relationship among the traits using an animal model.

Supplementation of Energy-Reduced Diets with Enzy Carboplus Shows a Positive Impact on Growth Performance and Nutrient Digestibility of Fattening Pigs

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The non-starch polysaccharide-digesting enzymes enhance nutrient absorption, improve feed efficiency, and promote growth performance in fattening pigs. This study evaluated the efficacy of ENZY CARBOPLUS, a xylanase- and glucanase-based enzyme supplement, in energy-reduced wheat-, rye-, and barley-based diets for fattening pigs from day 75 to day 145 of age (70-day feeding period). A total of 56 healthy fattening pigs (DanBred $\times$ Duroc) were allocated to four treatment groups, each receiving a different level of ENZY CARBOPLUS (0, 700, 1,400, or 2,100 Xylanase units (LXU)/kg feed). Growth performance (body weight, body weight gain, feed intake and feed-to-gain ratio) and health status were assessed. Apparent total-tract digestibility (ATTD) and metabolizable energy (ME) content of feeds were measured from day 103 to 107 of age. Statistical analysis was conducted using the software package SPSS. Results showed that compared to the control, supplementation at 1,400 and 2,100 LXU/kg significantly improved final body weight (+4%, +4.5%), overall body weight gain (+5.9%, +6.6%) and feed-to-gain ratio (-3.8%, -5.8%) respectively. The highest dose (2,100 LXU/kg) significantly enhanced the ATTD of crude protein (+2.6%), gross energy (+3.6%), crude ash (+19%) as well as calcium (+6.4%) and phosphorus (+7.6%) digestibility. No adverse health effects or mortality were observed. These findings demonstrate that ENZY CARBOPLUS at $\geq 1,400$ LXU/kg enhances growth performance and nutrient digestibility in fattening pigs, offering a viable strategy for optimizing feed efficiency in reduced-energy diets.

Metabolomic Analysis of Obesity-Related Changes and the Effects of an Anti-Obesity Supplement in Beagle Dogs

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In 2022, a survey by the Association for Pet Obesity Prevention in the United States found that 59% of dogs were overweight or obese. Obesity can lead to various health issues, including joint problems, diabetes, and heart disease, so preventing it is essential to your pet's overall health. We found that the levels of various metabolites, such as valine and tyrosine, change in response to body mass index changes, indicating obesity. It confirms that obesity is not simply an increase in fat but an increase in some metabolites due to metabolic disturbances in the body. In this study, we used Agilent's LC-QTOF instrument to observe changes in metabolites in the blood of beagle dogs. Specifically, blood was extracted from a group fed a regular diet, a group fed a high-calorie diet, and a group fed a high-calorie diet plus an anti-obesity supplement for metabolomics analysis. In addition, metabolites were matched with METLIN PCDL and qualitative analysis to evaluate their types and expression levels comprehensively, and the mass profiler professional program was used for statistical analysis. As a result, five metabolites were significantly increased in the supplemented group compared to the high-calorie diet group, and three metabolites were decreased. In the supplemented group, inflammatory markers Tetranor-12(S)-HETE and Octadec-9-ynoic acid were lower than in the high-calorie group. It confirms that adding an anti-obesity supplement to dogs fed a high-calorie diet can positively impact obesity management and health in pets.

Session 61

Theatre 8

Effect of 25-OH-cholecalciferol on performance and mineral metabolism of Holstein cows during the transition period

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The present study investigated the effects of 25-hydroxycholecalciferol supplementation on performance and mineral metabolism during the transition period in dairy cows. Forty-two multiparous Holstein cows were fed either cholecalciferol (D3) or 25-hydroxycholecalciferol (HyD; 25-OH-D3; Hy-D®, dsm-firmenich) as dietary vitamin D supplements. From 21 days before the expected calving until parturition, the D3 group received 0.475 mg/d of cholecalciferol, and the HyD group received 1.45 mg/d of 25-OH-D3. From parturition until 21 DIM, the D3 group received 0.625 mg/d of cholecalciferol, and the HyD group received 0.625 mg/d of D3 plus 1.0 mg/d of 25-OH-D3. Blood samples from the tail were taken at day -21 (22±5 days), -7 (6±2 days), -3 (2±1 days), 0 (day 0; 13±8 hours after calving), 1 (36±11 hours after calving), 10 (9±2 days), and 20 (16±2) relative to calving to analyze plasma metabolites. Additionally ear arterial blood samples were analyzed for blood ionized Ca at days -21, -7, -3, 0 (calving day), and 1. Data were analyzed using PROC MIXED with treatment, time of sampling, and the interaction between treatment and time as fixed effects. There was an interaction between treatment and time for DMI, and the HyD had lower intake before calving and higher DMI after calving. Plasma 25-OH-D3 was higher in HyD (98±4) compared to D3 (39±4 ng/ml; Lsmeans for treatment across time; P<0.001 for treatment). Arterial blood ionized Ca was higher for HyD as compared with D3 at the day of calving (P=0.002; 1.08 vs. 0.99 ± 0.02 mmol/L). Plasma total calcium, inorganic phosphorus, and magnesium were not affected by treatment. Plasma β-hydroxy butyrate level was significantly lower in HyD (0.61±0.02) compared to D3 (0.70±0.02 mM; Lsmeans for treatment across time; P=0.004 for treatment). In conclusion, feeding 25-OH-D3 to transition dairy cows induced a reduced feed intake before calving, a greater feed intake immediately after calving, as well as a higher arterial blood ionized Ca on the calving day compared with cows supplemented with cholecalciferol.

The effect of buffering dairy cow diets with micronised calcite coated with stearic acid on milk yield, ruminal pH, and blood gases

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This study was conducted to determine the effects of stearic acid-coated micronized calcite added to dairy cow diets on milk yield, rumen pH, and blood gases. A total of 40 Holstein dairy cows, 20 cows each, were divided into two groups: control and trial. The control group received only micronized calcite, while the experimental group received slow-release micronized calcite coated with stearic acid, with a particle size of 40 microns. Cows ingested Smaxtec Premium boluses for real-time rumen pH monitoring, accessible via computers and mobile phones. During the 30-day study, cows were milked three times daily, and milk yields were recorded using a herd monitoring program. Blood samples were collected from all cows weekly after milking, and blood gas parameters were analyzed with a blood gas analyzer. pH data were recorded every 10 minutes throughout the study using boluses. Rumen pH in the experimental group receiving slow-release rumen buffer was found to be significantly higher ($p < 0.001$) than in the control group. Milk yields were also significantly higher ($p < 0.05$) in the experimental group throughout the research. No significant difference was observed in blood gas results except for sodium ($p < 0.01$). In conclusion, this study demonstrates that the slow-release buffer produced from micronized calcite effectively maintains rumen pH over a long period of time when incorporated into the daily diets of dairy cattle, thus providing protection against rumen acidosis.

A specific magnesium oxide blend improves ruminal pH, milk production and milk quality in lactating dairy cows on grazed grass

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Improving rumen environment using buffering agents could contribute enhancing ruminant performance. In grazing systems, diet consistency can vary in line with fluctuations grass quality over the grazing season, leading to challenges in the rumen of dairy cows and associated changes in milk production. The aim of this experiment was to evaluate the effects of two neutralizing agents on rumen pH, milk production and milk composition on a typical Irish grazing system. Lactating Holstein-Friesian cows ($n=100$) were blocked by parity, days in milk and milk production prior to beginning of the experiment and randomly assigned to dietary treatments in which calcareous marine algae at 0.4% DMI (control) was compared with a specific blend of magnesium oxides at 0.4% DMI (MG). The different buffers were offered in a 15% CP pelleted fed through an automatic feeder as part of a Lely robotic milking system. The experiment lasted 90 days. Rumen boluses were used (six in each group) measuring ruminal pH every 10 minutes. Milk yield and composition (fat and protein) were recorded daily via Lely Horizon. Data were analyzed using a mixed-effects linear model, with treatment, parity as fixed effects and cow as random effect. Days in milk was used as a covariate. Cows offered MG had an increased ruminal pH by +0.2 ($P < 0.001$), milk yield by +0.8 kg/cow/d ($P < 0.05$). Milk protein tended to be 0.04% ($P = 0.07$) greater in cows offered MG compared to control while milk fat (4.34% vs 4.31%; $P > 0.05$, respectively for control and MG) remained similar. Magnesium oxides fed at 80g per cow per day had a greater effect on rumen pH and milk yield than calcareous marine algae at the same level of 80g per cow per day. These results indicate that MG inclusion in grazing diets for lactating dairy cows had a positive effect on rumen environment and milk production. Key words: buffer, rumen, milk production

Effect of time of application of ethyl esters of polyunsaturated fatty acids of linseed oil on growth performance of dairy calves

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The objective of this study was to determine the effect of time of application of ethyl esters of polyunsaturated fatty acids of linseed oil (EPUFALO) on growth performance and health of dairy calves. A total of 48 female Holstein Friesian calves (average weight 41.68 ± 4.57 kg; 11 days of life) were divided into 4 experimental groups, each consisting of 12 animals. The experiment was divided into two phases (20 days per phase) and lasted 40 days. The animal groups were; a control group (A) fed milk replacer (MR) without added EPUFALO, treatment group (B) received added EPUFALO in MR in the first phase and no EPUFALO during the second phase, Group (C) did not receive EPUFALO in MR in the first phase until the last phase while animals in group (D) had EPUFALO added in their MR throughout the experiment. The treatment groups received daily doses of either 0 mL (A), or 30 mL EPUFALO; a liquid mixture containing 64.5% of α -linolenic acid, 16.1% of linoleic acid, and 19.4% of oleic acid, as declared by the manufacturer (LeenVit Group Sp. z o.o., Jaworzno, Poland). The intake of MR and starter mixture (SM) was recorded daily, and body weight and body measurements were done before the start of the experiment and after every ten days. The animals were checked every morning and evening for signs of diarrhea. Addition of EPUFALO in the MR tended to increase the DM intakes of starter mixture and total DM. There was quadratic increase in the total DM and a tendency for quadratic increase in ADG due to EPUFALO. Heart girth and withers height increased linearly and were best in group B animals. In conclusion, addition of 30 mL/day of EPUFALO in MR during first month of dairy calf's life showed prospect for an improved growth performance.

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Theatre 12

The effect of saponins and citroflavoids based-feed additive in managing cryptosporidiosis in calves

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Cryptosporidiosis is the important infection of calf leading to economic losses due to diarrhea, impaired growth and mortality. It raises the public concern due to zoonotic potential while only few solutions are available with inconsistent efficacy. Alternatives, such as plant-derived solutions have been considered as promising solutions. This study aimed to evaluate the effect of the plant-based feed additive (CTXO) supplementation, standardized in saponins and citroflavoids, on the artificially infected calves with *Cryptosporidium parvum* during their first 50 days of life. 20 one-day-old Holstein calves, housed individually, were randomly assigned to 3 groups: IUC (n=7): standard feeding program and orally infected with *C. parvum* oocysts on day 6; CTXO (n=7): standard feeding program supplemented with CTXO at 3 g per head daily during trial and infected with *C. parvum* on day 6; UUC (n=6): standard feeding program and no infected with *C. parvum*. Clinical signs were performed on days 1, 3, 7, 14, 30, and 50. Fecal samples were collected on days 1, 5, 8, 12, 35, and 50 for microscopic analysis and on days 8, 12, 35, and 50 for PCR diagnostics. Body weight was recorded before trial and on days 14 and 50. UUC calves remained free from *C. parvum* infection and clinical signs during the trial. Clinical signs were found earlier and lasted longer in IUC calves (day 3 till day 50) than CTXO one (day 5 till day 14). Clinical severity was significantly reduced in CTXO group on day 5, 14 and 30 compared to IUC group. PCR diagnosis confirmed the presence of *C. parvum* in all IUC feces from day 8 to day 50, while *C. parvum* was detected only on day 8 and 12 in CTXO samples. UUC group had the highest average body live weight (61.72 ± 0.91 kg). CTXO calves were significant heavier (59.97 ± 1.18 kg) than those in the IUC group (49.23 ± 0.93 kg) at the end. This study showed the interest of saponins and citroflavoids supplementation in newborn calves to mitigate *Cryptosporidium parvum* infection and revealing a positive impact on performance. Further studies with larger sample sizes are warranted to validate these results.

Supplementing a *Bacillus*-based probiotic improves rumen digestibility and fermentation responses in growing beef heifers

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This experiment evaluated the effects of a *Bacillus*-based probiotic (BAC; *Bacillus licheniformis* + *B. subtilis*; Bovacillustm; Novonesis, Lyngby, Denmark) on feed intake, in situ ruminal degradability, rumen fermentation and microbiota responses of beef heifers consuming a forage-based diet. Fifteen ruminally-cannulated Angus-influenced heifers were ranked by body weight (BW; 303 ± 7 kg) and assigned to receive no BAC supplementation (CON; n = 7), or BAC at 3.0 g/day (n = 8). During the experiment (day 0 to 35), heifers were housed in individual pens and received water and bermudagrass hay (*Cynodon dactylon*) for ad libitum consumption, in addition to 1.270 g/d (as-fed basis) of a corn-based concentrate. The BAC was mixed daily with the concentrate and offered to BAC heifers from day 5 to 35. Blood samples were collected on days 0, 7, 14, 21, 28, and 35 (4 h after feeding). Rumen fluid samples were collected on days 0, 14, and 28 (4 h after feeding). Ruminal in situ disappearance parameters of forage dry matter (DM) and neutral detergent fiber (NDF) were evaluated by incubating polyester bags with 4 g of dietary hay on day 32 for 96 h. Data were analyzed using heifer as experimental unit, and values from day 0 as covariate when appropriate. Hay intake did not differ between treatments ($P = 0.59$). Heifers supplemented with BAC had less ($P = 0.02$) acetate:propionate in the rumen fluid (3.97 vs. 4.44). Heifers supplemented with BAC had less ($P \leq 0.05$) prevalence of the bacterial genera *Bacteroides*, *Pedobacter*, and *Ruminococcus*, but greater ($P = 0.05$) prevalence of *Paludibacter* in the rumen. Heifers supplemented with BAC tended to have greater ruminal disappearance rate of hay DM (3.36 vs. 2.65 %/h) and NDF (4.00 vs. 3.10 %/h), and had ($P \leq 0.05$) greater effective degradability of hay DM (42.3 vs. 40.2%) and NDF (52.0 vs. 50.1). Heifers supplemented with BAC had greater ($P = 0.02$) mean plasma concentrations of glucose (72.7 vs. 68.9 mg/dL) and less ($P < 0.01$) mean plasma concentration of urea N (5.63 vs. 6.62 mg/dL). Collectively, BAC supplementation improved rumen digestibility and fermentation responses in growing beef heifers consuming a forage-based diet.

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Theatre 14

Effect of isoquinoline alkaloids on health, blood parameter, gut inflammatory status and growth in beef cattle during the adaptation period

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Natural compounds, such as isoquinoline alkaloids (IQs), with systemic and local anti-inflammatory, immunostimulant and antioxidant properties, can be an effective strategy to counteract the immunosuppression and proinflammatory conditions typical of the arrival period in beef cattle farming. A study was performed to test the effect of those IQs during the arrival period (60 days) on health, cortisol level, gastrointestinal inflammation and growth on a total of 30 Aubrac bulls, allocated in two groups: i) Control, standard diet (n=15, live weight: 359.5 kg; age: 10 months); ii) IQs group, standard diet + 30 g/head/d of the IQs blend (n = 15, live weight: 374.4; age: 9.7 months). Daily, the individual rectal temperature as well as the general health status and veterinary treatments were recorded. At the start, middle and end points, the individual weights and blood, and faecal samples were taken. Blood cortisol level and the faecal calprotectin and myeloperoxidase content are currently under evaluation, while the faecal pH was recorded instantaneously. Statistical analyses were performed using SAS software, applying specific models. The IQs animals showed a significantly lower and more stable rectal temperature during the first sixteen days (38.7 vs 39.1°C) ($P < 0.0001$), as well as a reduction in its increase in the following days after the boost vaccination at day 17. Also, there was a positive effect along the entire trial (38.7 vs 39.9°C) ($P < 0.0001$). The incidence of bovine respiratory disease (10 vs 26% of the animals treated) ($P < 0.05$) was significantly lower in IQs group and there was a tendency toward a reduction in the total treatment needed (4 vs 11 treatment) ($P = 0.08$). There was significant increase in the average daily gain (ADG) of IQs group during the first seventeen days (1.436 vs 1.143 kg/head/d) ($P = 0.0184$), without significant effects on the final weight or total ADG. At the end, the faecal pH tended to be higher in the IQs animals (5.97 vs 5.80) ($P = 0.057$). Those positive effects highlighted at systemic and gastrointestinal levels, suggest a better resilience and ability to cope with inflammation and stress during the arrival period, with a possible impact also on growth performance.

Assessing the impact of *Saccharomyces cerevisiae* boulardii CNCM I-1079 on performance in healthy – non-antibiotic treated pre-weaned calves

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Several studies have shown positive effects of dietary inclusion of *Saccharomyces cerevisiae* boulardii CNCM I-1079 (SC-1079) on gut microbiota and immune system of calves during challenging conditions. This probiotic is largely used in humans for the treatment of acute-infectious enteritis and antibiotic-induced gastrointestinal disorders. However, there are limited results regarding overall performance of calves not facing diseases. A multi country trial with the same experimental protocol was conducted in Poland, Greece, South Africa, and Canada to investigate the effect of a dietary administration of SC-1079 live yeast (1×10^9 cfu/kg of complete feed offered daily) on performance of healthy calves that were not subjected to antibiotic treatment. Across the countries, the average proportion of healthy calves ranged between 28% and 58% in the four studies. The final dataset included only the healthy pre-weaned calves (n=40 supplemented and n=35 control) daily supplemented on average for 68 days. A mixed-model ANOVA was conducted to compare the two groups, using study as a random factor as the interaction between study and dietary treatment was not significant. SC-1079 supplementation in calves prior to weaning significantly increased total feed intake by 5.30 kg DM (5.6% increase compared to control, $P < 0.10$). It also improved their final body weight, contributing to an average increase of 52 g/day in daily weight gain, equivalent to an 8.0% improvement ($P < 0.10$). However, it did not significantly affect the feed conversion ratio (2.37 vs. 2.50, $P > 0.10$) or fecal score (1.50 vs. 1.58, $P > 0.10$). This consolidated analysis demonstrates SC-1079's positive impact on calves not treated with antibiotics before weaning.

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Poster 16

Nutritional interventions to alleviate heat stress in dairy cows: A meta-analysis

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This meta-analysis aimed to examine the impacts of dietary interventions (DI) on dairy cows subjected to heat-stress conditions. A total of 41 articles were used. The data were analyzed according to random-effects models using metafor package in R where the study served as random effects and DI as fixed effects. Yeast and minerals and/or vitamins predominate the interventions, contributing to 37.8% and 30.9% of the datasets. The results indicated that heat-stress cows treated with *Saccharomyces cerevisiae* (SC) exhibited higher milk yield (MY), fat-corrected milk (FCM), energy-corrected milk (ECM), dry matter intake (DMI), milk protein, digestibility, and VFA concentration but feed efficiency and body temperature were unaffected. The improved of MY was noted on all types of SC including SC extract, SC postbiotics, SC cell wall, and Chromium-SC. Treatment with minerals and vitamins improved MY, FCM, ECM, milk fat (kg), and milk protein and did not affect DMI, feed efficiency, and body temperature with Chromium exhibited the greatest MY increased. Higher MY was also observed on heat-stress cows supplemented with postbiotics, betaine, and prebiotics while anion-cation, fiber, lipid, phytobiotics, starch, and rumen undegraded protein did not affect production and milk composition. Haptoglobin was lower on cows received SC and most of feed additives. Covariables such as country of origins, breed, parity, and year of study did not show substantial effect on the major parameters. However, high heterogeneity ($I^2 > 75\%$) in most outcomes across studies warrant a bigger dataset. Overall, despite promising results of the efficacy of several DI to alleviate heat stress in dairy cows were confirmed, studies on several DI were limited and requires further investigation or product improvement to uncover their potential.

Corn-wheat diets supplementation with β -Mannanase enhances reproductive performance of lactating sows and growth performance of suckling piglets

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Corn and soybean meals are the most widely used plant-based ingredients globally in pig diet formulations, but they contain 6% to 17% non-starch polysaccharides that are indigestible by monogastric. Concurrently, exogenous enzymes were used in animal diet to reduce the negative effects of antinutritional factors and promote greater digestion and assimilation of nutrients thereby improving health and growth performance of pig. Thus, this study aims to evaluate the effects of dietary β -mannanase supplementation on reproduction performance in sows and growth performance in their offsprings. For a period of 21 days, a total of 21 sows (Landrace $\times$ Yorkshire) were randomly assigned to 1 of 3 treatments with 7 replicates each. Dietary treatments were as follows: basal diet (CON), TRT1: CON +0.05% β -mannanase, TRT2: CON +0.10% β -mannanase. Experimental data were analyzed in a complete randomized block design using the GLM procedures (USA). Linear and Quadratic polynomial contrasts were used to examine the graded levels (0.05% and 0.1%) of β -mannanase supplementation. Dietary MAN supplementation has linearly decreased ($p = 0.015$) the body weight loss of lactating sows. While dietary supplementation of MAN has linearly improved the body weight after weaning ($p = 0.001$) and average daily gain ($p = 0.001$) of suckling piglets, and it has quadratic improved the body weight after weaning ($p = 0.066$) of suckling piglets. The total tract digestibility of dry matter and energy was linearly increased ($p = 0.036$ and $p = 0.024$) with the graded level of MAN supplement. Furthermore, a linear effect was increased ($p = 0.0301$) ($p = 0.007$) on the milk fat profile with the dietary inclusion of MAN at the end of day 10 and after weaning. In summary, our finding indicates that the dietary inclusion of MAN supplement with corn-wheat-based diet had a beneficial effect on the nutrient digestibility and milk profile of lactating sows and improved the growth performance of suckling piglets.

Daily-Phase Feeding Strategy Modulates Gut Microbiota and Metabolic Pathways to Improve Feed Efficiency in Growing-Finishing Pigs

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Feed efficiency is one of the most important issues for sustainable pig production. Daily-phase feeding (DPF) is a form of precision feeding that could improve feed efficiency in pigs. Gut microbiota can regulate host nutrient digestion, absorption, and metabolism. However, which key microbes may play a vital role in improving the feed efficiency during DPF remains unclear. In the present study, we used a DPF program compared to a three-phase feeding (TPF) program in growing-finishing pigs to investigate the effects of gut microbiota on feed efficiency. A total of 204 Landrace $\times$ Yorkshire pigs (75 d) were randomly assigned into 2 treatments. Each treatment was replicated 8 times with 13 to 15 pigs per replicate pen. Pigs in the TPF group were fed with a commercial feeding program that supplied fixed feed for phases I, II, and III, starting at 81, 101, and 132 d of age, respectively, and pigs in the DPF group were fed a blend of adjacent phase feed from 81 to 155 d at a gradual daily ratio and phase III feed from 155 to 180 d of age. Daily feed intake and body weight were recorded by a computerized device in the feeders. Feces and blood samples were collected from 1 pig per replicate at 155 and 180 d of age. The results showed that the DPF program remarkably improved the feed efficiency at 155 d ($P < 0.001$) and 180 d of age ($P < 0.001$), with a significant reduction of the intake of crude protein ($P < 0.01$), net energy ($P < 0.001$), crude fiber ($P < 0.001$), ether extract ($P < 0.01$), and ash ($P < 0.001$). The daily-phase feeding program increased the abundance of *Prevotella copri* ($P < 0.05$) and *Paraprevotella clara* ($P < 0.05$), while it decreased the abundance of *Ocilibacter* ($P < 0.05$) at 155 d of age. The results of correlation analysis indicated that the differentially abundant microbiota communities were closely associated with 20 metabolites which enriched amino acid and phenylalanine metabolism. Our results suggest that specific microbes may contribute significantly to feed efficiency and could serve as potential probiotics in traditional feeding strategies for pigs.

A Sustainable Approach to Boost Growth and Mitigate Nitrogen Emissions in Pigs by a low protein diet supplemented with probiotics

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Herein, we conduct two experiments (Exp) to investigate the effect of *Bacillus* with low protein diet on ileal digestibility in growing pigs (Exp.1) and growth performance, nutrient digestibility, gas emission and blood profile in finishing pig (Exp.2). In Exp.1. A total of 12 growing pigs with initial body weight (BW) of 20.79 ± 0.35 kg were surgically cannulated at the ileum terminal and randomly assigned to one of three dietary treatments. The test diets were as follows: LP- low-protein diet (13% CP); HP- high-protein diet (15% CP); and an LP diet supplemented with a probiotic mixture (*Bacillus licheniformis*: 5.1×10^8 CFU/g; *Bacillus subtilis*: 5.8×10^8 CFU/g; *Bacillus sp.* 1×10^6 CFU/g). Both experimental data were analyzed in a complete randomized block design, with a 2×2 factorial design using the GLM procedure of SAS. The addition of probiotic to LP diet showed significantly ($p < 0.05$) increased apparent total tract digestibility (ATTD) of dry matter, nitrogen (N), essential amino acid (EAA, leucine, phenylalanine, histidine, lysine, methionine) and non-EAA (aspartic acid, serine, proline, glycine), and the apparent ileal digestibility (AID) of N, EAA (isoleucine, leucine, histidine, lysine, methionine) and NEAA (proline, alanine) in pigs. In Exp. 2. $n=150$ finishing pigs with initial BW of 54.88 ± 1.28 kg was randomly assigned to the same dietary treatments as shown above. Pigs fed 15% CP along with probiotic had significantly improved ($p < 0.05$) BW at weeks 0-5, and gain-to-feed ratio at weeks 5-10, and average daily gain (ADG) during overall trail period. Additionally, it enhanced N digestibility. A diet with 13% CP significantly ($p < 0.05$) reduced ammonia and N_2O concentrations. Both LP diets and LP diets supplemented with probiotics resulted significantly ($p < 0.05$) lower blood urea nitrogen levels. In conclusion, feeding an LP diet with a probiotic mixture is beneficial for improving EAA levels in growing pigs and enhancing growth performance in finishing pigs thereby reducing fecal gas emissions.

Session 61

Poster 20

Effects of hydroxytyrosol, undernutrition in late gestation and breed on tocopherols, retinol and cholesterol in colostrum and milk

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Undernutrition at the end of gestation is common in beef herds. Olive waste supplements, rich in hydroxytyrosol (HT), may play a positive role in calf health when exported in colostrum and milk. The objective was to evaluate the effects of undernutrition during the last third of gestation (100% vs. 60%), HT supplementation (Control vs. HT, 180mg/kg) (+50% α -tocopherol, +110% γ -tocopherol, -30% lutein), and breed (Parda de Montaña (PA) vs. Pirenaica (PI)) on the profile of tocopherols, retinol, and cholesterol in colostrum and milk of beef cows. Colostrum was manually milked <12 h post-partum, and milk was machine-milked at week 3 post-partum. The freeze-dried samples were treated with mild saponification, liquid-liquid extraction, evaporation, and redissolution. The quantification of the analytes was performed by ultra-high-performance liquid chromatography with a fluorescence detector (tocopherols) and a diode-array detector (retinol and cholesterol). The levels of α -tocopherol were higher in colostrum from T60% cows (29.5 vs 18.8 ± 3.1 $\mu\text{g/g}$) and in milk from PA cows (5.8 vs 5.0 ± 0.3 $\mu\text{g/g}$) ($P < 0.05$). The levels of γ -tocopherol in colostrum were higher in PA (1.7 vs 1.2 ± 0.2 $\mu\text{g/g}$, $P < 0.05$), and in the T60%-HT group compared to the T100%-HT and T60%-CONTROL groups (2.2 vs 1.2 vs 1.1 ± 0.2 $\mu\text{g/g}$, $P < 0.005$). Additionally, T60%-PA showed higher levels compared to T60%-PI (2.4 vs 0.8 ± 0.2 $\mu\text{g/g}$, $P < 0.0005$). Retinol levels were higher in PA in colostrum (15.9 vs 11.2 ± 1.5 $\mu\text{g/g}$), while they were higher in PI in milk (1.5 vs 1.3 ± 0.1 $\mu\text{g/g}$) ($P < 0.05$). Finally, cholesterol levels in colostrum were higher in the T100%-PI and T60%-PA groups compared to T60%-PI (1.8 vs 1.8 vs 1.3 ± 0.2 $\mu\text{g/g}$, $P < 0.05$). In conclusion, cows have mechanisms to protect their offspring from the effects of undernutrition, mechanisms enhanced by HT, upon improving colostrum's antioxidant capacity. Similarly, colostrum and milk of the PA breed, shows greater metabolic adaptability to oxidative stress. Funded by PID2020-113617RR-C21 FETALNUT.

Guanidinoacetic acid and its impact on carcass traits of Nellore young bulls finished in the feedlot

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Guanidinoacetic acid (GAA) is an intermediate metabolite synthesized from L-arginine and glycine in the kidneys and transported to the liver for creatine synthesis, essential for muscle energy metabolism. Dietary GAA has shown benefits for energy metabolism and muscle growth in non-ruminants, but studies in cattle, especially zebu, are scarce. Therefore, this study aimed to evaluate the effect of GAA on carcass traits of Nellore young bulls finished in the feedlot. Fifty-one Nellore young bulls (initial body weight [BW] = 500.5±38 kg of BW; 18±2 months of age) were randomly assigned to blocks by BW into four treatments: control, without GAA (CON), and GAA3, GAA6, and GAA9, with 0.3, 0.6, and 0.9 g/kg DM of GAA, respectively. After 74 days of feedlot, shrunk BW were recorded before slaughter. Hot carcass weight (HCW) was measured post-slaughter, and cold carcass weight (CCW) after 24 hours of chilling (4°C). Carcass yield (CY; %) was calculated as HCW/shrunk BW ×100. The left half carcass was divided into forequarter, thin flank, and hindquarter. Additionally, pH and temperature of the Longissimus thoracis muscle were assessed at 1- and 24 hours post-slaughter. Data were analyzed by SAS proc MIXED for polynomial regression with a significant level of $P \leq 0.05$. Increasing GAA levels did not significantly affect shrunk BW ($P \geq 0.245$), HCW, CCW, or CY ($P \geq 0.122$). Likewise, the distribution of primal cuts, post-mortem pH decline, and carcass temperature ($P \geq 0.100$) remained unaffected by GAA supplementation. Under the experimental conditions, GAA supplementation did not affect carcass traits in Nellore young bulls. Keywords: Creatine, Muscle, Primal cuts

Session 62

Theatre 1

European Research Infrastructures to Support Livestock Science: Whom and How?

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Research Infrastructures (RIs) are key to advancing scientific discovery, pooling high-quality resources and expertise to support long-term research. By offering access to advanced equipment, methodologies, and training, RIs foster collaboration across laboratories and countries, facilitating the development and sharing of new tools and methods. European member states set up a European Strategic Forum for Research Infrastructures (ESFRI) since more than 20 years ago, to identify high-priority methods in all research domains. European commission in turn is opening calls to support the development of Infrastructure projects and has funded temporary RI projects for phenotyping in livestock species such as cattle, pigs, and fish, but there remains a critical gap in sustainable RIs for livestock science. This session aims to popularize the concept of RIs within the livestock scientific community, encompassing various disciplines such as genetics, physiology, and pathology. It will highlight current RI projects relevant to the community, including those focused on phenotyping and functional genomics, such as PIGWEB, AQUAEXCEL, EuroFAANG, and SMARTCOW. The session will also showcase established RIs in areas like data science (ELIXIR), biomedical models (BBMRI) and model species (INFRAFRONTIER) to demonstrate their added value. Ultimately, this session will collect feedback from the scientific community to inform the development of a new RI that integrates phenotyping with functional genome annotation, bridging the gap between genotype and phenotype for both terrestrial and aquatic species.

The Current Landscape of European Research Infrastructures: Where Do Livestock, Aquaculture, and Insect Science Fit In?

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From a policy perspective, European research infrastructures play a crucial role in shaping the future of health, food security, and sustainability across sectors. This session will provide an overview of the current landscape of established research infrastructures, particularly in the context of livestock, aquaculture, and insect science. The presentation will explore how these infrastructures contribute to policy development, innovation, and sustainable practices within the broader health and food sectors. Drawing on examples of established infrastructures, the session will frame the role of livestock science within these networks, emphasizing the importance of cross-sector collaboration, data sharing, and alignment with EU policies and strategic goals. By highlighting the policy relevance and impact of these infrastructures, the session aims to demonstrate their critical role in advancing science and supporting the sustainable development of European agriculture and food systems.

Session 62

Theatre 3

Biobanking for a Healthier World – introducing BBMRI-ERIC

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Background/Objectives: BBMRI-ERIC is the European Research Infrastructure for biobanking and biomolecular resources, supporting health and life sciences research across 24 Member and Observer countries and IARC/WHO. With 25 National Nodes, 473 biobanks, and affiliated partners, BBMRI-ERIC provides a unique framework for enabling responsible access to high-quality biological samples, data, and biomolecular resources. As a distributed Research Infrastructure, it fosters collaboration, advances innovative methodologies, and enhances interoperability across countries. **Methods/Results:** BBMRI-ERIC offers a comprehensive portfolio of services spanning Ethical, Legal, and Societal Issues (ELSI), Quality Management (QM), Information Technology (IT), Biobanking Development (BBD), Public Affairs (PA), Outreach, Education & Communication (OEC), and Finance and Project Management (FPM). Hereby, BBMRI-ERIC engages its community and partners in more than 23 active EU projects, including various projects for advancing cancer research, precision medicine, rare diseases and others. BBMRI's federated access and analysis platform has become a strong asset and reference point for major EU initiatives (e.g., EHDS, 1+Mio, GDI, EUCAIM) ensuring GDPR-compliant access to biological and health data from over 7 million individuals across 32 countries. BBMRI has recently published its vision for the next decade, which emphasises a “One Health” approach that connects human, animal, and environmental health. **Conclusions:** This presentation will showcase how researchers can access BBMRI-ERIC's extensive resources, highlight opportunities for collaboration across disciplines, and will introduce the 10-Year Roadmap.

INFRAFRONTIER: Cutting-Edge Human Disease Modelling

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INFRAFRONTIER, the European Research Infrastructure for Modelling Human Diseases, is a non-profit European consortium dedicated to advancing the understanding and treatment of human diseases through state-of-the-art models. Coordinated by INFRAFRONTIER ERIC, more than 20 cutting-edge biomedical research institutes across Europe and beyond constitute the INFRAFRONTIER Research Infrastructure, providing access to state-of-the-art human disease modeling resources and associated FAIR data. The core services of INFRAFRONTIER include model generation, systemic phenotyping of mouse mutants in participating mouse clinics, as well as the archiving and distribution of mouse mutant lines through the European Mouse Mutant Archive (EMMA), which contains more than 9,000 mouse strains. INFRAFRONTIER also offers specialized services such as the generation of germ-free mice (axenic service) for microbiome studies and training in state-of-the-art cryopreservation and phenotyping technologies. Supported by European Commission funding, INFRAFRONTIER is currently developing next-generation precision mouse models, cutting-edge in vitro models, machine learning-assisted data analysis tools, and advanced curation capabilities. With its extensive expertise, innovative services, and commitment to collaboration, INFRAFRONTIER continues to play a pivotal role in driving biomedical research forward, ultimately contributing to the advancement of human health.

Session 62

Theatre 5

SmartCow in the AgroServ project: Giving access to research services to advance the agroecological transition of cattle husbandry

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The SmartCow project 2018-2022 (H2020 N°730924; <https://www.smartcow.eu/>) integrated key cattle research infrastructures (RIs) for the first time at European scale to help their operators to develop synergies and complementary capabilities. This resulted in improved and harmonised research services, and supported 24 research projects led by researchers from academic and private sectors. Research activities in SmartCow aimed to increase phenotyping capabilities while implementing the 3R principles (refine, reduce and replace) in cattle physiology and behaviour studies. SmartCow now belongs to the AgroServ project 2022-2027 (HE N°101058020 <https://agroserv.eu/>) that gathers 11 recognised research infrastructures to provide the scientific community with large, high profile research services to facilitate and enable interdisciplinary and transdisciplinary research in agroecology and foster open science and innovation. The first project involving SmartCow in AgroServ is called EcoLotus and will test the hypothesis of common Lotus as a sustainable crop and valuable forage for cows on agroecological farms. The interdisciplinary study will cover: 1) field performance of common lotus in Serbia and Poland, including soil quality, land coverage, yield and quality of green biomass and hay of five cultivars differing with their genetic and geographical origin; 2) chemical properties of common lotus plants (proanthocyanidins and carotenoids) grown in extensive and intensive agricultural conditions at two locations, 3) the effect of different doses of common lotus in forage for cows on milk quantity and quality; 4) methane release by cows feeding with and without different doses of common lotus. The study will be done at three facilities (EMPHASIS: Plant&Soil Pheno, Serbia; EMBRC-ERIC: HCMR, Greece; SmartCow: INRAE IEPL Pegase, France) by two research teams from Polish Academy of Sciences: Institute of Plant Genetics (Poznan) and the Kielanowski Institute of Animal Physiology and Nutrition PAS (Jabłonna).

Open Science needs a more open livestock research community – the view of the coordinator of the PIGWEB project

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Livestock production and its research are in a somewhat delicate relation with society. On the one hand, citizens question the management of animals in production settings, the environmental impact of livestock production, and the use of animals in livestock research. On the other hand, even though consumption of animal-derived foods has declined somewhat in the past decade, many consumers still (want to) consume these foods. These issues and the need for research and related infrastructure concern the three pillars of sustainability, but where do we, as a livestock research community, stand in our relationship with society? I would say: “too much aside”. We have to be much more pro-active in engaging an open and constructive discussion with society about the future of livestock production, its research, and thus its research infrastructures. It is not only with society, but also within our community that we have to work on the “whom” and “how”. Many of us have been trained in a way that if we have a research question, the answer is to be found in an experiment. Animal experimentation (and the use of research infrastructures) is typically our first response, rather than the last resort to find the answer to our question. It is up to us if we want to change this and it has implications on how we train future generations of scientists and on how we interact with society. As an animal and veterinary community, we publish more than 10,000 peer-reviewed papers each year. It is hard to believe that part of the answers we are looking for cannot be found there. The enormous body of literature illustrates that there can be (or: should be) value in old data. This also means that, for the experiments that we carry out now, the data and biological samples will be very valuable. Sharing this through FAIR data and biobanks is thus essential. This also concerns the sharing the research infrastructures we now have or that we need in the future. The author is coordinator of the PIGWEB project, which received funding from European Union’s Horizon 2020 research and innovation program under Grant Agreement No 101004770. He expressed his personal view on the issue, which may or may not be shared by other PIGWEB participants.

Session 62

Theatre 7

GenoPHENix – a European research infrastructure to study genome and phenome interactions for sustainable animal production

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The European Strategy Forum on Research Infrastructures (RI) identified critical gaps for farm animal genotyping, phenotyping, conservation of genetic resources, and breeding necessitating a consolidated pan-European approach. To address this, the GenoPHENix RI builds on 1) the international FAANG (Functional Annotation of Animal Genomes) initiative and the INFRADEV EuroFAANG concept development project to advance genotype to phenotype research in farmed animals, and 2) the INFRAIA projects AQUAEXCEL3.0, PIGWEB and SmartCow that have been instrumental in advancing efficient and ethical species-specific phenotyping methods for farmed animals. The GenoPHENix RI aims to create a pan-European, multi-species, multidisciplinary platform for animal genetic resources, phenotyping, and sustainable breeding, focused on improving animal health, welfare, resilience, and efficiency. GenoPHENix will support cutting-edge research in animal science while contributing to Replacement, Reduction and Refinement (3Rs) principles in animal experimentation and help the livestock and fish sector to develop sustainable production systems. GenoPHENix RI will improve the leadership and excellence of European farmed animal research community by achieving 1) shared experimental capacity and access for genotyping and non-invasive large-scale phenotyping of farmed animals in complementary environmental conditions; 2) standardisation and FAIRisation of animal genome and phenome data; 3) expanded biobanking capabilities and access to animal genetic resources and cellular models, and 4) synergies with existing projects and other RIs to consolidate European farm animal research. GenoPHENix will provide over 300 experimental and laboratory services and a training program for young scientists. Next to the consolidation of the FAANG data portal, GenoPHENix aims to build new repositories on animal biosamples data and provide open access to phenotyping data.

Platforms and Methods for Sharing and Collaboration on Agricultural Genome to Phenome Using Public and Confidential Data

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Effective data sharing and collaboration are increasingly critical for validating research findings, enabling further studies, and integrating multiple datasets for joint analysis. However, these efforts are often hindered or entirely blocked due to limited visibility and accessibility of public data and the confidential or proprietary nature of private industry data. To bridge this gap and advance Agricultural Genome to Phenome (AG2P) research, with the ultimate goal of accelerating genetic improvement in agriculture, we have adopted a tiered approach: (1) Whenever possible, we promote the sharing of publicly available data. (2) When public sharing is not feasible, we develop encrypted data-sharing solutions to protect privacy while enabling research access. (3) In cases where neither raw nor encrypted data can be shared, we facilitate collaborative research approaches such as federated learning and transfer learning. Beyond methodology development and evaluation, we are providing educational resources on effective data sharing and collaboration in AG2P, as well as associated statistical and data science skills, to various audiences, including undergraduates, graduates, breeding professionals, and data-sharing stakeholders, such as field-based breeders. By expanding access to genomic and phenomic datasets through this multifaceted strategy, we aim to remove key barriers to public research data sharing and industry data utilization in agricultural research. Eliminating these obstacles is essential for advancing the field and achieving the long-term objectives of our project.

Session 62

Theatre 9

Creating common structure and data access services for animal agriculture in Europe in collaboration with ELIXIR

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There is a need to create a common structure and data access strategy for all the datasets generated for agricultural animals to consolidate research efforts and minimise redundancy across Europe. This includes both genomic and phenotype data and all the associated metadata relating to the animals and samples collected and how and where they are stored. ELIXIR (<https://elixir-europe.org>) coordinates, integrates and sustains bioinformatics resources across its member states and enables users in academia and industry to access services that are vital for their research. Through concept development for the EuroFAANG research infrastructure (RI) project we have explored the potential for a shared strategy for management and standardisation of animal agriculture data with ELIXIR. Through the ELIXIR Domestic Animals Genome and Phenome Focus Group we aim to meet the desire of the European research community and industry stakeholders for an agricultural animal science focused ELIXIR Community, filling a long-standing gap in the data coordination landscape. A highly standardised data submission and management system is currently in place in the form of the FAANG data portal (<https://data.faang.org/projects/EuroFAANG>). The data portal underpinned the data resources for the six H2020 EuroFAANG projects. For the full long-term impact of these, and other farmed animal science projects, to be realised access to the data that they are generating through the data portal must be extended beyond the duration of the funding for these projects themselves. Concept development for the EuroFAANG RI project has maintained the durability of the FAANG Data Portal for an additional three years ensuring the sustainability of a key resource for the farmed animal science community. Additionally, through the EuroFAANG RI project, and the development of GenoPHENix RI, we are working with ELIXIR on a concept for the GenoPHENix Data Portal that would incorporate submission and management of new data types including qualitative data in the form of, for example, phenotypic measurements and image data.

Strengthening Collaboration Between European Research Infrastructures and the Private Sector

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European research infrastructures are essential for advancing research and innovation in terrestrial, aquaculture, and insect breeding. As the European Forum of Farm Animal Breeding (EFFAB) and the Farm Animal Breeding & Reproduction Technology Platform (FABRE TP), we represent both public and private sector stakeholders, working to bridge the gap between research and application. Ensuring that infrastructures effectively serve the needs of breeding and animal science requires stronger collaboration with the private sector, addressing key challenges such as data accessibility, intellectual property management, and long-term sector engagement. This session will explore strategies to enhance public-private cooperation, making research infrastructures more accessible, industry-relevant, and sustainable. Through case studies and discussion, we will highlight best practices for fostering meaningful partnerships that drive innovation and ensure research infrastructures remain key enablers of progress in animal breeding and sustainable food production.

Session 62

Theatre 11

The EuroFAANG Research Infrastructure as platform to support new projects in farmed animal G2P research
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The EuroFAANG research infrastructure (RI) project aims to advance farmed animal genotype-to-phenotype (G2P) research, facilitating widespread collaboration and access to key services and cutting-edge resources to drive innovation across Europe. Six Horizon 2020 projects have synergized with the objectives of the EuroFAANG RI project to build an RI offering G2P services for a wide range of European stakeholders. The completed projects GENE-SWitCH (pig and chicken), BovReg (cattle), and AQUA-FAANG (six aquaculture species) collectively generated a vast amount of functional annotation data, metadata and protocols, as well as resources such as tissue samples, cell lines, and tools to refine genomic selection approaches and/or more precise identification of targets for genome editing. The ongoing projects GERO NIMO (monogastrics), RUMIGEN and HoloRuminant (ruminants) continue to develop tools that can be made available to the community via an RI, e.g. biobanks of tissues and cells from genome edited animals (Rumigen). Several projects have identified the access to refined phenotyping data and the lack of a centralized, organized 'one-stop shop' for priority cell lines and other in vitro models (e.g., organoids) as important current bottlenecks that, once available, will be of great benefit to future research endeavors.

Challenges to be addressed by a European Research Infrastructure for livestock science

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The session pointed out to some general challenges for a European Research Infrastructure (ERI). First, data anonymisation is needed to facilitate data sharing with industry, considering that industry collaboration is key to maximise the impact of an ERI on the livestock sector. Then, developing synergies with other ERIs is needed to benefit from their expertise, for instance in the field of phenotyping or biobanking, and to demonstrate to EU and Member States that there is no redundancy between a newly proposed ERI and existing ones. This is particularly important to motivate investment of Member States and of the EU commission as well, in order to support the implementation of a new RI. Embarking the research community is also key for an ERI, particularly by setting a regular workflow of new ideas and methodologies between RIAs and the ERI, in order to capitalize on results from RIA and to enhance services of the RI, following a continuous improvement approach. More generally, the most difficult challenge may be for the RI to address the threats that the livestock sector is facing, regarding climate footprint, global health and biodiversity erosion. A collaboration with social sciences for a transition towards circular bioeconomy is likely to be needed. Feedback from the academic and industry participants to the session is expected to help identify hypotheses or solutions to be tested thanks to ERI services. This should particularly involve the mechanisms supporting the elaboration of phenotypes from genotypes in a set of environmental conditions, at individual or population scale. Finally, while waiting for the new ESFRI roadmap to be published in 2026, the scientific community and the industry are invited to become accustomed to the method of work of an ERI and should collaborate more than ever to address the challenges above mentioned.

Session 63

Theatre 1

Young EAAP session – supervision, work-life balance

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The first part of our Young EAAP session will interactively address challenges and exchange opinions related to supervision. Supervision is an essential part of being a young researcher, either as being supervised or the supervisor. Both roles frequently come with challenges. For example, a supervisor can be perceived as too controlling or, vice versa, not caring enough. Similarly, a student being supervised by a young researcher can demand too much time, or is difficult to engage. In this interactive session, we will share views and stories on good and bad supervision experiences, as well as advice on how to tackle challenging situations. The second part of the session will address the topic of work-life balance. Young adults often face the challenge and pressure of multiple things being simultaneously expected from them: excelling in their careers, building a family, keeping up with hobbies and social life. The situation could be additionally aggravated since the goals often clash with each other. In academics, excelling career often comes with moving to different countries far from the social safety net and friends that puts work-life balance under more stress. During the session, we will go deeper into this topic by networking and share experiences from people that have been in such situations and could share advice on how to best deal with them.

A genetic pathway to reduce the impact of eosinophilic myositis in French cattle breeds

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Seizures in slaughterhouses due to eosinophilic myositis (EM) lesions are fortunately rare in France (~1‰ of females slaughtered between 2 and 10 years old) but show significant breed variability. In Blonde d'Aquitaine, this rate exceeds 5‰, with major economic consequences, partially covered by regional sanitation funds. In this breed, a 20% seizure reduction via genetic selection (from 6‰ to 5‰) could save over €300k annually. In the GMyosEo2 project, we: 1/ Built a phenotypic database (affected vs. non-affected animals) from compensated cases and slaughterhouse data across five French regions; 2/ Established a network of high-prevalence farms to create a reference genotyped population; 3/ Studied blood biomarkers to predict EM lesion development; 4/ Analyzed the genetic determinism of susceptibility/resistance to EM, developed a genetic evaluation, and conducted genome-wide association studies (GWAS) to identify associated genomic regions. Studied blood biomarker analysis did not reveal predictive parameters for EM lesions. It could be interesting to study other candidate biomarkers. Genetic analyses in Blonde d'Aquitaine and Normande breeds estimated heritability between 3 and 4%, focusing on farms with at least one seized female. Routine genotypes (EuroGMD 50k) were imputed to whole-genome sequences using Run 9 of the 1000 Bull Genomes Project. GWAS, performed with a linear mixed model adjusting for non-genetic effects, identified several highly significant genomic regions ($-\log(p)$ between 8 and >20). Candidate gene exploration is ongoing to better understand the genetic basis of EM susceptibility/resistance and support genomic selection to reduce seizure-related losses. The GMyosEo2 project is funded by APIS-GENE.

Session 64

Theatre 2

Prospection of resilience indicators and genetic analyses using longitudinal data in dairy sheep

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Resilience, defined as the aptness of an animal to be minimally affected by a disturbance or to return quickly to the expected level of functioning, is a trait of interest to livestock farmers. More resilient animals are associated with less handling and lower veterinary expenses, as well as with improved longevity. The aim of this study was to define, for the first time in dairy sheep, general resilience indicators using longitudinal daily milk yield records and to estimate their genetic parameters. After the quality control of the initial database of daily milking records, this study focused on the analysis of 357,372 records from a total of 1,777 first lactation ewes belonging to 4 Assaf sheep flocks of the ASSAFE association. A quantile regression was used to fit individual lactation curves. By using the deviations from these fitted curves, three resilience indicators previously applied in dairy cattle were estimated: the natural logarithm of variance (LnVar), the lag-1 autocorrelation coefficient (r_{auto}) and the skewness of the deviations (skew). Estimates of genetic parameters, heritability (h^2), and genetic correlations (r_g) between all possible pairs of indicators, and between indicators and average daily milk yield (ADMY), were obtained using the Gibbsf90+ software. Based on its h^2 (0.28 ± 0.07), LnVar would be the most appropriate indicator for assessing overall resilience in the Assaf breeding program. The r_g between the different indicators show that they could be considered genetically different traits ($r_g = [0.44-0.60]$). However, r_g between ADMY and resilience indicators suggest that selecting animals uniquely for high production could compromise the overall resilience of the animals. Further studies will be needed to confirm the validity of the indicators proposed here by estimating the r_g with other resilience-related traits such as longevity, fertility and health.

Genome-wide associations and potential candidate genes for additive-genetic and dominance effects of tail and body characteristics in Merinoland sheep

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Breeding on short tails is the only sustainable solution to combine both health and welfare perspectives, i.e., minimising faecal contamination and avoiding tail docking. In this regard, it is imperative to fully understand the underlying genomic mechanisms for tail length (TL) and tail characteristics, and to infer respective (co)variance components. In consequence, we performed an in-depth genomic analysis including TL, tail circumference (TC), number of caudal vertebrae (nVERT), tail abnormalities like block vertebrae (BLCKV) and wedged vertebrae (WDGV), as well as body length (BL) and body weight (BW) in Merinoland sheep using whole-genome sequencing (WGS). Therefore, 254 lambs from a specific selection and mating experiment to create phenotypically and genetically diverse subgroups for TL were phenotyped at birth and used for WGS. The statistical model for genome-wide associations and estimation of genetic parameters considered both the variant based additive-genetic relationship matrix and the dominance relationships. Variant-based heritabilities were large for TL (0.85), BL (0.93) and BW (0.85), moderate for TC (0.21) and nVERT (0.29) and close to zero for tail abnormalities. Dominance effects on TC and BW were negligible, but moderate dominance contributed to the phenotypic variation of TL (14.95%) and BL (14.90%). Breeding value correlations were strong and positive between TL and TC, between TL and nVERT and between TL and BL respectively BW. Correlations between TL and BLCKV and between TL and WDGW were negative, indicating a higher prevalence of tail abnormalities when breeding for short tails. Genome-wide association studies for additive-genetic and dominance effects identified 726 significant variants within potential candidate genes with known functions on the development of morphological traits, bone structure or vertebrae characteristics and on the skeletal growth, like CAMK1D and PPP6R3. The quite large additive-genetic variance and direct heritability for TL indicate great potential for genetic reduction of TL, but associated risks address the occurrence of tail abnormalities and reduced BW and BL.

Session 64

Theatre 4

Genetic analysis of detection and movement traits in laying hens using ArUco marker-based video tracking data

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Genetic analysis of behavior is challenging due to the labor-intensive nature of data collection. Modern video-tracking algorithms enable individual-level behavioral data collection, but consistent identification remains an issue, as physical similarities can lead to misassigned phenotypic records. This issue can be mitigated using ArUco marker-based video tracking. In laying hens, time spent in litter areas and movement patterns can serve as welfare indicators. Breeding values for these could help prevent unintended indirect selection. This study examined the feasibility of using ArUco tracking data for the genetic analysis of detection and movement traits in 1,441 Dekalb White hens across 12 groups (~130 per group). Each group was recorded for seven days, 11 hours per day. Individual positions were obtained for every frame. After filtering, over 400 million detections were recorded, averaging 8,710 per hen per hour, meaning hens were detected 34% of the time. Three hourly traits were genetically analysed: "Minutes detected," "Detected or not," and "Distance walked," all within the litter area. Without pedigree data, hens were genotyped using a 60K SNP chip, and genetic parameters were estimated using GREML. Variance components were estimated with univariate and bivariate linear mixed models, accounting for permanent environmental effects. Due to hourly trait definitions, heritability was low but strongly significant ($h^2 = 0.05-0.08$). Detection traits had higher coefficient of genetic variance (CVA = 0.04-0.19) than Distance walked (CVA = 0.003). Estimated breeding values (EBVs) showed moderate reported accuracy: Distance walked (0.68), Detected or not (0.68), and Minutes detected (0.71). The two detection traits had a strong genetic correlation ($rg = 0.97$), while Distance walked was negatively correlated with them ($rg = -0.36$ to -0.73). These results support the feasibility of estimating genetic parameters and obtaining accurate EBVs using ArUco marker-based video tracking data.

Stress hormones in hair as genetic indicators of stress response and disease resilience in pigs

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The potential of stress hormones in hair as genetic indicator traits for (disease) resilience was investigated. Detailed performance and disease resilience data were collected on 65 batches of healthy Yorkshire*Landrace barrows (n=4068) from 7 companies, by exposing them to a natural polymicrobial disease challenge at ~40 days of age. Hair was collected from the last 15 batches (n=863) just before exposure (H1), as well as its regrowth (H2) 42 days later. Levels of cortisone, cortisol, DHEA, and DHEA-S were determined. Using genotypes on >400K SNPs, heritability estimates were moderate for cortisol in H1 (0.33±0.10) and H2 (0.23±0.09), for cortisone in H2 (0.27±0.10), for DHEA-S in H1 (0.31±0.10), and for DHEA in H2 (0.15±0.09). Estimates for the other combinations were <0.04. For cortisol, a major QTL was identified near the glucocorticoid receptor gene that explained 45% of genetic variance in H1 and 38% in H2. This QTL was not associated with a causative mutation previously identified in this gene for cortisol in blood. Levels of some stress hormones were genetically correlated with some disease resilience traits in H2 and to a lesser degree in H1, but standard errors were large. An extra copy of the minor allele at the major gene (frequency=9%) was also favorably associated with some disease resilience traits. These findings demonstrate that stress hormones in hair from young healthy pigs are potential genetic indicator traits for response to stress and disease resilience. Also, stress hormone levels in hair from older pigs have potential as resilience phenotypes collected on pigs in commercial farms. Supported by Genome Canada, PigGen Canada, RDAR, and USDA-NIFA.

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Theatre 6

Genetic analyses of posture traits automatically recorded from video data of pigs

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Larger, cost-effective production units have led to less human-animal interactions in pig production. Together with rising consumer demand for animal welfare, this makes pig behaviour more relevant to monitoring in breeding programs. Breeding companies should ensure that selection does not alter behaviour negatively and that animals are calm, sociable and harmless, thriving in future production systems. However, measuring behaviour on the scale required for modern breeding programs presents challenges. An automatic recording system for behaviour from video data has been developed. The aim of the current study was to perform the first genetic analysis of inference data from this system. This includes posture traits and their relevance to production traits as a step towards identifying potential proxy traits for more complex behaviours. Modules for automatically detecting, tracking and identifying pigs were applied to 8 days of video data from 700 boars of a sire line. From this deployment, the following traits were predicted: Average minutes spent standing, sitting, eating, drinking and lying per day. Overall, the heritabilities for posture traits ranged from 0.06 – 0.12, with large standard errors. Genetic correlations to average daily gain, feed conversion ratio, loin depth at 120 kg and backfat at 120 kg were estimated, but no significant correlations were identified. However, the correlations ranged from -0.86 to 0.83. For instance, a negative genetic correlation was estimated between minutes spent sitting and average daily gain, suggesting that sitting behaviour may be linked to poor growth. This could be indicative of other health issues, like lameness. Similarly, bivariate genetic analyses between posture traits and ear and tail lesions were performed, but due to low frequency of lesions, the analyses did not converge. Looking to the future, prediction models for complex behaviours are under development for recording behaviours like tail- and ear biting and belly nosing. This study received funding from the Fund for Research Fees for Agricultural Products and Agricultural Agreement Research Fund (FFL/JA) through project number 321409.

Introduction of a novel random regression model for litter weights

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In litter-bearing species such as pigs, rabbits, sheep, dogs, or mice, measuring litter weight as a summary trait instead of individual birthweights is more cost-effective and less labor intensive. However, the genetic variance of litter weight depends on litter size because it summarizes all individual weights in a single value per litter. Previously introduced models cannot sufficiently account for the confounding of litter weight with litter size, nor did they include a sire effect. As a remedy, we propose a random regression model for total litter weight (LW), where the genetic effects of dams and sires are modeled as linear random regression slopes on litter size with potentially different variances. Restricted maximum likelihood estimation of variance components was conducted separately for four diverse mouse lines and results were compared. In total, there were 11,430 LW from 175,446 pups. The sire genetic variance component was significant in two lines. The random regression model implies that the heritability of the trait and reliabilities of estimated genetic effects depend on litter size, in contrast to previously used models. When averaged over each strain-specific distribution of litter sizes heritability for total LW was moderate to low at 0.27 ± 0.00 in the unselected control line FZTDU, at 0.43 ± 0.04 in the growth-selected line DU6, and 0.12 ± 0.00 and 0.12 ± 0.00 in the high-fecundity lines DUC and DUK, respectively. The proposed random regression model unravels the detailed use of the information on average birth weight enshrined in total litter weight data. The random regression model potentially can be applied in litter-bearing livestock to make selection for birth weight more cost-effective by using litter weight data.

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Theatre 8

Genetic correlation between environmental sensitivity to unrecorded challenges and economically important traits in a male pig line

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Pigs in production systems face both short- and long-term perturbations, including management practices, pathogen load, social interactions, and extreme weather etc., which impact productivity and welfare. Given the importance of resilience in modern production systems, it is necessary to develop selection methods that contribute to its improvement. In stressful days, there are more or less sensitive animals. This differential sensitivity (resilience) can be detected in the consumption of animals. We used daily feed intake (DFI) data from Piétrain pigs to detect different animal response profiles to disturbances. Variance components were estimated using the single-step GBLUP, using a series of multivariate reaction norm animal model with two phenotypes (DFI and each of the traits of economic importance), with the probability of an unrecorded challenge on the given day (p) included as an environmental descriptor for DFI only, because DFI is recorded daily but the other traits are not. This study revealed that the genetic correlation between the slope of DFI across a stress gradient and traits of economic importance for Piétrain varied across different environmental (stress) conditions. Results showed a strong negative genetic correlation (-0.82) between the slope of DFI and age at 100 kg (AGE) under non-stressful conditions ($p=0$), meaning pigs that grow faster (reach 100 kg sooner) tend to be more sensitive. As stress increases, this correlation weakens (from -0.82 at $p=0$ to -0.39 at $p=1$), with sensitive pigs not being capable to maintain growth, leading to increased AGE due to energy shifts from growth to maintenance. A strong positive genetic correlation (0.90) was observed between the slope of DFI and feed conversion ratio (FCR) under non-stressful conditions. The genetic correlation between the slope of DFI and FCR reduces as stress increases (from 0.90 to 0.62 at $p=1$). Selecting animals for improved feed efficiency (lower FCR) reduces sensitivity to environmental challenges. Conversely, selecting pigs for faster growth increases sensitivity.

Multi-trait social genetic analysis of ear damage, growth and mortality in pigs

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Social genetic models estimate the direct genetic effect of animals on their own phenotype and the social genetic effects on their pen mates' phenotypes. These models allow breeders to estimate the total heritable variance available for genetic improvement, and to select social animals that favourably affect their pen mates' phenotypes. Here we used a dataset of pigs ($n=21,715$) with ear damage (ED) scores to estimate direct and social genetic variances, which correspond to the victim and the performer effect of ED respectively. Furthermore, to estimate social genetic correlations, we combined the social genetic model for ED with those of average daily gain (ADG) and weaning to finishing mortality (W2FM) in bivariate models. In addition, in all our models we tested the importance of accounting for the effect of a pig's birth litter on the phenotype of its pen mates ("social litter effect"). We show that ear damage is a heritable trait with a (direct) h^2 of 0.03 (s.e.=0.006), and a ratio of total heritable variance over phenotypic variance (T_2) of 0.34 (s.e.=0.099), showing that most heritable variation in ED originates from social effects. We estimated a h^2 of 0.25 (s.e.=0.014) and T_2 of 0.51 (s.e.=0.064) for ADG, and a h^2 of 0.011 (s.e.=0.003) and T_2 of 0.012 (s.e.=0.008) for W2FM. Though T_2 was not higher than h^2 for W2FM, this results from a strong negative correlation ($r=-0.67$, s.e.=0.186) between the direct and the social genetic effects rather than a lack of social genetic effects. We found a moderate favorable genetic correlation ($r=-0.35$, s.e.=0.25) between the social genetic effect of ADG and ED, suggesting that pigs with a genetic predisposition to perform less ear biting also increase their pen mates' growth. We found no significant genetic correlation between ED and W2FM. Furthermore, we found that not accounting for the social litter effect biases the T_2 upwards for ED and ADG. In conclusion, all three traits showed significant social genetic variation, and the estimated social genetic correlation between growth rate and ear damage was favourable.

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New boar taint phenotypes for selection against boar taint

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In order to meet animal welfare requirements, non-castration can lead to the development of boar taint in whole males which results in the pork food quality breach. Genetic selection can address this problem, and selection up until now is performed based on phenotypes currently associated with this odor (i.e., androstenone, skatole and indole (ASI) with $h^2 \sim 0.5$). However, it has been recently postulated that broadening this phenotypic base, considered to cover major components but not all, could be beneficial for selection against this defect. This preliminary study was based on the previous identification of 13 volatile organic compounds (VOC) responsible for discrimination of boar taint by laboratory sensory evaluation (LSE). To evaluate the potential of this new phenotyping method, an extended dataset of 1272 boars including LSE ($n=1269$), ASI ($n=308$) and VOC ($n=127$) was established. Firstly, variance components of each variable were estimated and penalised by their estimated sampling errors to compute LSE genetic contributions. Genetic prediction equations (i.e., boar taint indexes) were established based on selection index theory. Then, final variance components were estimated for LSE and the 2 boar taint indexes (i.e., based on ASI and VOC) and resulted in final genetic parameters of these indexes. The genetic correlations of LSE-ASI index (0.87) and LSE-VOC index (0.96) showed that VOC were more representative of boar taint than ASI. Moreover, if confirmed, given these results and heritability of VOC index (0.65), using VOC for genetic selection could lead to a higher response to selection compared to LSE ($h^2=0.15$) and ASI index ($h^2=0.51$). In conclusion, this preliminary study indicated that using VOC, as a broader and more characteristic group of phenotypes, may result in a more focused selection against boar taint, and could be an alternative to ASI.

Swine inflammation and necrosis syndrome as a proxy for skin damage

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Lesions and injuries to the extremities of the pig's body parts, like the tail and ears, are commonly associated with biting. Lesion signs identified in 3-d-old piglets suggest that injuries might be caused by Swine Inflammation and Necrosis Syndrome (SINS), which can occur without any interaction with other pigs. This study investigated the genetic background of SINS in different body parts and its genetic correlation with Skin Damage (DMG) after weaning. We analyzed about 7k females born from February 2022 to June 2023 on three farms in the Netherlands. At birth, we scored SINS (no SINS=1, and SINS=2) on the ears, tail, and teats. Tail docking occurred after phenotyping. We defined the SINS total as the number of affected parts. The same animals were scored for DMG on the ears, tail, and flank on average three times during the rearing phase (no DMG=4, slight DMG=5, severe DMG=6, and very severe DMG=7), resulting in around 20k DMG records. We defined DMG total as the sum of scores in the three body parts. Single and two-trait animal models were used to estimate heritabilities and genetic correlations. Those models included a maternal effect for SINS. Direct (maternal) heritabilities for SINS were equal to 0.03 ± 0.02 (0.05 ± 0.04) for the ear, 0.03 ± 0.01 (0.01 ± 0.00) for the tail, and 0.08 ± 0.01 (0.04 ± 0.05) for total score. Direct heritability for DMG was equal to 0.07 ± 0.01 , 0.07 ± 0.01 , and 0.09 ± 0.01 for ear, tail, and total, respectively. SINS and DMG total were highly correlated with different body parts (≥ 0.70). The genetic correlations between SINS total and DMG in the ear, tail, and total were 0.78 ± 0.01 , 0.20 ± 0.05 , and 0.76 ± 0.01 , respectively. For DMG in the ear and total, the genetic correlation with SINS total was high. As the animals had their tail docked, this impacted the prevalence of DMG in the tail, resulting in a low correlation of this part of the body. Overall, our study indicates that reducing DMG through selection can be achieved by selecting against SINS during an earlier stage.

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The role of the blood transcriptome in the prediction of porcine immunity traits

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The blood transcriptome is closely linked to swine immunocompetence. Incorporating gene expression data in genomic prediction models is thus expected to improve the accuracy of predicting immunity-related traits. This study explores the potential of whole blood RNA-seq data to predict health-related phenotypes in a commercial porcine population. 255 eight-week-old Duroc pigs were phenotyped for several health-related traits encompassing adaptive and innate immunity and stress indicators. Animals were genotyped with a commercial chip and imputed to whole genome sequence level (~8 million SNPs). Gene expression levels were obtained by whole blood RNA-seq. Modelling strategies considered in this study were: GBLUP, which used genomic data; TBLUP, which used transcriptomic data; GTBLUP, which considered both as independent random effects; and the models GTCBLUP, GTCBLUPi and MBLUP, which accounted for the shared genetic component between both effects using different strategies. GTCBLUP and GTCBLUPi both conditioned transcriptomic data on SNP genotypes to make them independent, while MBLUP used a weighed strategy to both random effects. The inclusion of the transcriptome information improved model fitting for all studied traits. Moreover, models considering transcriptomic effects generally captured a higher proportion of phenotypic variance and demonstrated better accuracies in phenotype prediction. Among models considering the interaction between genomic and transcriptomic effects, only GTCBLUPi and MBLUP improved phenotype prediction accuracy, whereas GTCBLUP performed similarly to GTBLUP. Only the GTCBLUPi model showed a tendency towards better breeding value prediction, but the high dispersion prevented conclusive results. In conclusion, whole-blood RNA-seq data improves both model fitting and phenotype prediction of immunity and other health-related traits in pigs, the gain in accuracy depending on adequately modelling of the overlap between genetic and transcriptomic effects.

Phenotypic and genetic analysis of locomotion and social traits in turkeys based on long-term automated tracking with ArUco markers

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Automated monitoring of locomotion traits can help to detect animal health and welfare issues and to provide insight into the social tendencies of animals. In this study, we performed a genetic analysis of locomotion and social tendency traits in turkeys based on automated tracking with ArUco markers. We developed a system with 31 cameras to track 296 turkeys housed in a pen of 86 m². We attached a backpack with four ArUco markers to each turkey and tracked these markers from 5 AM to 5 PM for 7 days. From the observed tracks, we derived four traits related to locomotion and social tendency: (1) the proportion of time spent in locomotion, (2) speed during locomotion, (3) the average number of pen mates nearby (in a 1-meter radius), and (4) the density of pen mates ahead during locomotion, as a weighted sum of the pen mate density within 5 meters ahead. The average time spent moving ranged from 6.8% to 13.89% across turkeys and days, with speeds recorded between 0.36 to 0.56 m/s. The mean of pen mates nearby was ~10, with a standard deviation of ~0.6 across turkeys. Using an animal model for repeated records we found significant genetic variance for all traits, with heritability estimates of 0.15, 0.08, 0.23 and 0.23 for the four traits respectively, emphasizing the potential for selective breeding in turkey for such traits. We also observed high repeatability values, ranging from 0.65 to 0.96, indicating stable and systematic individual differences across all traits. Overall, this study represents the first automated analysis of turkey locomotion in a social context. The findings contribute to a deeper understanding of turkey locomotion dynamics as influenced by social interactions, paving the way for future research aimed at enhancing animal welfare and optimizing production systems in commercial turkey farming.

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Genomic Insights into AHPND Resistance in *Litopenaeus vannamei*: Heritability, Prediction Accuracy, and Candidate Loci

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Acute Hepatopancreatic Necrosis Disease (AHPND), listed by the World Organization for Animal Health (WOAH), has caused severe economic losses to the white leg shrimp (*Litopenaeus vannamei*) industry. To investigate its genetic basis, two challenge tests were conducted using two specific-pathogen-free (SPF) shrimp lines at Shrimp Improvement Systems in Florida. Using 37,285 single nucleotide polymorphisms (SNPs), we estimated moderate to high heritabilities for binary survival (BS) ($h^2 = 0.27-0.54$), indicating that genetic improvement for AHPND resistance is feasible. Genetic correlation analysis showed strong positive associations between survival genomic estimated breeding values (GEBVs) and growth traits, suggesting selection for survival could enhance growth. In contrast, survival GEBVs showed a moderate negative correlation with bacterial load, implying reduced pathogen susceptibility through selection. Prediction accuracies varied by validation approach: forward-in-time validation yielded lower accuracy ($r_a = 0.40$ for the fast-growth line, 0.13 for the slow-growth line), while backward-in-time validation improved accuracy ($r_a = 0.66$ and 0.38, respectively). K-means clustering cross-validation provided intermediate accuracy estimates but suggested potential bias in genetic effect estimation. These results emphasize the need for optimized genomic selection models. Multi-line and single-line GWAS identified loci associated with AHPND resistance, including genes linked to cytoskeletal regulation, epithelial integrity, extracellular matrix organization, and immune function. Notably, a SNP on LG 11 (AX-249610386) was consistently identified, highlighting a key genetic factor. This study provides insights into disease resilience in *L. vannamei*, supporting improved selective breeding and genomic selection strategies in shrimp aquaculture.

Inflammatory response in males from two rabbit lines divergently selected for resilience

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Adaptation to the environment is a key factor in animal welfare. Rabbits are highly sensitive to heat stress, which can increase susceptibility to disease. Inflammation is a physiological response to infection or tissue damage, with tumor necrosis factor- α (TNF- α) and C-reactive protein (CRP) serving as key biomarkers. Their levels indicate the severity of inflammation and disease. This study aimed to evaluate TNF- α and CRP levels in male rabbits from two divergently selected lines during winter and summer. A total of 40 (TNF- α) and 18 (CRP) males (50% per line) from generation 17 of a divergent selection experiment for litter size variability (HO and HE lines) were used. Rabbits were 4.5–10 months old, averaging 3.53 kg. Blood samples were collected twice: in September (heat stress) and February (thermal comfort). Samples (3 ml) were drawn from the auricular and central artery in the morning, centrifuged at 4000 rpm for 20 min, and serum was stored at -80°C until analysis. TNF- α and CRP levels were measured using ELISA kits. Statistical analysis included the effect of line-stress interaction (HO-stress, HO-comfort, HE-stress, HE-comfort), weight as a covariate, and animal as a random effect, using Bayesian methodology. The HO line exhibited lower TNF- α levels than the HE line under heat stress (56.8 vs. 69.1 pg/ml; $P = 98\%$) and a similar trend in thermal comfort (58.2 vs. 66.3 pg/ml; $P = 88\%$). Neither line was significantly affected by thermal conditions. Regarding CRP, the HO line had lower levels under heat stress (39.9 vs. 52.5 mg/ml; $P = 86\%$), but not in thermal comfort (43.0 vs. 41.3 mg/ml; $P = 57\%$). Both lines showed a trend towards CRP modification under heat stress ($P > 80\%$). The HO line exhibited a lower inflammatory response than the HE line, particularly under heat stress. CRP results complement TNF- α findings, and further samples are needed to confirm these results. We thank the institutions (European Union and Generalitat Valenciana) involved in AGROAL-NEXT/2022/037 that have provided the necessary funds (PRTR-C17.I1) for the execution of the project.

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Poster 16

Body temperature as indicator of robustness in the final phase of a divergent selection experiment in mice

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The outcomes of a divergent selection experiment for environmental variability of birth weight in mice have previously been presented. It was demonstrated that genetic control of environmental variability of birth weight is achievable through selection. Selection for homogeneity has been shown to improve litter size, embryo and foetal survival, birth and weaning survival, fertility, reproductive longevity, and the response to feed restriction, identifying signs of robustness and welfare. This selection method is already being applied in livestock species. The body temperature is a physiological indicator of health and stress in various species. The aim of this study was to analyse the body temperature of mice from two divergent lines as an indicator of robustness in the final phase of the selection experiment. The body temperature from low (L-line) and high variability line (H-line) was measured in 2 consecutive generations by infrared thermography (FLIR E6xt) at 7 moments: before mating, at mating, at 10 minutes, at 24 hours and 19 days after mating, at birth, at separation and after 10 minutes only for females. Differences between lines at the different measurement times were studied using the proc glm of SAS programme. Subsequent to mating, higher temperatures were observed, stabilising thereafter in both sexes. The temperature of L-Line males was significantly higher in the first generation at 19 days post-mating (35.3°C vs 33.9°C) and at separation (36.2°C vs 35.0°C). Significant differences were only observed at 10 minutes after male separation, with higher temperature in H-Line females (36.9°C vs 36.4°C). At birth, a significant stress-reducing event occurred if body temperature is considered as a stress indicator. However, no significant differences were observed between lines. Based on the benefits shown by the selection method, we will move to refine the experimental design to further investigate the benefits of uniformity in other traits. The selected mice lines remain available as animal model to perform studies in diverse scientific disciplines.

Genetic parameters for uniformity of birth weight in an Iberian pig population

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The heterogeneity of birth weight (BW) leads to increase in the farm management costs and in mortality during weaning, affecting the animal welfare. Selection for uniformity in pigs is of interest and its main objective is to gain an advantage not only in trait uniformity but also in reducing piglet mortality. The main selection criteria in this population have been litter size, but also piglet weight and piglet survival. Sánchez Romero Carvajal (SRC) company has implemented a recording system for individual birth weight in their farms since 2022. The objective of this study was to estimate genetic parameters for BW uniformity in the SRC Iberian pig population to promote its inclusion in the global selection index. Data contained 16705 records of piglets BW from 1731 sows from two linked farms. The pedigree file contained 2827 individuals. A heteroscedastic model was applied including sex (2 levels), parturition number (12 levels), litter size (13 levels), batch (23 levels) and feeding system (2 levels) as fixed effects. The litter effect beside the maternal genetic effect were included as random effect. Same effects were included for the mean trait and its variability. The analysis was performed using ASReml 4.4 and GSEVM. The genetic coefficient of variation for the residual variance (GCV) was 0.24 (0.05) being in the range of other pig populations. The genetic correlation between the mean BW and its variability was not different from zero meaning that no effect on the mean trait is expected when selecting for uniformity. Regarding fixed effects, females were more consistent than males, litter sizes from 4 to 11 showed less BW variability (with an increasing trend) than extreme values, and as parturition number increased from 1 to 7, so did residual variance. The next steps will be to determine the weight of this new selection objective in the global genetic index. Grant number: CPP2023-010423 (iberTOP).

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Poster 18

Identification method for unknown disturbances in growing pigs: a case study

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There are two ways to approach pig's robustness based on longitudinal data. One is to analyze the pattern of these data without previous knowledge on events that may have affected the animals, such as LSR. Another is to search for indicators of disturbances in the data and then perform assessment of robustness with that prior knowledge. Axiom boar testing station was equipped with electronic feeders (EFS) allowing daily monitoring of feed intake (DFI) and body weight (BW). For on-farm monitoring, alarms indicators, i.e. detection of abnormal events, were calculated from deviation of DFI or BW compared to the previous days for individual and pen level. In the case of low impact at individual level or missing human recording, alarms may not be emitted. The method UpDown (UD) was developed to identify disturbances and to estimate their intensity and duration with a high specificity at batch and/or pen level. UD was developed using simulated data and was validated using real data from a test farm. This study aimed at comparing the overlap of events detected by UD with alarm indicators recorded at Axiom boar testing station. Individual weight and individual feed intake from EFS of 7456 growers bred between 2019 and 2024 in 74 batches were analyzed with UD R-package with at least 4 pens per batches and 7 animals per pen. To identify disturbance with high technical impact, threshold parameter of UD was set at 95%. 5 batches were detected as disturbed for DFI and 0 batches but 3 pens detected for BW. As expected for a genetic farm with high sanitary status, a few groups appears as disturbed with 95% of individual affected. All groups detected by UD approach had alarms available, confirming a disturbance. UD detected global disturbances and their duration when alarm information caught only the most important variation happening usually on one day. UD is useful also to detect disturbance a posteriori independently of events registration. Such information could be used to interpret results from LSR from a more practical perspective.

Evaluation of skinfold thickness as phenotype for breeding against psoroptic mange susceptibility in Belgian Blue cattle

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The Belgian Blue (BB) cattle breed is the cornerstone of Belgium's beef industry due to its superior lean meat yield. However, this breed is highly susceptible to psoroptic mange, a skin disease caused by the ectoparasitic mite *Psoroptes ovis*. This condition results in severe dermatitis, intense itching, crusted skin lesions, hair loss, and secondary bacterial infections, significantly impacting animal welfare and causing economic losses. Anecdotal reports suggest a potential link between skinfold thickness and psoroptic mange susceptibility, as BB is described to have relatively thin skin. Furthermore, skinfold thickness is an easily measurable trait that can be assessed non-invasively and could be incorporated into a breeding program aimed at reducing mange susceptibility. Although previous research on 790 animals (period 2018 – 2019) revealed no significant correlation between skin thickness and lesion extent (LE) ($r = -0.04$), a refined phenotype collected on a new set of 260 cattle (period 2024 – 2025) showed more promising results. Skinfold thickness was measured at two dorsal sites using a Harpenden Skinfold Caliper (highly correlated measurements, $r = 0.85$). Mean skinfold thickness was 10.8 mm (SD = 1.5 mm) and ranged from 6.3 to 14.4 mm. Skinfold thickness showed a weak negative correlation with LE ($r = -0.17$), suggesting a potential relationship between thinner skin and higher lesion severity. A linear model indicated a negative effect of skinfold thickness on lesion extent ($p = 0.052$). Notably, significant differences in skinfold thickness were found between farms ($p = 0.00084$). These preliminary findings suggest that skinfold thickness could play a role as phenotype in the upcoming breeding program against psoroptic mange susceptibility in BB cattle.

Breeding of E.-coli resistant pigs: The end of post weaning diarrhoea?!

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Post weaning diarrhoea and oedema disease are porcine diseases, which cause significant economic losses. Both diseases emerge from infection with pathogenic *E. coli* bacteria. The decisive factor for severe infection courses is the presence of so-called adhesion factors (fimbriae). Those fimbriae match with special receptors in the mucous membrane of the small intestine and are decisive for the mass reproduction of *E. coli* bacteria. The presence of these receptors depends on the genotype of the animal. One approach to reduce diseases caused by *E. coli* bacteria of the fimbriae-type F 18 is to breed homozygous (A/A), resistant pigs. Animals with such genetic expression do not develop receptors; fimbriae of pathogenic *E. coli* cannot link and therefore pigs are resistant. The aim of our project (2022-2024) was to test the efficacy of *E. coli* resistant pigs on the occurrence of post weaning diarrhoea. To this end, the genetic status of all sows in the herd at our organically certified experimental station was determined, and the homozygous sows were selectively mated with homozygous sires in order to obtain homozygous offspring. After a suckling period of six weeks, we examined a total of 804 weaned piglets for four consecutive days and noted their faecal score to assess their susceptibility to weaning diarrhoea caused by *E. coli*. The faecal score was defined as follows: 0 = firm/shaped; 1 = no defined shape; 2 = watery. Statistical analysis of the frequency of the scores was done using SAS 9.4 proc glimmix, with a model including the effects of genotype, day, sex, birth weight, age at weaning, season and the interaction of genotype*day. There were no significant differences in the frequency of post weaning diarrhoea between homozygous and heterozygous piglets. With passing time, the occurrence of post weaning diarrhoea decreased significantly (day 2 > day 3 > day 4). Age at weaning had a significant effect on the frequency of post weaning diarrhoea, with less piglets receiving a score 2 when the weaning age was higher. To summarise the results of our study, the genotype of the piglets had no influence on post weaning diarrhoea but other factors had.

Nutrigenomics in Livestock: Advancing Precision Feeding and Animal Health

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Nutrigenomics, which explores the relationship between nutrition and the genome, is shaping the future of animal production. While dietary components influence gene expression, an animal's genotype also determines how nutrients exert their effects, contributing to the complex interplay between host genetics, the microbiome, and nutrition. Understanding these interactions enables precision feeding strategies tailored to livestock genetics and physiology, improving feed efficiency, disease resilience, and sustainability. Among various research areas in nutrigenomics, microRNAs—small, non-coding RNAs that regulate gene expression—are gaining attention for their regulatory functions and potential as biomarkers of health and disease. Circulating microRNAs in blood are emerging as promising diagnostic tools, allowing early detection of physiological imbalances in livestock and enabling targeted interventions to enhance welfare and productivity. In vitro models for livestock species such as chickens, pigs, cattle, and horses provide insights into how dietary components shape immune responses. These models help investigate nutrition-genetics interactions, particularly with bioactive compounds like probiotics and functional feed additives. Such research supports reducing antibiotic use and improving animal health through precision nutrition. Despite advances, implementing nutrigenomics faces hurdles, including the complexity of gene-nutrient interactions, species-specific variations, and translating findings into farm applications. Additionally, responsible use of large-scale genomic data raises ethical and regulatory concerns. This talk will explore the latest advances in nutrigenomics, emphasizing its potential to redefine animal nutrition, enhance health and welfare, and support sustainable production systems.

Session 65

Theatre 2

Exploring the link between blood transcriptome and plasma lipidome in pigs

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The present work is part of the METAPIGEN project, focused on exploring the relationship between porcine immunity and energy metabolism to identify biomarkers that improve immunocompetence without impairing production efficiency. A moderate genetic determinism of the porcine plasma lipidome has been stated in previous studies. The aim of the present study was to further investigate the genetic basis of porcine plasma lipidome by evaluating the genomic regions associated with the abundance of different lipid metabolites and their colocalization with regulatory regions of the blood transcriptome. Untargeted lipidome data from plasma samples of 300 commercial Duroc pigs was obtained through mass spectrometry, detecting a total of 982 metabolites. Genotypes of these animals were imputed at genome level from a commercial 70K GGP porcine array. A Genome-Wide Association Study (GWAS) was performed between the 8.5M filtered polymorphisms and the normalized lipid data using GCTA. Additionally, blood transcriptome RNA-seq data was obtained for 255 of these pigs and used to perform an expression GWAS analysis. A total of 123 genomic regions comprising 62,582 polymorphisms were associated to the abundance of 104 lipid metabolites. The genomic region with the strongest association was found on chromosome 8, associated with three different lipids. Several QTLs were associated to at least five lipid metabolites in porcine chromosomes 2, 8, 10, and 17. Further analyses allowed identifying 408 candidate genes within these regions, potentially involved in immunity, energy metabolism, or lipid metabolism. Colocalization analyses revealed the overlap between lipidome-associated genomic regions and blood transcriptome cis-eQTLs associated to the expression levels of 229 genes involved in metabolic or immunological functions, such as ST-3GAL1 or members of the FADS family. Our study found genomic loci and candidate genes linked to the porcine lipidome, highlighting the genetic interplay between lipids, metabolism and immunity.

Impacts of different crude protein levels on growth performance, cecal transcriptome, and cecal microbiota of weaning piglets

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This study aims to investigate the effects of different dietary crude protein (CP) levels on growth performance, cecal transcriptome, and cecal microbiota of weaning piglets. A total of 96 weaning piglets ([Landrace × Yorkshire] × Duroc) were randomly assigned to 3 groups: H group = 20% CP in the early phase and 18% CP in the late phase, M group = 18% CP in the early phase and 16% CP in the late phase, L group = 16% CP in the early phase and 14% CP in the late phase. Each group had 4 replicate pens of 8 piglets per pen. One-way analysis of variance with Tukey's honestly significant difference test was used for analyzing the statistical differences. The significance was defined as $P < 0.05$. Final body weight and average daily gain were lower than in the L group than in the other groups ($P = 0.008$). The feed conversion ratio was lower in the M group than in the L group ($P = 0.01$). Compared to the H group, transcriptome analysis identified 570 upregulated and 286 downregulated genes in the M group, and 82 upregulated and 119 downregulated genes in the L group. Heatmap and principal component analysis of differentially expressed genes revealed distinct clusters among the groups. Pathway enrichment analysis showed that genes related to cytoskeleton in muscle cells and cAMP signaling were upregulated in the M group, while genes associated with retinol and nitrogen metabolism were upregulated in the L group ($P < 0.001$). The proinflammatory response was downregulated in the M and L groups ($P = 0.027$ and $P < 0.001$). Microbiota analysis revealed bacterial richness and the abundance of *Lactobacillus amylovorus* DSM 20531 was higher in the M group ($P < 0.05$). Procrustes analysis indicated significant consistency between the cecal transcriptome and microbial composition ($P < 0.01$). In conclusion, differential modulation of cecal transcriptome and microbiota is observed in weaning piglets fed different dietary CP levels. Reducing the level of dietary CP from 20% to 18% in the early phase and 18% to 16% in the late phase exerted no effect on the growth performance of weaning piglets.

Session 65

Theatre 4

Modeling microbiome information in the genetic evaluation of finishing traits in pigs

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The pig gut microbiome can influence the pig's growth and feed efficiency. Given its potential impact, incorporating microbiome data into genetic models may increase the accuracy of breeding value estimation for those traits. In this study, we aimed to (1) estimate the proportion of phenotypic variance explained by the microbiome and (2) assess whether including microbiome information improves the prediction accuracy of breeding values for five finishing traits. A total of 1,391 pigs were analyzed, each with microbiome and genomic data from a 50K SNP panel. Phenotypic records were available for backfat carcass (BF), feed intake (FI), average daily gain from birth to slaughter (ADG_Bir), loin depth (LD), and average daily gain during the finishing period (ADG_Fin). After quality control, 48.4K SNPs and 4.6K operational taxonomic units (OTUs) were kept. A mixed model was applied to each trait, modeling the animal effect using a genomic relationship matrix (G). Additionally, models either included or excluded a microbial relationship matrix (M), resulting in two models per trait: G_wM and G_woM. For validation, data from the last generation (~20%) was masked, and prediction accuracies were estimated using the linear regression method. Except for LD, incorporating microbiome data reduced heritability estimates, suggesting a confounding effect between host genetics and microbiome influence on the traits. Furthermore, for most traits, microbiability explained approximately 30% of the phenotypic variance, often equaling or surpassing heritability estimates. For validation analyses, except for BF, including microbiome information improved prediction accuracy for all traits. Compared to G_woM, relative increases in prediction accuracy ranged from 14% for ADG_Fin to 21% for ADG_Bir. In conclusion, this study highlights the important contribution of microbiome information in explaining the phenotypic variance of finishing traits and its potential to improve the accuracy of breeding value estimation. In the presentation, we will explore how these results can be applied in the breeding program and the challenges involved.

Changes in the hepatic proteome of broilers induced by high-level extruded spirulina (*Limnospira platensis*) and enzyme supplementation

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Limnospira platensis, commonly known as Spirulina, is widely used as a feed supplement to enhance animal health and production. Studies on liver proteomics in broilers fed Spirulina inclusion diets are scarce. We investigated the effects of extruded Spirulina dietary inclusion, with or without enzyme supplementation on hepatic function in broiler chickens using proteomics. Differential protein expression analysis identified 25 differentially abundant proteins across four dietary treatments: control (CTR diet), SP (15 % Spirulina incorporation), SPE (15 % extruded Spirulina), and SPEM (15 % extruded Spirulina + 0.21 % enzyme mixture). Seven proteins had decreased abundance in the Spirulina-supplemented diets, while eight proteins had increased abundance, compared to CTR diet. Data are available via ProteomeXchange with the identifier PXD059769. Proteins with increased abundance in the control group were primarily associated with protein and lipid metabolisms, while those with increased abundance in Spirulina-supplemented diets were linked to carbohydrate metabolism and ATP production. These findings suggest that Spirulina dietary inclusion, particularly when combined with extrusion and enzymatic pre-treatments, modulates key metabolic pathways, potentially enhancing nutrient utilization and overall health in broilers. Nonetheless, further studies are required to elucidate these mechanisms and assess their broader implications for animal health and production. Acknowledgments: This research had financial supported by Fundação para a Ciência e a Tecnologia grants, Lisbon, Portugal (UIDB/00276/2020 to CIISA, LA/P/0059/2020 to AL4AnimalS, UI/BD/153071/2022 to M.S., UIDB/04129/2020 to LEAF). Also by the Portugal2020 project (P2020/17/SI/70114/2019) and associated researcher contract to M.C..

Session 65

Theatre 6

The interrelationship between Phosphorus utilization and metagenome in two high-yielding laying hen strains under low P supplementation

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Phosphorus (P) is an important nutrient in poultry nutrition. Organic P stored as phytates in plants is poorly utilized by non-ruminant animals. To compensate for this, poultry diets are often supplemented with either mineral phosphate or phytase enzymes. Given that mineral phosphate reserves are projected to be depleted, it is crucial to identify strategies for improving P utilization (PU) in poultry. Microorganisms in the gastrointestinal tract of laying hens might be involved in phytate degradation and PU. This study aimed to characterize the ileal microbiome of two high-yielding laying hens (Lohmann Brown Classic, LB; Lohmann LSL Classic, LSL) under low P supplementation and to investigate the association between ileal microbiota composition and PU variability. Shotgun metagenomics was applied to analyze the taxonomic and functional profiles of the ileal microbiome. Significant differences were observed between LB and LSL groups, indicating a strain-dependent effect on the ileal microbiota. At the species level, *Turicibacter* sp. TJ11 and *Streptococcus alactolyticus* were significantly more abundant in LB hens, while *Limosilactobacillus vaginalis* and *Limosilactobacillus reuteri* were enriched in LSL hens. In LB hens, *Turicibacter* sp. TJ11 and *Streptococcus alactolyticus* showed positive correlation with ileal InsP6 and P. In contrast, *Limosilactobacillus reuteri* in LSL hens was positively correlated with the concentration of plasma P and Ca as well as the ileal InsP6 and P. Functional analysis using Kyoto Encyclopedia of Genes and Genomes database revealed that pathways associated with Inositol phosphate metabolism were more enriched in LB hens, while pathways involved in mineral transport were more abundant in LSL hens. Our findings demonstrated the impact of strain on the composition of gut microbiota in laying hens fed a low-P diet. Furthermore, under low P supplementation, LB and LSL hens may utilize distinct physiological mechanisms to fulfill their P requirements.

The third generation sequencing reveals spatial variation of microbial composition of airborne bacteria in intensive dairy farm

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The intensification of livestock farming has led to increased bacterial bioaerosol emissions, posing potential health risks to both animals and humans. This study aimed to investigate the bacterial community composition, abundance, diversity, and variation in different functional zones of cattle farms to assess their impact on public health and environmental quality. We employed the third generation sequencing to sequence 16S rRNA sequences to analyze air samples, identifying a diverse range of bacterial phyla, including Proteobacteria, Firmicutes, Verrucomicrobia, Bacteroidetes, Fusobacteria, Actinobacteria, Deinococcus-Thermus, Cyanobacteria, and Acidobacteria. Notably, Firmicutes and Proteobacteria were predominantly enriched in the samples, with genera such as Staphylococcus, Enterobacteriaceae, Acinetobacter, Enterococcus, and Bacillus being particularly abundant. These bacteria are known to be associated with various infections and chronic diseases. Correlation and canonical correspondence analysis (CCA) revealed that environmental factors, particularly ultraviolet radiation (UV) and global horizontal irradiance (GHI), significantly influence microbial species distribution with R^2 of 0.774 ($P < 0.05$) and 0.769 ($P < 0.05$). We further calculated the alpha and beta diversity of microbiome in these samples and observed that fermenting manure (F), fresh manure (X) and piled-up manure after fermentation (D) samples have the highest alpha diversity, while PC1 from beta diversity i.e. weighted PCoA explained 32.66% of the variance in the data. Interestingly, it was observed that the relative abundance of Kocuria genus is significantly different between groups waste management area (FW) and the milking parlor (NT) (t-test, $p < 0.013$). Our findings underscore the importance of understanding the complex microbial ecosystems in livestock farming environments and highlight the need for targeted interventions to mitigate the public health risks associated with bacterial bioaerosols.

Session 65

Theatre 8

Multi-OMICS integration approach to assess the effects of prenatal nutrition in beef cattle: Insights into meat, subcutaneous fat and muscle

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This study investigated the long-term metabolic consequences of prenatal nutrition in Nelore bulls. A total of 126 pregnant cows were assigned to three dietary treatments: NP (mineral supplementation only), PP (protein-energy supplementation during the last trimester), and FP (protein-energy supplementation throughout pregnancy). At the finishing phase's conclusion, longissimus dorsi (muscle and meat) and subcutaneous fat samples were collected from 63 offspring. Muscle tissue was used to perform transcriptomics (RNA Sequencing), while meat and fat samples were subjected to targeted metabolomics analysis. Data dimensionality was reduced using Weighted Gene Co-expression Network Analysis. Significant gene modules ($p < 0.05$) and metabolite modules ($p < 0.1$) were functionally enriched using clusterProfiler and MetaboAnalyst, respectively. A holistic metabolic pathway analysis was conducted using MetaboAnalyst to evaluate the overall impact of prenatal nutrition in beef cattle. Pathways with an adjusted p -value < 0.05 were considered significant in all analyses. Across the -OMICS datasets, 11, five and nine significant modules were identified in the NP, PP, and FP group, respectively. The NP showed a greater influence on lipid-related biological processes and was strongly associated with protein metabolism. In contrast, the PP was primarily linked to stress resilience, immune function, and nutrient delivery pathways, with the FP displaying similar associations. Prenatal nutrition significantly influenced key metabolic pathways and hub components in important meat-producing tissues, including those involved in lipid and protein metabolism, immune function, nutrient delivery, and stress resilience. These findings offer valuable insights for meat production chain in beef cattle.

Colostrum and milk replacer intake influence blood metabolites in Holstein calves

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This study examined nutritional interventions and their impact on blood metabolites over time in Holstein calves. In the first 12h after birth, 60 bull calves (43.1±3.6 kg BW) were fed either 2L (Failure (F); 100g of IgG) or 6L (Successful (S); 300g of IgG) of colostrum replacer (CR) to contrast adequacy in transfer of passive immunity (TPI). Colostrum protocols were alternated at birth. Within 3 d, calves were transported to the research facility, where serum IgG was measured. Calves were blocked by CR protocol, arrival date and age, and within block randomly assigned to two milk replacer (MR) volume treatments: moderate (M; 0.250Mcal of ME/kg of BW^{0.75}) or low (L; 0.135Mcal of ME/kg of BW^{0.75}). This resulted in a 2×2 factorial design with 4 treatments: FM, FL, SM, SL (n=15/trt). Calves were individually housed, fed twice daily until d42, gradually weaned over two weeks and fully weaned by d57. Calves had ad libitum access to straw and water, and from d22 on, a pelleted starter was offered. To assess immunity, energy balance, and health, blood samples were collected weekly until wk 5, and bi-weekly thereafter until wk 11. Data were analyzed using PROC GLIMMIX in SAS, considering fixed effects of CR, MR, time, and their interaction, with block as a random effect. Time was included as a repeated statement. Serum IgG was greater in S calves (P<0.01). Calves in the S group, compared to F, had higher total protein (TP, P<0.01), glucose (P=0.02), and γ -glutamyl transferase (P<0.01), suggesting improved TPI and energy status. β -hydroxybutyrate concentration was influenced by MR intake (P<0.01), with L calves having higher levels, suggesting greater reliance on starter feed. Calves in the M treatment, resulted to have higher IGF-1 concentrations (P<0.01). Interactions between CR and MR feeding were noted for cholesterol (P=0.02) with SL calves having the lowest concentration. Creatinine (P<0.01) was higher in FL calves, while SM calves had the highest TP (P=0.04). These findings emphasize the importance of colostrum and MR supply in shaping the metabolic profile of calves, which may influence their short- and long-term development and health.

Session 65

Theatre 10

Changes in the rumen microbiome composition from the growing to fattening stages in Hanwoo steers

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This study aimed to comparatively analyze the rumen microbiome composition during the growing, early fattening, and late fattening stages in Hanwoo steers. A total of 52 Hanwoo steers were raised from the growing to fattening stages and were fed a diet consisting of rice straw and concentrate. Steers in the growing stage were fed a 50:50 ratio of rice straw and concentrate, while those in the early and late fattening stages were offered diets with 21:79 and 13:87 ratios, respectively. From the 52 Hanwoo steers, rumen samples were collected 3 hours after morning feeding using the stomach tubing method at the three stages: the growing stage (11 months of age, n=52), early fattening stage (18 months of age, n=52), and late fattening stage (24 months of age, n=52). Total community DNA was extracted from the 156 rumen samples (52 animals × 3 stages = 156) and then analyzed using 16S rRNA gene amplicon sequencing on the PacBio Sequel II system. The 16S rRNA gene amplicon sequences were processed and analyzed using the QIIME2 pipeline. Further analyses of sequencing data were performed using the web-based MicrobiomeAnalyst platform. The linear discriminant analysis of effect size was used to analyze differentially abundant taxa among the three stages. The Shannon and Simpson indices were significantly higher in the late fattening stage than in the growing stages (p<0.05). The principal coordinates analysis based on Bray-Curtis's dissimilarity indicated significant differences among the three stages (p<0.05). Among the three stages, *Prevotella ruminicola* was the most abundant in the growing stage, while *Butyrivibrio fibrisolvens* was the most abundant in the late fattening stage (p<0.05). *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Streptococcus lutetiensis*, *Lactobacillus ruminis*, and *Lachnobacterium bovis* were the most abundant in the early fattening stage among the three stages (p<0.05). The results of this study indicate that the rumen microbiome composition is influenced by different growth stages and the forage-to-concentrate ratio.

Differential transcriptome profile associated with water and heat stress tolerance in Rasa Aragonesa ewes
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Water scarcity and rising temperatures caused by climate change have a major impact on extensive and semi-extensive sheep production systems in the Mediterranean basin. In this study, high-throughput RNA sequencing was used to investigate transcriptomic profiling changes in blood, between 14 Rasa Aragonesa ewes with high (HT) and 14 low tolerance (LT) to water and heat stress (according to neutrophil/lymphocyte ratio) measured in a previous experiment. After adaptation, blood samples were collected from these 28 animals in Tempus tubes stabilised to prevent RNA degradation. RNA was extracted using the MagMAX™ kit for Tempus stabilised tubes and sequenced using Illumina MiSeq technology. Quality control was performed with FASTQC, and poor-quality adapters and bases were removed using TRIMMOMATIC. Alignment to the Rambouillet v2.0 sheep reference genome was conducted with STAR, while expression quantification was performed using HTSeq. Differentially expressed genes (DEGs) were identified with EdgeR, and functional enrichment analysis was carried out using Gene Set Enrichment Analysis (GSEA). The RNA-Seq analysis generated 1,658,061,150 clean reads and identified four differentially expressed genes (DEGs), with RASSF10 downregulated and FRMD4A, CCT6A and ADAMDEC1 upregulated in low-tolerant compared to high-tolerant ewes. GSEA of all expressed genes in the blood identified 27 overrepresented pathways at FDR 5%, predominantly related to biological process. Although these genes have no direct association with tolerance to water and heat stress, they may represent novel or indirect mechanisms contributing to water stress tolerance. Investigating these genes and the processes they regulate will enhance our understanding of the genomic mechanisms underlying water and heat stress tolerance in ewes.

Optimizing Nellore Beef Production in Tropical Climates: Multi-omics Insights into the Long-Term Effects of Creep-Feeding

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Tropical climate, with significant fluctuations in forage availability and quality, pose ongoing challenges to efficient beef cattle production. While the Nellore breed is naturally adaptable to these conditions, nutritional strategies protocols can improve performance and carcass/meat quality aspects. To explore this issue, we assessed the impact of creep-feeding during the cow-calf phase on the molecular profiles of the Longissimus thoracis muscle in feedlot-finished Nellore cattle. The study involved twelve 414-day-old feedlot-finished Nellore cattle, with six receiving creep-feed and six without, supplemented up to 240 days. Muscle samples from pre- and post-slaughter were analyzed to evaluate, respectively, impacts on the transcriptome (RNA-Seq); and metabolome, and lipidome profiling (MS/MS via MRM). Discriminant genes and small molecules were identified through multi-block sPLS-DA, with optimized variable retention based on CV error rates ($k = 3$, $n = 500$). Functional enrichment analysis was performed. In two components were identified 67 genes, 41 metabolites, and 85 lipids (across 7 classes) as key discriminators. Pre-slaughter transcriptional changes in PRKAG2 (AMPK), STEAP3 (Fe⁺ metabolism), and BCL2A1 (apoptosis suppressor). Creep-feeding cattle showed PRKAG2 down- and STEAP3 upregulation at pre-slaughter, indicating lower lipolytic activity as an adaptive energy response, possibly enhancing anaerobic glycolysis and pH decline post-mortem evidenced by higher levels of ATP, ADP, glyceraldehyde-phosphate, and fructose-phosphate, favoring proteolytic activity. Additionally, BCL2A1 downregulation correlated with increased sphingomyelins, sphingosines, and phospholipids in creep-feeding cattle, indicating higher proteolysis. The upregulation of DNMT1 in non-creep-feeding, potentially regulated by STAT3/TP53 TFs, can suggest epigenetic control of muscle/adipose energy metabolism responses of creep-feeding associated to post-weaning high-energy diets.

Exploring IgA-coated bacteria as biomarkers of rumen health during dietary transitions in cattle

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Maintaining rumen health is essential for the productivity and well-being of dairy cattle, yet non-invasive biomarkers for its assessment remain limited. Immunoglobulin A (IgA) is a key mucosal antibody, which can bind bacterial cells. However, its specificity toward pathogenic versus commensal bacteria is poorly understood. This study measured IgA-coated bacterial populations in saliva, rumen fluid, and feces during a dietary transition to a high-grain (HG) diet, which improves productivity but increases the risk of subacute ruminal acidosis (SARA). Additionally, phytogenic feed additives (PHY) were tested for its potential to alleviate SARA-related dysbiotic symptoms. Eight rumen-cannulated, non-lactating Holstein cows were studied in a cross-over design. After one forage-only (FOR) week, they transitioned over one week to a 65% HG diet, maintained for four weeks. All diets included PHY or no PHY. Sampling occurred at FOR and HG weeks 1 and 3. IgA-coated bacteria were quantified via flow cytometry and sorted by magnetic-activated cell sorting (MACS), followed by 16S rRNA sequencing. HG feeding altered IgA-coating patterns. In feces, IgA-positive MACS-sorted fractions showed reduced *Roseburia*, *Dorea*, *Raoultibacter*, and *Anaerostipes*, while rumen samples showed *Succiniclasticum* and *Methanobrevibacter* depletion and *Thermoclostridium* enrichment. In rumen fluid and saliva, IgA-coated *Acetobacteroides* decreased, while *Blautia*, *Prevotella*, and *Syntrophococcus* increased. PHY supplementation maintained diversity in IgA-coating patterns during the transition to HG feeding, particularly by limiting the increase of IgA-coated *Streptococcus* in saliva. This suggests a modulation of acidogenic taxa dynamics, highlighting PHY's potential in mitigating dietary-induced dysbiosis. The results open up promising opportunities for non-invasive health monitoring and potential targets for innovative prevention strategies.

Session 65

Poster 14

Effect of supplementation during the pre-weaning phase on the plasma proteome of Nellore calves

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Creep-feeding is a nutritional management strategy used to provide supplemental feed to suckling calves in a restricted-access. Its goal is to produce heavier animals at weaning, improve carcass quality and increase meat marbling. It may also improve calf health and reduce mortality. There is a lack of research evaluating the effects of creep-feeding on gene expression and metabolism in Nellore cattle (*Bos indicus*), the most important genetic group in Brazil's beef herd. This study aimed to identify differences in protein abundance, biological processes, and metabolic pathways in the plasma of Nellore calves that were either supplemented with an energy-protein diet or not during the pre-weaning phase. Ninety-six non-castrated male Nellore calves, offspring of four bulls, were divided into two groups: SM (n= 48) – no creep-feeding; CF (n= 48) – creep-feeding. Plasma proteomes were generated by Label-free LC-MS/MS shotgun, with blood samples randomly collected from six individuals in each group. The raw mass spectrometry data were analyzed using PatternLab software for total protein identification. PLS-DA analysis was used to assess whether the sample groups could be statistically discriminated, and the t-test was applied to identify proteins with abundance differences. Differentially abundant proteins with a VIP score > 1.0 were further investigated regarding protein-protein interactions (PPI), as well as biological processes (BP) and signaling pathways (PW), using the STRING software. Body weight, average daily gain, and rib-eye area differed between the groups (CF > SM: p < 0.05). A total of 84 proteins were identified, with 49 showing differences in abundance (log2FC ≥ [2.0] and p adj. ≤ 0.05), and 30 having a higher impact on the discriminant model. The PPI analysis highlighted the BP: Defense response; Immune response; and Response to stress, as well as the PW: Regulation of complement cascade; Hemostasis; and Immune system. These results support the use of creep-feeding in cattle to strengthen the immune system and, consequently, improve performance and animal welfare.

Changes in histone modification induced by maternal poor nutrition in fetal calf liver

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[Background] Maternal undernutrition (MUN) during gestation disrupts fetal liver and muscle development, potentially through epigenetic mechanisms such as DNA methylation and histone modifications. Our previous studies on Japanese Black (JB) fetal calves revealed significant hepatic metabolic and transcriptomic alterations due to low nutritional treatment, but the role of histone modifications remains unclear in MUN effect on beef cattle. This study investigated histone modifications in JB fetal liver using chromatin immunoprecipitation sequencing (ChIP-seq) with anti-H3K4me3 and anti-H3K27 antibodies. [Methods] Pregnant cows received either 60% or 120% of the standard dietary energy and nutrients according to the Japanese Feeding Standard for Beef Cattle (2008 ed). Liver sample preparation for ChIP-seq was performed, followed by next-generation sequencing (NGS) and data annotation. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to identify functional implications and correlations with previous gene expression data. [Results] Gene promoter H3K4me3 is associated with biological terms, such as Cortisol synthesis and secretion, PPAR signaling pathway, Cushing syndrome, and Aldosterone synthesis and secretion. Meanwhile, gene promoter H3K27me3 were associated with Glutamatergic synapse, Gastric acid secretion, and Synaptic vesicle cycle. Promoter H3K4me3 levels in fetuses of low nutrition (LN) were higher in IRF2BP2, PPP1R3B, QRFP, and GDF15, but lower in APOA5 and ANGPTL4. The H3K27me3 levels in LN were higher in KHDRBS1, SREBF2, and SRSF4 than high nutrition (HN) fetuses. Furthermore, genes with >1.5-fold change exhibited the anticipated up/down-regulation from elevated/reduced H3K4me3 levels. [Conclusion] This study provides insights into epigenetic regulation in response to MUN, potentially contributing to the identification of molecular biomarkers for optimizing beef cattle nutrition and meat quality.

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Poster 16

Effect of residual feed intake on the fecal microbial community of Hanwoo steers

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The objective of this study was to investigate association of residual feed intake with the fecal microbial community of Hanwoo steers. A total of 63 Hanwoo steers (21 months of age) were raised on the same farm and fed the same TMR diet. All steers were ranked based on their RFI values and then divided into two groups: the L-RFI group (low RFI, top 10% efficient steers, n=6) and the H-RFI group (high RFI, the bottom 10% inefficient steers, n=5). Fresh fecal samples were collected before the morning feeding, and microbial DNA was extracted using the repeated bead-beating plus column method. The bacterial V3-V4 region of the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. Bioinformatic analyses were performed using the QIIME2 and MicrobiomeAnalyst 2.0 platforms. The Linear discriminant analysis (LDA) Effect Size (LEfSe) analysis was used to evaluate differences in major taxa between the two RFI groups. No significant differences were observed in the alpha diversity indices (Shannon and Simpson) between the two RFI groups. Principal coordinate analysis based on the unweighted and weighted UniFrac distance matrices revealed no significant differences in the microbial community structure between the two RFI phenotypes. Genera *Acetivomaculum* and *Kandleria* were more abundant in the H-RFI group than in the L-RFI group ($p < 0.05$). *Acetivomaculum* primarily produces acetate as a fermentation product, while *Kandleria* mainly produces lactate and some acetate. Inefficient steers may have lower rumen digestibility than efficient steers, which could lead to higher excretion of undegraded feed components into the colon. These two genera seem to increase by fermenting feed components that remain undegraded in the rumen and pass into the colon. On the other hand, *Akkermansia* was more abundant in the L-RFI group than in the H-RFI group ($p < 0.05$). *Akkermansia* helps maintain the integrity of the intestinal barrier and supports immune function by enhancing intestinal mucus secretion. This improvement in gut health may be associated with enhanced feed efficiency in steers. Overall, these findings suggest that fecal microbial taxa could serve as potential biomarkers for estimating feed efficiency in Hanwoo steers.

Longitudinal Analysis of DNA Methylation in Dairy Calves

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DNA methylation plays a crucial role in gene regulation and development, yet its temporal dynamics in live-stock remain largely unexplored. This study investigates genome-wide DNA methylation changes in dairy calves over five key developmental stages: at birth, six months, one year, 18 months, and after first parturition. Blood samples were collected at each time point and analysed using the RUMIGEN methylation array. Missing values were imputed using the MICE algorithm. Principal Component Analysis was performed to explore variance in methylation patterns, revealing clustering trends over time. A linear mixed-effects model was applied to assess the impact of age on CpG methylation levels, with Bonferroni correction for multiple testing. Of the 8,572 CpG sites analysed, 35.04% exhibited increased methylation, while 64.96% became hypomethylated. Gene mapping identified several genes associated with differentially methylated CpG sites, providing functional insights into key biological processes. In cellular regulation, genes such as KCNN3, STK17A, and PARP1 influence excitability, apoptosis, and DNA repair, impacting udder health, while PPAT is crucial for cell growth during lactation. In mammary gland development, NFIA, BCL2L11, and TUSC3 play key roles, with CCDC102A and FAM3D showing potential involvement in this process. In immune response, GATA3 and IRF2BPL contribute to immune function and may influence mastitis susceptibility, while STK17A and PARP1 also play immunological roles. Lastly, in milk production, MYO9B may regulate milk secretion, and the microRNA bta-mir-2284c influences gene expression related to milk synthesis. These findings provide new insights into the epigenetic regulation of growth, immune function, and lactation in dairy cattle, highlighting potential biomarkers for future breeding and management strategies.

Session 66

Theatre 2

The impact of chronic heat stress on DNA methylation and gene expression in laying hens

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Environmental stressors, such as heat, can induce epigenetic modifications, including changes in DNA methylation, which may influence gene expression and contribute to phenotypic variability. This study examined the effects of chronic heat stress on liver DNA methylation in laying hens and investigated whether these changes may mediate genes expression. We used Reduced Representation Bisulfite Sequencing (RRBS) for genome-wide, single-base resolution DNA methylation profiling to analyze patterns of heat-induced methylation variation. We used RNA sequencing (RNA-seq) data to analyze differential gene expression between control and heat stress hens. A total of four hundred NOVOgen commercial brown egg layers were housed in two temperature-controlled chambers (200 hens each) under thermo-neutral conditions (22°C). At 27 weeks of age, the temperature of one chamber was increased to 32°C for four weeks. Liver samples were collected at 31 weeks of age from 20 animals per group for RRBS and RNA-seq analyses. Differential methylation analysis identified 4824 DMC (Differentially Methylated CpG sites) and 418 DMR (Differentially Methylated Regions). The majority of the affected CpGs were hypermethylated and distributed across most chromosomes. In total, 1120 genes were associated with DMCs. The transcriptomic analysis revealed 3578 differentially expressed protein coding genes (DEG) with 1747 genes upregulated and 1831 downregulated under heat stress. Comparing these two gene lists, we found that 269 of these DEGs harbored DMCs in promoter, exon, or intron regions. Interestingly, the large majority of these were upregulated and 27 of them were transcription factors. The functional enrichment analysis of these genes highlighted an enrichment for biological processes and molecular functions related to regulation of transcription, RNA metabolic process, and DNA-binding. Funding : ANR ChickStress, GERO NIMO (H2020 GA No 101000236).

Transgenerational response to an endocrine disruptor ingestion: phenotypic, genetic and epigenetic analyses in quail

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Typical prediction of an offspring's phenotype usually focuses on the inheritance of parental alleles. However, variations across generations can also result from the transmission of non-genetic factors. Epigenetic marks such as DNA methylation offer a dynamic molecular signature of an individual's history, as they can carry the memory of the individual's environmental past, and probably of its ancestors. In this study, we aim to analyze in quail (*Coturnix japonica*) the impact of an initial supplementation with an endocrine disruptor, the genistein, on the phenotypes and epigenotypes of subsequent generations. To better disentangle the complex interplay between genetic and epigenetic effects, we designed a "mirror" breeding plan to ensure a balanced genetic structure between the group of animals issued from the treated ancestors (called epilene +) and the control group (epilene -). By recording multiple phenotypes (growth, breeding, production, behaviour) across three generations, and performing Reduced Representation Bisulfite Sequencing on blood samples for 1344 individuals, our goal is to investigate the transgenerational inheritance of an environmental effect through changes in DNA methylation profiles. Here we present the preliminary results obtained from phenotypic analysis (analysed with multiple linear regression and GLMM) and RRBS data (analysed with classical tools: BISCUIT, TrimGalore, DSS). Phenotypic analyses have shown multi and transgenerational effects for several phenotypes of interest including body weight. We are now looking to identify methylated patterns linked to phenotypic changes between generations and between groups. Funding: Région Occitanie, INRAE Animal Genetics Division, GEroNIMO (H2020 GA No 101000236).

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Theatre 4

Characterization of DNA methylation patterns and genomic regulation of DNA methylation of skeletal muscle in three commercial pig breeds

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Commercial pig breeds are intensively selected for specific phenotypic differences in fertility, growth, feed efficiency, and meat quality. Understanding the molecular basis of these traits has accelerated genetic improvement over the past decades. Advancements in sequencing technologies now enable the study of molecular phenotypes, such as DNA methylation, a dynamic epigenetic modification that plays a crucial role in transcriptional regulation. In this study we investigate breed-specific DNA methylation patterns in skeletal muscle. Biopsies were collected from longissimus dorsi of Duroc (N = 151), Landrace (N = 162), and Yorkshire (N = 167) pigs underwent reduced representation bisulfite sequencing (RRBS). Reads generated were quality-checked (FASTQC), trimmed (TrimGalore), aligned to the pig genome, deduplicated, and analyzed for methylation (Bismark). CpGs with ≥10 reads in >80% of pigs per breed, and individuals with ≥50% coverage of filtered CpGs were used for downstream analysis. Using tensorQTL, we associated cis-regulatory variants (±1 Mb from CpG) with methylation variation, identifying 35,052, 35,275, and 19,259 independent meQTLs in Duroc, Landrace, and Yorkshire, respectively. Most identified meQTLs mapped to open sea regions (distant from CpG islands) and gene bodies. Breed-specific regulation was predominant, with minimal overlap in CpG-SNP associations across breeds (none shared by the 3 breeds, 19-43 shared by breed pairs). Among ~71K unique eCpGs, only 3,632 were shared across all breeds, primarily in open sea and gene body/intergenic regions. These findings highlight the distinct epigenetic regulation across commercial pig breeds, suggesting a relevant role of DNA methylation in economically important traits. Future work will incorporate production traits and gene expression to investigate the effect of methylation patterns in different traits relevant for sustainability and profitability in pig production. Furthermore, the potential integration of multi-omics data in phenotype estimation will be evaluated.

Unravelling gene switches for the regulation of litter size through the study of swine with high and low prolificacy

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Between 2012 and 2022, the genetic potential for total number of born piglets increased 2.8 per litter in commercial pigs. Alentejano pigs, from Southern Portugal, have low litter size with an average of 5 piglets providing an opportunity to investigate different gene interactions related with litter size and characterize the biological mechanisms associated with this trait. An experiment was carried out to investigate the effect of breed and season in the gene expression levels of the uterus of Alentejano (AL) and commercial Landrace x Large White gilts at early gestation. Per breed the levels of thyroid hormones and gene expression of uterine tissue collected in three experimental conditions, diestrous (not inseminated), estrous (inseminated, 2 time point of gestation) for two seasons (bi-factorial design) were compared. Results showed that the effect of breed and season was significant, and T3 and T4 serum concentration are higher in AL gilts ($p < 0.001$). The results at gene level showed significant differences allowing to identify candidate genes for further downstream analyses. The identified gene networks reveal connections between the endocrinological and the neurological system. In conclusion, through the study of breeds that have not been intensively for a given trait enables the investigation at molecular level of how genetic variation determines the epistasis and pleiotropic effects on polygenetic traits.

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Theatre 6

Finding evidences for genetically transmitted epigenetic effects in Spanish Holstein-Frisian due to heat stress

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Climate change is challenging livestock production systems. Heat stress has been described as a factor that trigger epigenetic changes in dairy cows. These changes may or may not be transmitted across generations depending upon when those epigenetics changes occur and the existence of reprogramming. The objective of this work was to identify evidences of inter-transgenerational effects in Spanish Holstein-Friesian cows affecting production traits of the three first lactations associated to heat stress during gestation. Production data of 1,268,564 cows from 2010-2023 provided by CONAFE were used. The underlined hypothesis is that epigenetic marks are generated by the exposition of great-granddams (G0) to heat stress while they were conceiving grand-dams affecting their primordial cells of grand-dams (G1) as fetus and transmitted onto dams (G2) primordial cells and finally onto cows (G3) thus conditions that triggered the epigenetic marks do not persist along generations. Statistical models used EBVs for milk, fat and protein yields as well as conception rate at first insemination of G3 cows as dependent variable. Two models G2 or G1 EBVs, calving date of G2 or G1 and thermal loads as a broken-line function influencing the conception and gestation of G2 or G1 dams, respectively. Thermal loads were represented by the average of degrees over the average daily comfort temperature (24°C) around conception (30, 60, 90 d afterward) and the end of gestation (30, 60, 90 prior to calving). Losses caused by inter-transgenerational effects on protein and fat were more relevant than on milk production. Losses during the first lactation of G3 cows were the least relevant. G3 cows whose grand-dams were conceived and exposed 90 d to more than 24°C of average temperatures produced -20 kg/C° of 305 milk, -1.32 kg/C° of protein and -1.85 kg/C° of fat. These figures represent losses of 1.3%, 2.8% and 3.5% over the mean for milk, protein and fat along the gradient of temperatures. Results highlight existence of epigenetic effects due to thermal stress. Epigenomic will help to assess transmission of epigenetic marks. This work has received funding from H2020 under grant agreement n°101000226 (RUMIGEN).

Constructing haplotype-separated assemblies for Norwegian Red cattle

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High-quality reference genomes improve our understanding of genome structure and enables the identification of genetic variants associated with traits of interest. We have constructed the first set of haplotype-separated assemblies for Norwegian Red (NR) cattle using long-read sequencing and a trio-binning method. This method uses parental sequences to label offspring reads from the maternal and paternal haplotypes, creating fully haplotype-resolved assemblies, which give a more accurate representation of complex SVs and haplotype-specific alleles than assemblies representing both haplotypes in a collapsed manner. Three trios were selected from the NR population. Short-reads (Illumina) were collected from parents and long-reads (PacBio, ONT) were collected from offspring. Computational simulation of parental sequences from genome-wide panels of SNP genotypes was also tested as an alternative way of generating parental data. Offspring long-reads were assembled with Hifiasm using both Illumina reads and simulated sequences as parental input. All six haplotypes, assembled with Illumina reads as parental input, show high continuity (N50 > 73 Mbp) and completeness (BUSCO > 95%). Mapping of bovine satellites suggests that more of the cattle centromere has been assembled than in previous references. Counting the number of haplotype-specific k-mers in the final assemblies shows that only a small fraction [0.46 – 2.52%] are found in the wrong haplotype, suggesting successful separation of offspring sequences into haplotypes. Using simulated sequences as parental input produces assemblies with similar quality and haplotype-resolution as using Illumina short-reads. This enables global haplotype assembly without additional sequencing of parental genomes in livestock species where genotyping is done routinely. A genome-graph will be constructed from the collection of haplotype assemblies, providing a valuable resource for studying genetic diversity and structural variation across the NR population. Funding: European Union's Horizon 2020 research and innovation program, grant no. 101000226 (Rumigen), Geno and NMBU.

Session 66

Theatre 8

Considering variances and correlations of offspring breeding values shows beneficial effects on genetic gain and antagonistic correlations

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Determining gametic Mendelian Sampling variances of potential parents allows to predict the variance of breeding values of their future offspring. Several studies have shown that considering these predicted variances in selection decisions can increase realized genetic gain. Most of those studies considered only a single trait. Our objectives were to investigate if in the case of multi-trait selection including variances and correlations of breeding values of future offspring in selection decisions can increase genetic gain, and what the impact of this approach is on the genetic correlation between traits. To achieve this, we used the gametic Mendelian Sampling variances and correlations, which explain observed genetic variances within traits and correlations between traits among the offspring of individual animals. Given that individual differences in gametic Mendelian Sampling correlations have been observed, we hypothesized that using those correlations in selection decisions may affect future antagonistic genetic correlations. We considered one generation of selection, by simulating one parental and one offspring generation. We directly simulated bivariate distributions of breeding values for two traits, and then performed selection of the parents based on: breeding values (current practice), or selection based on the so-called Index5, which in addition to the breeding values also included individual gametic Mendelian Sampling variances and/or individual gametic Mendelian Sampling correlations. Including Mendelian Sampling variances and correlations in selection resulted in up to 2% more genetic gain compared to ordinary selection on breeding values, while it was able to change the unfavourable genetic correlation in the next generation from -0.30 to -0.25. When the correlation between traits was ignored, the correlation was unaffected and less genetic gain was achieved. These results provide a first indication that use of individual Mendelian Sampling correlations in selection decisions can be beneficial for genetic gain and may help to mitigate effects of antagonistic genetic correlations.

Estimating true Identity By Descent relationships using high density marker data

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Molecular genetic measures of relationship and inbreeding, such as the genomic relationship matrix (GRM), are unbiased if the markers are not under selection. However, genomic selection and genomic optimal contribution selection (GOCS) use the markers to manage relationships, which violates the assumption of marker genotypes being randomly sampled given the relationship structure. GOCS restricting GRM relationships, which measure genetic drift relative to reference allele frequencies, favors the loss of rare alleles, since this limits genetic drift. GOCS restricting molecular coancestries, which measure homozygosity, favors intermediate allele frequencies, since this minimizes marker homozygosity. The latter however implies substantial genetic drift. In linkage analyses, the markers are merely used as indicators of paternal or maternal inheritance, which is not affected by the markers being under selection. Given maternal/paternal inheritance, a Gla relationship matrix can be set-up, which resembles the pedigree relationship matrix A, but uses from the marker data inferred segregation indicators instead of 50/50 probabilities. Where segregation indicators are unknown, still 50/50 probabilities may be used. In gene dropping simulations using the map of BTA15 and a Norwegian Red Cattle (NRF) pedigree, Gla relationships were closer to the true relationships than other genomic estimates of relationship and inbreeding. When all animals were genotyped, the correlation between Gla and true relationships was 0.99. In commercial NRF data, Gla relationships were highly correlated to pedigree relationships (0.97), because segregation indicators were often unknown and because the cow has relatively many chromosomes such that segregation patterns over the entire genome more closely resemble those of pedigree-based expectations. The GRM relationships show a lower correlation to A (0.81) and to Gla (0.83), which may in part be because GRM relationships also reflect family structures that predate the pedigree's founder population.

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Theatre 10

Do national and regional differences matter? On the societal and ethical dimensions of genomic innovation in four European countries

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The GeroNIMO project aims to integrate sustainability and breeding of farm animals through genomic innovation. Work Package 5 addresses the ethical and societal aspects of breeding innovations, with a focus on genome editing. Although there has been some debate on the societal dimensions, the debate on genome editing has mainly involved experts rather than the general public. Furthermore, animals have played a minor role and the discourse has been largely technical and scientific. In addition, ethical discussions have often followed a consequentialist logic, whereas the literature shows that there are also other types of arguments/ concerns, e.g. related to virtues, duties or relations. To address these gaps, we conducted focus group discussions with general public in four countries, preceded by semi-structured interviews with experts in animal breeding, advocacy and policy-making. The interviews aimed to explore national dimensions of the public debate on genomic innovation and animal husbandry in general. Eight focus groups were then conducted in Germany, the Netherlands, France and Slovenia with over 70 participants, representing rural and urban groups. Participants were selected based on criteria of age, gender, level of education and meat-eating habits. Discussions covered topics including the relationship between humans and animals, the future of agriculture and genomic breeding innovations. This approach not only measured public opinion on these issues, but also contributed to a broader public dialogue. The focus groups revealed moderate differences in attitudes towards genome editing between the four countries. Germany and the Netherlands appeared to be more open to new technologies, while Slovenia and France were more sceptical. A particular concern in Slovenia was the protection of local breeds. Despite the differences, there were commonalities, particularly regarding the importance of animal welfare. The presentation will present the results and discuss how national and regional concerns can be integrated into public debate and European policy on innovations in animal breeding.

The 'Room of Acceptance': what defines Europeans' views on genetic adaptation of animals?

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The societal acceptance of genetic adaptation in ruminants is a critical factor influencing the success of breeding innovations within the agricultural sector. This presentation explores the "Room of Acceptance" method employed in the RUMIGEN project to assess public perspectives on breeding approaches across eight European countries. Utilizing the EuropeSay method and the EngageSuite online platform, structured citizen consultations engaged 771 participants in deep, reflective discussions about genome and epigenome-enabled breeding in ruminants. The "Room of Acceptance" method offers a novel approach to understanding societal boundaries and conditions for acceptance, revealing the values and motivations behind public opinions, which in turn can inform breeding strategies. This talk will address how the method's insights contribute to aligning breeding strategies with societal values, ensuring that innovations not only advance scientific and agricultural goals but also resonate with public expectations. By emphasizing the value-laden nature of acceptance criteria, This talk aims to bridge the gap between scientific innovation and societal readiness, giving insight into how future innovations might be implemented. The results indicate a broad spectrum of societal views, with acceptance levels influenced by perceived ethical considerations, transparency in breeding practices, and alignment with broader sustainability goals. Key findings include a general openness to genetic adaptation when framed within ecological and animal welfare contexts, contrasted by reservations regarding perceived unnaturalness and potential long-term impacts.

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Theatre 12

Epigenetic Biomarkers and Regulatory Mechanisms of Heat Stress Sensitivity in Dairy Cows

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Heat stress affects dairy cow performance, yet the underlying epigenetic mechanisms driving individual responses remain poorly understood. This study aims to identify heat stress-induced DNA methylation variations in the blood methylome that may influence regulatory regions and associated phenotypes. Thirty-six Holstein cows in late lactation/gestation were monitored during the summer in Western France and classified based on their sensitivity to acute heat stress, considering environmental conditions, behavioural and physiological indicators. Two distinct groups with contrasting heat stress sensitivity were identified ($n \geq 8$ per group). Blood samples were collected before and after an acute heat stress event (average daily THI > 74 for four consecutive days). Blood methylomes were analysed using reduced representation bisulfite sequencing and differential analysis was conducted comparing cows before and after heat stress in high-sensitivity (HS) and low-sensitivity (LS) groups. HS group showed a higher number of differentially methylated cytosines (DMCs) compared to LS group (616 vs. 33 DMCs, respectively). Among the 616 DMCs, 32,79% colocalised with regulatory elements and several were colocated with genes involved in T cell receptor signalling and immune response regulation. Interestingly, an intronic hypomethylated DMC was identified in a super-enhancer of the AKT1 gene that plays a crucial role in T cells fate and function and also induces HSF1 expression; a key regulator of the heat shock response, enhancing its transcriptional activity under heat stress. These results suggest that DNA methylation changes in regulatory regions, especially those related to immune pathways, could play a role in individual differences in heat stress resilience. Intergenerational effects are being investigated using the DNA methylation BeadChip (EpiChip). This project has received funding from the European Union's Horizon 2020 Programme for Research & Innovation under grant agreement n°101000226 (RUMIGEN).

From Genomic Applications to Societal Perception: Discussion and Feedback

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The final discussion of the “From Genomic Applications to Societal Perception” session will involve a wide range of subjects from epigenetics, genomics, quantitative genetics, and societal dimensions in implementing genomic advancements in livestock. Building on insights from presentations covering DNA methylation under environmental stress, transgenerational effects, and ethical considerations across Europe, this interactive conversation will explore how these scientific breakthroughs can be applied in real-life practices to meet sectoral and consumer needs. Participants will dive into the practical applications of genomic research—examining how genomic applications can guide breeding strategies to improve resilience and diversity while aligning with consumer expectations of improved sustainability and animal welfare. The discussion will tackle key challenges, from bridging the gap between research and farm-level application to addressing cost concerns, regulatory hurdles, and public perceptions of genomic technologies. By bringing together different actors from the livestock sector, this session fosters a collaborative exchange of ideas on how genomic and epigenetic innovations can drive value in European livestock production.

Session 66

Poster 14

Assessing the genetic contribution to intramuscular fat content in local Krškopolje pig

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Krškopolje pig is known for its advantages in meat quality traits, particularly its high but variable intramuscular fat (IMF) content. However, the genetic contribution to IMF using genotypic data has never been studied before and is crucial for evaluating the feasibility of developing a breeding program for the Krškopolje pig. DNA samples along with phenotypic data on carcass and meat quality have been continuously collected from Krškopolje pigs, out of which a total of 356 animals were selected. Samples were genotyped using the Choice Genetics custom-SNP array. SNP-based heritability, as well as genetic correlations between IMF, backfat thickness, and muscle thickness were estimated using the GREML method in GCTA software. Three models were used to estimate the genetic and environmental contributions to the studied traits, incorporating either fixed effects of 1) farm and age, 2) farm per season and age, or 3) farm per season. Preliminary analyses showed that all models exhibit similar heritability estimates for IMF (0.36-0.38), and backfat thickness (0.28-0.29). The genetic correlations between IMF and backfat thickness were -0.21 ± 0.28 for the model 1, and -0.08 ± 0.30 for models 2 and 3. The heritability for gluteus medius muscle thickness was 0.29 for model 1, and 0.19 and 0.21 for models 2 and 3, respectively. The genetic correlations between IMF and muscle thickness were 0.04 ± 0.26 , 0.03 ± 0.33 , and 0.16 ± 0.31 for model 1, 2, and 3, respectively. All models present relatively similar preliminary heritability estimates, but there is considerable uncertainty in the genetic correlations due to large standard errors. These estimates were, however, obtained from a relatively small number of animals but currently include all available phenotypic data for the Krškopolje pig. Therefore, additional phenotypic data should be further collected to improve the reliability of the estimates. Acknowledgements: Funding by Slovenian Research and Innovation Agency (grants P4-0133, Z4-60178, J4-3094) and project GeroNIMO (EU H2020 GA no. 101000236) are acknowledged.

Enteric methane emissions from cows at different stages of lactation grazing perennial ryegrass pastures in spring

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Current enteric methane (CH₄) emission estimates for dairy cows in New Zealand are based on a standard CH₄ conversion factor of 21.6 g CH₄/kg dry matter intake (DMI), however, feed type and quality alter CH₄ production. Emerging data show that CH₄ production from cows in pasture-based systems varies seasonally. This variation is attributed to changes in pasture quality and composition; but it is often confounded by the cows' stage of lactation. The objective of the study was to quantify CH₄ emissions from cows at different stages of lactation offered spring perennial ryegrass-based pasture (PRG). Forty multiparous Holstein-Friesian dairy cows were assigned to a 1) early (mean $\pm$ SD; 32 $\pm$ 9 days in milk; n = 20), or 2) late lactation (186 $\pm$ 14 days in milk; n = 20) herd. Cows were individually fed zero-grazed PRG for 3 weeks via a Calan Gate feeding system equipped with GreenFeed technology. Milk production, DMI and CH₄ emissions were measured daily, milk composition and liveweight were measured weekly. Statistical analyses were conducted using R (R Core Team), with a linear mixed-effects model (lmer), followed by ANOVA and pairwise comparisons using 'emmeans' package; data are presented as mean $\pm$ SEM. There was no effect of stage of lactation on daily CH₄ yield (17.51 $\pm$ 0.29 vs 17.33 $\pm$ 0.28 g/kg DM, P = 0.66 for cows in early and late lactation respectively). However, compared to cows in late lactation, cows' in early lactation had a lower CH₄ intensity (134.31 $\pm$ 5.28 vs 184.26 $\pm$ 5.17 g/kg milksolids, P < 0.01; 10.41 $\pm$ 0.42 vs 14.63 $\pm$ 0.41 g/kg energy correct milk, P < 0.01) and a greater daily CH₄ production (348.63 $\pm$ 6.43 vs 330.33 $\pm$ 6.30 g/d, P = 0.05). These data indicate that the current CH₄ conversion factor in the New Zealand national inventory is overestimating CH₄ production from cows grazing good quality spring PRG, and this response is not dependent on stage of lactation. Further work is needed to evaluate pasture composition and lactation stage in other seasons to develop CH₄ conversion factors specific to New Zealand's pasture-based dairy systems.

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Theatre 2

Automated Head-Chamber System (GreenFeed) to Measure Methane Emissions from Dairy Cows Fed Fresh Pasture in Metabolism Stalls

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To develop effective methane (CH₄) mitigation strategies for ruminants, appropriate methodology to quantify CH₄ emissions is crucial. One such method is the Automated Head-Chamber System (AHCS) (GreenFeed, C-Lock), which measures CH₄ and CO₂ mass fluxes from the animal's breath. While AHCS is commonly used in indoor facilities where animals are fed a total mixed ration (TMR), and scheduled and voluntary visitations are well established, its application in cattle fed fresh forages has focused on voluntary access, in longer-term studies. The objective of this study was to demonstrate the use of AHCS under a short-term scheduled visitation regime in forage-fed cows to determine whether it can provide representative emissions estimates over time. A subset of data was used from two 10-d metabolism stall studies, where cows were offered fresh cut perennial ryegrass-based pasture (PRG) twice daily. Experiment A included early-lactation cows consuming Spring PRG (n = 10) and experiment B included late-lactation cows consuming Autumn PRG (n = 8). The scheduled visitation regime involved presenting the AHCS to each animal 12 times in 72 h; every 6 h, offset by 2 h each 24 h, resulting in data representing every 2 h of a 24 h profile. During experiment A, cows consumed 20.1 $\pm$ 0.5 kg DMI (μ $\pm$ SEM) and produced 26.7 $\pm$ 0.6 kg ECM. Cows emitted 367 $\pm$ 13 g CH₄ per d, 17.3 $\pm$ 0.7 g CH₄ per kg DMI, and 13.8 $\pm$ 0.5 CH₄ per kg ECM. In experiment B, cows consumed 13.7 $\pm$ 0.4 kg DMI and produced 12.7 $\pm$ 0.6 kg ECM. Cows emitted 265 $\pm$ 15 g CH₄ per d, 19.2 $\pm$ 0.8 g CH₄ per kg DMI, and 20.4 $\pm$ 1.0 g CH₄ per ECM. In both experiments, CH₄ emissions exhibited a bimodal distribution, with a temporal lag reflective of the post-prandial emission pattern typical of cows offered fresh forages twice daily. The diurnal profile and daily averages for CH₄ emissions from the short 3-d scheduled visitation regime are representative of emission estimates gained from voluntary visitation regimes in longer term grazing or forage tie-stall experiments during the two different periods. These data support the use of the AHCS methodology using scheduled visitations with cows fed fresh forages in short term metabolism stall studies.

Variability of Methane Emissions from Beef Cattle in the Western United States

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Ruminants produce methane (CH₄) primarily through enteric fermentation, a process influenced by the nutritional quality of their diet. Data on CH₄ emissions from extensive production systems and grazing cattle remain limited. This study aimed to quantify CH₄ emissions from cattle raised in the Western U.S., where cows are typically fed during the winter and graze native rangelands from spring to fall. A total of 22 Angus × Hereford cows were randomly selected and acclimated in the spring to Greenfeed Pasture System monitoring units (C-Lock) to measure CH₄ emissions (g/day). During the acclimation period, cows were maintained as a group and provided meadow hay ad libitum (8.5% CP, 56% TDN). Once acclimated, cows and monitoring units were moved to the Northern Great Basin Experimental Range to assess CH₄ emissions during the grazing season. Monitoring units were strategically placed near water sources and programmed to record up to eight visits per cow daily, using alfalfa pellets as an enticement. Cow body weight (BW) and body condition score (BCS) were recorded, and their locations and grazing behavior were tracked using virtual fence collars (Vence, Merck). Preliminary CH₄ emissions data from the acclimation (spring; five weeks) were analyzed using a repeated measures model, accounting for the week and period of the day (morning, afternoon, evening, and night), with a cow as a covariate. Preliminary results indicated effects of both week ($P = 0.03$) and period of the day ($P < 0.0001$) for CH₄ emissions. Emissions gradually increased over time, peaking in week 5 (210 g/cow daily) compared to week 1 (180 g/cow daily). Emissions varied throughout the day, with the lowest emissions recorded in the morning (143 g/cow), intermediate levels in the afternoon (193 g/cow), and the highest emissions in the evening (221 g/cow) and night (218 g/cow), with no significant difference between the latter two periods ($P = 0.77$). These preliminary findings highlight substantial variation in CH₄ emissions over time and within different periods of the day, emphasizing the need to account for temporal fluctuations when evaluating CH₄ emissions of grazing cattle.

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Theatre 4

Unrestricted grazing shifted the rumen microbiome community and recorded lower enteric methane emissions in dairy cattle compared to restricted or zero-grazing

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The rumen microbiome's role in enteric methane (CH₄) is nutrition-dependent, key to reducing emissions in grazing systems. We conducted a grazing trial across three periods in a year- April – May (P1), June – July (P2), and August – September (P3). In each period, 45 Holstein Friesian dairy cows were assigned to 15 blocks, with three grass-based systems: restricted zero-grazing (ZG), restricted grazing (RG), and unrestricted grazing (UG). An additional block of three rumen-fistulated cows was included, with a 3×3 Latin square design rotating the fistulated cows over the treatments. This trial assessed enteric CH₄ emissions and rumen microbiota in dairy cows across ZG, RG, and UG systems and multivariate analysis and statistical tests were performed to identify significant differences ($P < 0.05$). GreenFeed (C-lock) measurements showed the lowest CH₄ yield (g CH₄/kg DS) in UG (P1: 14.0, P2: 16.9, P3: 17.7), followed by RG (P1: 17.9, P2: 20.1, P3: 21.4), and highest in ZG (P1: 22.8, P2: 21.9, P3: 23.6) ($P < 0.001$). Similar trends were observed for CH₄ production (g CH₄/cow/day) and intensity (g CH₄/kg FPCM) ($P < 0.001$), except for CH₄ intensity, which did not differ between RG and ZG in period 2. Microbiome analysis from fistulated cows in the final trial weeks in each period showed treatment-driven variations, with pRDA revealing significant genus-level clustering ($P < 0.001$). Phylogenetic diversity differed significantly ($P < 0.05$) across alpha and beta indices. A mixed-effects model confirmed lower alpha diversity ($P < 0.05$) in the UG group compared to RG and ZG. Differential abundance analysis identified 60, 54, and 66 differentially abundant taxa in RG vs. UG, RG vs. ZG, and UG vs. ZG, respectively. The UG group had a lower ($P < 0.05$) relative abundance of *Butyrivibrio*, *Fibrobacter*, and *Ruminococcus* genera than RG and ZG. Our findings confirm that UG not only achieves lower enteric CH₄ yields but also induces a distinct rumen microbiome profile with decreased diversity and specific genus abundances, suggesting a potential mechanism for emission reduction in grass-based systems by increasing the amount of grazing hours.

Evaluating CH₄ emission consistency in cows and its relationship with rumen microbiotaS. Van Gastelen¹, H. Van Laar¹, E. E. A. Burgers¹, E. Zaccaria¹, S. K. Kar¹, L. Sebek¹¹ Wageningen Livestock Research, Animal Nutrition, PO Box 338, 6700AH Wageningen, Netherlands

Six cows with high CH₄ ranking (HMR) and 6 cows with low CH₄ ranking (LMR), based on previous CH₄ yield (on average respectively 24.0 and 17.2 g CH₄/kg DMI; cows belonging to top and bottom 15% quartile), were used. At the start of a 28 weeks follow up LMR and HMR cows had 39.7 and 35.9 kg/d FPCM, 116 and 103 DIM and were on average in 3rd parity. Cows were group fed a silage-concentrate mix plus concentrate in milking parlour and Greenfeed system (GF). Measurements were milk yield, CH₄ emission by GF, and feed intake in milking parlour and GF. Data were analysed by using a repeated measurements model with fixed effects of CH₄ ranking (MR), week, and interaction, and cow as repeated subject, with an AR1 covariance matrix. Each week rumen fluid was collected with a oesophageal tube from two fixed cow pairs of HMR and LMR. Rumen fluid samples were pooled per pair, and microbial DNA was extracted for microbiome analysis using 16S rRNA gene amplicons sequencing. Results showed that MR had no effect ($P > 0.05$) on body weight (725 kg), feed intake (GF: 1.34 kg DM/d, parlour: 0.86 kg DM/d), milk yield (30.7 kg/d), milk composition, and CH₄ production (485 g/d). Methane intensity (g/kg FPCM) was lower ($P=0.03$) for LMR (13.4) compared to HMR (14.8). Week influenced all measured parameters ($P<0.01$), with no MR $\times$ week interaction ($P>0.05$). Ruminal bacteria and archaea showed overlapping groups, strongly influenced by MR pairs, as revealed by principle component analysis. MR group differences remained stable over time, as partial redundancy analysis explained 4.44% (bacteria) and 2.76% (archaea) of variation ($P<0.05$), with no MR $\times$ week interaction ($P>0.05$). Differential abundance analysis found 14 bacterial but no archaeal genera with a different (adj- $P<0.05$) relative abundance, highlighting distinct bacterial profiles associated between HMR and LMR, though limited archaeal detection may have influenced this result. In conclusion, although absolute levels of CH₄ intensity changed over time, the difference (9.5%) between HMR and LMR remained similar. The distinct bacterial profiles between HMR and LMR indicate a relationship between MR and rumen microbiome.

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Theatre 6

Prediction of rumen microbial contributions to feed efficiency in beef cattle using machine learning

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Methane (CH₄) is the second most important greenhouse gas (GHGs) that contributes to climate change, and it is the most significant source of emissions from ruminants in the Canadian agriculture sector. Enteric CH₄ is produced through rumen microbial fermentation and a recent FAO report (ICPP 2021) has identified targeting rumen methanogenesis in cattle as one of three key climate change mitigation strategies. In this study, we used datasets including 16S rRNA gene sequencing, qPCR quantification, genotyping, and measurements of volatile fatty acids (VFAs), feed conversion ratio (FCR), and residual feed intake (RFI), and applied multiple advanced machine learning models to analyze the data. Using microbial datasets generated from 400 beef cattle for initial testing, we split the data into training (75% samples) and testing (25% samples) datasets and employed Random Forest, XGBoost, Convolutional Neural Networks (CNN), Long Short-Term Memory (LSTM), and Transformer algorithms to predict the contributions of each taxon to feed efficiency. Models based on microbial relative abundance demonstrated good predictive performance for FCR (AUC > 0.8), with Random Forest and XGBoost outperforming deep learning approaches (CNN, LSTM, Transformer). Key microbial taxa, such as NK4A214_group and Christensenellaceae_R7, were identified as important contributors to high and low FCR. In contrast, RFI models exhibited overfitting across all algorithms (AUCtest < 0.7, AUCtrain = 1), with VFA data providing better predictive power than microbial features (AUC > 0.7). These findings highlight that microbial features are more predictive of FCR than RFI, with Random Forest and XGBoost showing superior performance. The findings provide critical insights into determining the microbial targets for improving feed efficiency and subsequently reducing methane emission in cattle.

Impact of Methodological Parameters (CO₂ Flushing Time and Headspace Volume) on Gas and Methane Kinetics in Two In Vitro Rumen Fermentation Systems: AnkomRF and Gas Endeavour

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This study examined how experimental conditions, specifically CO₂ flushing time (60, 30, 10 min) and bottle headspace volume (70 vs. 170 mL), affected gas production (GP) and methane (CH₄) kinetics in two commercial in vitro fermentation systems (AnkomRF and Gas Endeavour). In vitro incubations were conducted with rumen fluid collected from three lactating cows, mixed with Menke buffer (1:2), and incubated for 24 hours. Each treatment was run in duplicate across three incubation series, plus blanks, for a total of 90 simulations. Both systems recorded GP kinetics; CH₄ was measured by gas chromatography at the end of incubation, and Gas Endeavour additionally monitored CH₄ kinetics. GP and CH₄ production were also predicted from volatile fatty acids using specific stoichiometric relationships. No significant differences were observed between the two systems for total GP (214±23 mL/g DM), although the shape of the kinetics differed. However, measured GP was significantly lower than stoichiometric expectations (258.4±43 mL/g DM; $P < 0.01$). Decreasing the bottle headspace resulted in a significant increase in GP ($P < 0.05$) in the Gas Endeavour system but a decrease in AnkomRF. Shortening the CO₂ flushing time significantly ($P < 0.05$) lowered GP. At 24 hours, measured CH₄ (38.4±11.3 vs. 35.2±3.4 mL/g DM in AnkomRF and Gas Endeavour, respectively) was below stoichiometric predictions. Similarly, reducing headspace led to a significant increase in CH₄ production in both systems, while shortening CO₂ flushing time significantly decreased CH₄ production. In conclusion, controlling CO₂ flushing time and headspace volume significantly influences gas and methane measurements in vitro. Standardizing these parameters is essential for ensuring accurate comparisons between different fermentation systems. Research supported by PNRR, “National Research Centre for Agricultural Technologies – Agritech” – CN2, Missione 4, Componente 2, Investimento 1.4, CUP C93C22002790001 grant.

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Theatre 8

Feed intake, growth rate, and greenhouse gas emissions from pregnant dairy heifers fed best practice diets

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Dairy production often faces unexpected weather events, such as droughts, which can severely affect crop yields and roughage availability. Consequently, it is essential to assess readily accessible alternative feed options. Moreover, effective climate action necessitates the estimation and/or reduction of anthropogenic greenhouse gas emissions, highlighting the importance of understanding the effects of alternative feed sources on emissions and production. This study assessed the individual feed intake (BioControl system) and enteric methane emissions (GreenFeed system) in dairy heifers fed diets based on two different roughage sources. The experiment included 2 weeks of collecting covariate data, 1 week of adaptation to the diets, followed by 4 weeks of data collection. Rations comprising either pure grass-clover silage or a 50:50 (on dry matter basis) grass-clover silage:whole-crop wheat silage mix were randomly allotted to two groups (mean age 18.3 ± 1.16 months) of 14 pregnant Swedish Holstein heifers and 8 Nordic Red heifers. Furthermore, backfat thickness was assessed using an ultrasound, and the average daily gain was calculated based on body weight changes. Data were analyzed using a linear mixed-effects model in R Studio, incorporating covariate data. Over the four-week period, no differences were observed in feed dry matter intake (grass-clover silage: 10.4 kg/day, grass-clover/whole crops silage mix: 9.6 kg/day, ± 0.41 kg/day), enteric methane emissions (grass-clover silage: 212 g/day, grass-clover/whole crops silage mix: 221 g/day, ± 3.6 g/day), average daily gain (grass-clover silage: 1478 g/day, grass-clover/whole crops silage mix: 1518 g/day, ± 57.1 g/day) or backfat thickness change (grass-clover silage: 0.19 mm, grass-clover/whole crops silage mix: 0.52 mm, ± 0.47mm). These findings suggest that both roughage sources can support similar growth performance and result in similar methane output, providing the farmers with flexibility in ration formulation for pregnant dairy heifers. The results also contribute to an accurate assessment of enteric methane emissions of replacement livestock.

Red and brown seaweed mixture for dairy cows fed canola meal or soybean meal: Effects on enteric methane emissions and milk performance

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The objective of this study was to evaluate the effects of including a dried mixture of red (*Gracilaria* spp.) and brown (*Laminaria* spp.) seaweed (SEAMIX, Synergaze Inc., Calgary, AB, Canada) in dairy cow diets based either on soybean meal (SBM) or canola meal (CM) on enteric CH₄ (production, yield, and intensity, and milk performance (production and composition). For this purpose, twelve multiparous Holstein cows were used in replicated 4 × 4 Latin square design (35-d period; 14-d adaptation) with a 2 × 2 factorial arrangement of treatments. Cows were fed ad libitum (5% refusals on an as-fed basis) a TMR containing (as the main protein source) either CM or SBM without or with the SEAMIX (0.18% of DMI). Canola meal (25% of diet DM) was included in the TMR at the expense of SBM and soybean hulls. Milk production and milk composition (7 days) and CH₄ emissions (3 days, respiratory chambers) data were analyzed using the MIXED Procedure of SAS with the fixed effects of meal, seaweed and their interaction, and significant effect was declared at $P \leq 0.05$. Regardless of the meal source, supplementing cows with SEAMIX reduced DMI by 6%. Feeding SEAMIX reduced milk and ECM yields (5% and 6%, respectively) in cows fed CM but not in cows fed SBM (meal × SEAMIX interaction). Feeding SEAMIX decreased enteric CH₄ production, yield, and intensity by an average of 33%, irrespective of the meal. Replacing CM with SBM in the diet reduced methane production, yield, and intensity by 9% on average. Compared with feeding SBM without SEAMIX, feeding both CM and SEAMIX reduced enteric CH₄ production, yield, and intensity by 41, 39 and 38%, respectively. This study shows that including SEAMIX in dairy cow diets at the rate of 0.18% of DMI is an effective strategy to mitigate enteric CH₄. Feeding CM (25% of diet DM) instead of SBM caused small decreases in enteric CH₄, regardless of the expression unit. Seaweed exerted additive-mitigating effects on enteric CH₄ (production, yield, and intensity) when supplemented with CM.

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Theatre 10

Ruminal fermentation characteristics of diets formed by grazing lambs on Pampa native grasslands

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This study aimed to quantify ruminal fermentation parameters of grazing lamb's diet on Brazilian Pampa biome grasslands, and of a high tannin legume (*Desmodium intanum*, DI) present in these grasslands. Twenty-four weaned lambs were submitted to three feeding treatments: stall-fed (Control), Pampa grasslands with low proportion of legumes (LL), and with high proportion of legumes (HL). The animals in the Control were fed with grass hay (72% DM), ground corn (16,6%), soybean meal (10,6%) and calcareous (0.8%) to reach a similar growth rate as the other treatments. The intake of grazing lambs was assessed through the collection of daily fecal production and in vitro digestibility analysis of forage samples collected by grazing simulation. The proportion of C3 and C4 species in the diet of the grazing lambs was estimated by the carbon isotope ($\delta^{13}C$, ‰) in the feces. The methane (CH₄) and volatile fat acid (VFA) production were carried out using the in vitro gas production technique. Total VFA concentration did not differ among treatments ($P = 0.222$), but there were differences in the type of VFA. Both pasture treatments produced lower isovalerate and propionate concentrations ($P \leq 0.014$) and higher acetate-to-propionate ratios ($P < 0.001$) than the Control. Control treatment had a higher fractional rate of gas production and faster fermentation of non-fibrous carbohydrates (P1), but these results were no different between pasture treatments. Both pasture treatments resulted in higher total gas production and better fermentation of fibrous carbohydrates (P2). Interestingly, no significant differences in methane production were observed across the three treatments. However, when analyzing only DI leaves, it was observed the lowest value for total gas and methane production. The greater propionic production in Control did not lead to a reduction in CH₄ production. This highlights that there is an important potential of Pampa biome species, especially DI, for methane mitigation.

Effect of Aminoreach® Ruminants Supplementation on Milk Yield, Composition, and Protein Utilization Efficiency in Dairy Cows in a low protein diet

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The objective of this study was to evaluate the impact of reducing diet crude protein (CP) on milk yield, milk composition, and protein utilization efficiency and evaluate Aminoreach® Ruminants (AMR) in a low CP diet. While it may improve protein use efficiency (PUE), reduction of diet CP is hypothesized to decrease lactation performance. Addition of AMR, which aims to improve rumen degradability of fiber bound protein, is hypothesized to prevent this performance decrease, increasing PUE. Thirty-six multiparous Holstein cows (31.7 kg/d milk yield, 150 DIM) were used in a 3 × 3 Latin square design experiment with 28 d periods and 3 treatment diets. Cows were blocked according to parity, DIM and lactation performance. Treatment diets were Control (15.3% CP), Low CP (14.5% CP), and Low CP + AMR (20 g/d AMR, Cargill Animal Nutrition). Diet CP was set by varying the ratio of high (18.9% CP) and low CP (13.9% CP) concentrate on top of a shared basal diet (14.9% CP). Daily feed intake and milk yield were recorded, milk composition was determined during the last week of each period. Data from the last week of each period were analyzed using a linear mixed effects model in R, with treatment, period, block, treatment x period as fixed effects and cow as a random effect. Treatment did not affect feed intake (24.0 kg DM/d, $P = 0.76$). Milk yield decreased from 31.3 kg/d (control) to 30.6 kg/d (low CP) but not with AMR in the low CP diet (31.1 kg/d). Milk protein content (3.72%) and yield (1.13 kg/d) were not affected ($P \geq 0.60$) by treatment, whereas milk fat content increased ($P < 0.01$) from 4.66% for control to 4.88% for the low CP diets, with fat yield increasing in AMR vs control ($P < 0.05$). Milk urea levels decreased ($P < 0.01$) from 16.9 mg/dL in control to 14.2 mg/dL in the low CP diets. The PUE increased ($P < 0.01$) from 31.6% in the control to 32.5% in the low CP and to 33.2% for low CP + AMR. In summary, decreasing diet CP improved PUE but reduced lactation performance. Supplementing the low CP diet with AMR prevented this performance impact, while further improving PUE demonstrating the value of Aminoreach® Ruminants for a future-proof dairy production.

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Theatre 12

Evaluation finishing pig performance, nitrogen excretion in manure and carcass traits with varying levels of lysine supplement in reduced- nitrogen diets

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This study was conducted to evaluate the effects of lysine (Lys) supplementation in low-protein diets on growth performance, nitrogen excretion in manure, and carcass characteristics in finishing pigs. A total of 48 three-way crossbred finishing pigs ((Yorkshire × Landrace) × Duroc) with an average body weight of 83 ± 0.5 kg were used in a 35-day feeding trial (4 treatments × 4 replicates × 3 pigs per replicate), and 12 finishing pigs (4 treatments × 3 replicates × 1 pig per replicate) were used in a 14-day metabolism trial. The experimental diets were corn-soybean meal-based, and the treatments were formulated as follows: Con) CP: 14% + Lys: 0.91%, T1) CP: 13% + Lys: 0.91%, T2) CP: 13% + Lys: 0.98%, and T3) CP: 13% + Lys: 1.06%. The results showed that the T2 and T3 groups had the highest average daily gain (ADG) and feed efficiency (G:F). No significant differences in digestibility were observed among the treatment groups ($P > 0.05$), while nitrogen excretion in manure decreased with increasing lysine levels in low-protein diets. After slaughter at an average body weight of approximately 110 kg, the effects of dietary lysine levels on carcass yield (carcass weight, backfat thickness, and intramuscular fat thickness) and meat quality (meat color, pH, cooking loss, and proximate composition) were analyzed. The results indicated that lysine supplementation in low-protein diets had no significant impact on carcass yield or meat quality ($P > 0.05$). These findings suggest that supplementing lysine in low-protein diets does not negatively affect the growth performance of finishing pigs while potentially enhancing environmental sustainability. The results of this study are expected to contribute to the development of environmentally friendly and economically efficient feed formulation strategies. Acknowledgements: This study was supported by the Rural Development Administration research project (PJ017491)

Life Cycle Assessment of feed additives: Environmental Return on Investment across species and product contexts

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Recent literature highlights the role of feed additives in mitigating climate change through improved feed efficiency, optimized feed formulation, enhanced animal health, and reduced nitrogen (N) excretion. However, the climate change impact of their production can be higher than other feed raw materials (e.g. cereals, oilseed meals, cereal byproducts). This study evaluates the balance between the carbon footprint benefits of using feed additives at the farm level and their production impact. Life Cycle Assessments (LCAs) at plant gate were conducted for three feed additives (a multi-carbohydrase complex for broilers, lysolecithin for broilers and lyso-phospholipids for shrimps) following ISO 14040/44, PEFCR guidelines and FAO LEAP recommendations. Data Quality Rating (DQR) was at least of “very good” quality, which ensured the robustness of the LCAs. The climate change impact at farm gate was evaluated using environmental databases (GFLI, Agrifootprint) and performance data (feed intake, body weight gain). Studies were conducted under commercial or experimental conditions, comparing diets with and without additives, including reformulation of diets. System boundaries varied, accounting feed ingredients, on-farm emissions, energy consumption, feed processing, transport, and manure management. Functional units were kg CO₂-eq/kg liveweight of broilers or shrimp. The Environmental Return on Investment (EROI) was calculated as the ratio of the carbon footprint avoided at the animal product level to the impact generated by additive production at the plant level. Results showed reductions in climate change impact ranging from -4% to -15% (kg CO₂-eq/kg BWG). The EROI ranged from 12 to 115 across studies, indicating that for an investment of 1 t CO₂-eq due to feed additive production, their use could result in savings of 12 t to 115 t CO₂-eq on the value chain. These outcomes are attributed to the additives’ modes of action, including diet reformulation, performance improvements, and health benefits. These findings emphasize that, while reducing emissions from feed additive production is essential, their overall contribution to sustainability significantly compensates for their production carbon footprint.

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Theatre 14

Are low carbon farms sustainable in sheep production across Europe?

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“Is improving sheep farms’ carbon footprint (CF) positively linked to the overall farm sustainability?” One of the objectives of the LIFE Green Sheep project is to monitor sheep farms across five countries to evaluate their CF and sustainability indicators (environment, economics, social) with different precision level. The project involves 1,284 demonstrative farms, which undergo an initial environmental and sustainability evaluation (42 input data), and 260 innovative farms, which have more in-depth evaluations (280 input data) and monitoring. For the sustainability evaluation, each pillar (ie environment) is defined by several issues (ie climatic impact), which are then translated into indicators (ie GHG emission). Local references are used to rank each indicator on a scale of one to five. No weighting is applied to the indicators. The sustainability grid includes 18 indicators for demonstrative farms and 33 for innovative farms. As the results are divergent, the focus is on innovative farms. The best farms for CF results (called ‘top’) are those with a rank of five for GHG emissions per functional unit (FU). For dairy farms, top farms have the same rank on the environmental (3) and social (2,6) pillars as the average, while their economic rank is significantly lower (2,7 vs 3,3). An assessment of the ranking details of parameters within each pillar shows that the main negative differences are in farm efficiency and contribution to employment. For meat farms, top farms have a significant higher social rank (3,1 vs 2,6) than the average, while the opposite is true for the other two pillars. Conversely, they have a lower ranking on the two economic (3,1 vs 3,5) parameters, and in the environmental (2,5 vs 2,8) pillar, two out of five parameters are negative. Preliminary results show that sustainability is a multi-factor evaluation, and even within the environmental pillar, GHG is only one of the issues.

In vitro evaluation of different forage to concentrate ratio diets for ruminant feeding

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Dairy cow diets often vary in their forage to concentrate (F:C) ratios across farms, influencing both production and environmental outcomes. High-concentrate diets remain common for achieving high production, while some farms opt for balanced or high-forage diets, resulting in different energy densities. This study evaluated the effect of five F:C ratios on predicted in vivo methane (CH₄) production, digestibility, and fermentation parameters using an automated in vitro system, and aimed to select the optimal ratio for Nordic dairy systems. The tested F:C ratios were 100:0 (100F), 80:20 (80F), 60:40 (60F), 40:60 (40F), and 20:80 (20F). The 100F diet consisted of only grass silage, while the others included barley and rapeseed meal. All diets were balanced for crude protein (20% DM), but ether extract and neutral detergent fiber content declined with increasing concentrate. F:C ratio affected total gas and CH₄ production, which increased linearly ($P < 0.001$) with higher concentrate inclusion. Organic matter digestibility (OMD) followed a quadratic pattern ($P < 0.001$), peaking in 60F and 40F diets and declining in 100F and 20F. Although F:C ratio had no effect on pH or total volatile fatty acid concentration, acetate proportion declined linearly ($P < 0.001$) with concentrate inclusion, while iso-butyric and butyric acid proportions increased. The results suggest that F:C ratios of 60:40 or 40:60 optimize OMD while minimizing CH₄ emissions, balancing production efficiency with environmental sustainability. However, the observed trends are influenced by the high fermentability of the substrates and the limitations of the closed in vitro system, which does not account for differences in ruminal retention times between forage and concentrate. Previous studies reported lower reliability of such systems, particularly for diets with higher concentrate inclusion. Therefore, while the findings provide valuable insights into the effects of F:C ratio on CH₄ production and digestibility, the results should be interpreted with caution and cannot replace in vivo experiments.

Session 67

Poster 16

Evaluation of methane mitigation by increasing the incorporation of starch mainly from maize in the diet of dairy cows

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As part of France's commitments to the Paris Agreement, a significant reduction in greenhouse gas emissions is necessary to achieve the goal of "net-zero emissions" by 2050. Cattle farming, particularly dairy cows, is a major source of enteric methane (CH₄) emissions, a greenhouse gas with a high warming potential. Optimizing animal nutrition offers a promising approach to mitigate CH₄ production which needs to be estimated. This meta-analysis study aims at evaluating the impact of increasing diet incorporation of starch, primarily derived from maize, on reducing CH₄ production in Holstein dairy cows. A database was created from studies published in peer-reviewed journals compiling data assessing the effect of increasing the diet starch proportion from maize (over 2.5 % of increase) on CH₄ production. In this database (npub=21, nexp=63), diets include 37.9 ± 9.3% of concentrate with 20.8 % starch (ranging from 7.4 % to 38.2 %). In these experiments, cows ate 21.7 ± 3.0 kg DM/d. The milk yield was 31.7 ± 4.9 L/d with milk fat and protein content of 4.1 ± 0.6 % and 3.3 ± 0.3 % respectively. The CH₄ emission rate, yield and intensity were 429.9 ± 72.1 g/d, 20.1 ± 3.3 g/kg DMI and 14.0 ± 2.1 g/kg ECM, respectively. A mixed linear model incorporating the publication as a random effect was used to establish the response of CH₄ emission rate and intensity to diet increase in starch proportion using the slope estimate. Using all the dataset, the decrease in CH₄ emission rate or intensity were estimated at -27 g/day and -1.0 g/kg ECM respectively for an increase of the diet starch content of 10 points. When selecting experiments over 12% of starch in the diet for a more accurate linear response (npub=20, nexp=55), these estimates increased to -39 g/day and -1.6 g/kg ECM. These reductions remain relatively modest and are associated with zootechnical constraints, notably an increased risk of acidosis, requiring careful diet management.

Decrease of milk carbon footprint using dent-starch silage maize in dairy cattle feed

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A major challenge for livestock farming is to reduce its impact on climate change. This can be achieved in several ways, including changes in herd feeding. The firm Pioneer has developed a range of maize silage, double hybrid dent maize, which can be harvested late in the season, leading to higher %DM and starch content than flint-dent maize hybrid. This study aimed to compare the effect of 2 maize hybrids (Pioneer's P8888, and a control flint-dent maize, Matthew) included in the diet of dairy cows, on enteric methane emissions and milk carbon footprint. Both maize varieties were grown on the Grignon experimental farm with the same cropping practices. Mass yields were compared at harvest. P8888 yields were +1.1 to +2 tDM/ha higher than Matthew's, with 41.5% DM (vs 36.6%). A trial was then conducted on 12 Holstein dairy cows, using a complete Latin square experimental design with cross-over. The cows were fed either an experimental diet (ED with P8888) or a control diet (CD with Matthew). All other diet ingredients were identical. ED had +0.8% starch and -1.7% NDF compared to CD. Gross CH₄ emissions, feed intake, milk production levels and quality, and changes in live weights were measured individually. CH₄ emissions were measured using the SF₆ tracer technique. Results were subjected to ANCOVA analysis using R. ED significantly reduced methane emissions rate, yield, and intensity by 6%, 6%, and 5% respectively, without changing any other zootechnical performance parameters. ED and CD were then extrapolated at the farm scale with PerfAgroP3 software, a modelling and decision aid tool developed by CEREOPA. PerfAgroP3 was used to model farm operations and evaluate farm performance, including milk carbon footprint. ED produced a 2.4 to 2.6% reduction in the milk carbon footprint, dropping from 1.058 to 1.031 kg CO₂eq/l, compared to CD. This reduction was mainly due to a decrease in enteric methane emission (78%) and to the increased maize yield (22%). Although this reduction is relatively modest, it is particularly interesting as it offers a technically accessible, relatively low cost, and low carbon solution for breeders.

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Poster 18

Effects of different probiotic supplementation in lactating sows under heat stress

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Probiotics, particularly *Lactobacillus* species, have gained attention for their potential to enhance gut microbiota stability, modulate inflammation, and improve reproductive performance under challenging environmental conditions. The *Lactobacillus reuteri* and *Lactobacillus plantarum* strains were isolated from the feces of prolific lactating sows during heat stress. The stability of the two isolated strains was tested *in vitro*. In this study, we evaluated the effects of supplementing *L. reuteri* and *L. plantarum* in the diets of lactating sows during heat stress. The primary objectives were to assess their influence on reproductive performance, inflammatory responses, oxidative stress, and microbiota composition. A total of thirty sows were randomly assigned to three dietary treatments: (1) a control group (CON) receiving a standard lactation diet and (2) a *L. reuteri*-supplemented group (LR) receiving the basal diet supplemented with *L. reuteri* and (3) a *L. plantarum*-supplemented group (LP) receiving the basal diet supplemented with *L. plantarum*. The trial was conducted during the summer months when heat stress conditions were prevalent. The body weight and backfat thickness were not affected by the treatments, however, the feed intake tended to increase ($p=0.082$) in the LP group. The litter size and litter weight remained unaffected, but piglet weight at weaning tended to increase ($p=0.056$) in the LP group. There was no effect on levels of IL-10 and IL-1 β , however, the concentration of TNF- α tended to decrease in the LP treatment. Oxidative stress markers including TAC, SOD, and MDA, were unaffected by treatments. The supplementation of probiotics did not affect zonulin and occludin in the blood. The hair cortisol concentration was not affected by the treatments. The abundance of microbiota at phylum, family, and genus level was unaffected. In conclusion, supplementation with *L. reuteri* and *L. plantarum* did not show an obvious improvement in lactating sows performance during heat stress unless the minor increase in feed intake and piglet weight at weaning.

Field and Feasibility Tests of Probiotic Spray for Reducing Ammonia Emissions from Swine and Poultry Manure

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The noxious gases emitted from swine and poultry manure are a global concern because of their detrimental effects on human health and animal welfare. Herein, we conduct two subsequent experiments (Exp) for four weeks: feasibility and field manure test to determine whether spraying microbial loaded surfactant (*Lactobacillus Plantarum*) could suppress gas emission, solvita index and fecal copper and zinc concentration in swine and poultry manure. For the feasibility test (Exp.1), fresh fecal samples were randomly collected from 216 laying hens and 60 finishing pigs at the end of each week. The samples were divided into four groups and placed in 10 L containers labeled as CON, TRT1, TRT2, and TRT3. The CON group was sprayed with a phosphate-buffered saline (PBS) solution, while the other three groups were treated with 0.1% liquid probiotics (LP, TRT1), 0.1% powdered probiotics (PP, *Lactobacillus plantarum*, TRT2), and a combination of 0.05% LP and 0.05% PP (TRT3). In the field manure test (Exp.2), animals were divided into 4 groups, housed (n=15/ pig house and 54 birds/room), and directly sprayed with the same surfactant. Over a 4-week period, both test animals were fed a standard basal diet (as recommended by the NRC). At the end of week 2, both tests showed significantly ($p<0.05$) reduced ammonia emissions when sprayed with 0.05% LP and PP. Additionally, feasibility test results of TRT3 showed significantly ($p<0.05$) reduced ammonia (NH₃), hydrogen sulphate (H₂S), and carbon dioxide (CO₂) emission at the end of week 3. Unlike feasibility test results, field manure test showed only significant differences ($p<0.05$) in NH₃ and H₂S emissions when sprayed with 0.05% 0.05% LP and PP. The results of this study suggest that spraying microbial-loaded surfactants, particularly the combination of 0.05% LP and 0.05% PP, is an effective strategy for reducing harmful gas emissions and metal concentrations in swine and poultry manure. We suggest that this approach could contribute to enhancing animal welfare by mitigating the environmental pollution.

Session 68

Theatre 1

Implications of Rearing Broiler Progeny Without Parental Presence: Insights on Early-Life Programming

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The gut microbiota performs a number of important functions for the host. Thus, it is important for the host that symbiotic gut bacteria are vertically transmitted efficiently from hens to progeny. In nature the main mechanism for the vertical transmission of gut symbiotic bacteria is the direct interaction between parents and offspring. However, in commercial settings broiler chicks hatch and grow in the absence of adults. We and others have shown this results in a delayed colonization and maturation of the gut microbiota. To understand the implications of growing chicks without adults we artificially exposed newly hatched chicks to adult cecum content which contains a large number of phylogenetically diverse adult bacterial strains. Following treatment, the cecum bacterial community was completely transformed while the jejunal and crop communities were less effected. Some differences in community composition remained for the full 5-week growth cycle. Short chain fatty acid levels in the cecum of treated chicks were different from that of control chicks. Especially, propionate levels were very different between treated and control chicks for the full 5 weeks, implying chicks grown in commercial conditions did not fully utilize nutritional fibers found in their feed. We also found gene expression in cecum and bursa host tissue was affected by treatment. Interestingly, treated chicks had lower weight during the first two weeks but caught up with control chicks later on. Finally, we exposed newly hatched chicks to mouse cecum content. We found that mouse bacteria were unable to colonize newly hatched chicks emphasizing the importance of host adaptation for colonization and proving that even the bacteria found in the guts of newly hatched chicks are not environmental bacteria but rather chicken adapted. To conclude, raising chicks in the absence of adults affects chicken physiology and future studies are needed to determine the full implications of this practice and to develop mitigation strategies.

In ovo nutriepigenetic modulation of liver transcriptome: inter- and transgenerational insights from a chicken model

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Epigenetic modifications, which can result from various environmental factors affecting the organism, play a key role in regulating gene expression. By modulating gene activity, these changes contribute to the development of phenotypic traits and may have long-term biological implications within and across generations. Therefore, this research aimed to investigate inter- and transgenerational effects of in ovo embryo stimulation on liver transcriptome using a chicken model. On day 12 of incubation, Green-legged Partridge-like chicken eggs were injected with synbiotic PoultryStar® (Biomim) and choline. Three F1 groups were established: (1) control receiving 0.9% NaCl (C), (2) synbiotic (SYN), and (3) synbiotic + choline (SYNCH). In F2 and F3, groups SYN and SYNCH were further divided: (A) solely injected in F1 and (B) repeated injected in each successive generation. Liver samples (n=39) were collected at 21 week post-hatch. RNA-seq libraries were sequenced on Illumina NovaSeq 6000 platform (PE150). Reads were mapped to the Gallus gallus reference genome (bGalGal1.mat.broiler.GRCg7b). Differential expression analysis was performed with DESeq2 with GO and KEGG enrichment via ShinyGo 0.82. In general, in ovo injections of bioactive substances resulted in changes in the expression of specific genes in the liver of mature birds across generations. DEGs were enriched in various pathways including those related to metabolic and immune pathways. A more in-depth analysis will investigate how in ovo synbiotic and choline stimulation may influence processes in the liver via the identified pathways. Funding: National Science Centre, Poland (2020/37/B/NZ9/00497). Bioinformatics analysis: Poznań Supercomputing and Networking Center resources.

Session 68

Theatre 3

Impact of high CO₂ concentration at the beginning of egg incubation on chicken metabolism and meat defects

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In practice, hatcheries let CO₂ concentration reach 1% at the beginning of egg incubation, limiting energy costs and improving hatching rates. However, the long-term consequences of this practice on the meat-type chicken metabolism, health and meat quality were scarcely explored. Therefore, this study aimed to analyse the effects of high CO₂ concentration between days (I) I3 and I11 of incubation (up to 1%, CO₂ group) compared to 0.1% CO₂ concentration (Controls) on the embryo and chicken growth, chicken plasma metabolites and meat quality, especially meat defects related to muscle oxygen supply, depending on the animal sex. The 300 control eggs and 300 eggs treated with a transitory increase in %CO₂ were incubated together before I3 and after I11 and separately between I3 and I11 of incubation. Embryo tissue and body weights (BW) were measured at I11 and I15. After hatching, 80 males and 80 females per group were reared together, before being weighted and slaughtered at 40 days of age. After slaughter, meat processing quality and meat defects were evaluated. Embryo and tissue sampling during incubation showed that CO₂ embryos were heavier at I11 than controls, which was still found at I15, with a trend for greater heart relative weight at this age (p=0.07). Hatching rates were high and did not differ between groups (93%). No apparent health issue was detected throughout the growth period. At 33 days of age, glycaemia and lactatemia of CO₂ males only were greater than those of control ones. Finally, at slaughter, BW did not differ between groups, but the breast muscle of CO₂ chickens was redder compared to controls. The incidence of the meat defect wooden breast was lower in CO₂ males (76%) than in control males (88%), but greater in CO₂ females (76%) than in control ones (51%). These results suggest that embryo hypercapnia has short-term and sex-dependent long-term physiological and metabolic effects impacting health and meat quality, for which the underlying mechanisms remain to be explored.

Early-Life Hypoxia Conditioning: Impact on Vascularization, Growth, and Woody Breast in Broilers

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Woody breast (WB) myopathy is a significant welfare and economic concern in broiler production, primarily affecting fast-growing strains. This study investigated the effects of early-life hypoxia conditioning on WB incidence, growth performance, and meat quality. Fertile Cobb500 eggs were incubated under standard oxygen conditions (21% O₂) or mild hypoxia (17% O₂) from embryonic day 5 (E5) to 12 (E12). High-resolution episcopic microscopy imaging of E13 breast muscle tissue revealed that hypoxia-treated embryos exhibited enhanced vascularization, with increased arterial plexuses compared to controls. This suggests improved oxygen and nutrient delivery to the muscle. Post hatch, 120 male broilers from each treatment were monitored weekly for growth performance, feed consumption, and blood parameters (hematocrit and hemoglobin). At slaughter, WB incidence and meat quality traits were evaluated. WB incidence was significantly lower in the hypoxia group (36.1%) compared to controls (50.9%, $p = 0.0266$). While incubation treatment did not affect final body weight, broilers with WB were 300g heavier than those without myopathy. Meat yield analysis revealed that hypoxia-treated broilers had a slightly higher relative breast weight ($p = 0.034$) with no adverse effects on overall growth. However, WB-affected broilers exhibited significantly greater breast muscle mass and drip loss ($p < 0.0001$), reinforcing the link between rapid muscle growth and myopathy. Additionally, initial pH was slightly lower in hypoxia-treated broilers, suggesting subtle metabolic adaptations. Although hypoxia-induced angiogenesis in the breast muscle enhances oxygen delivery and reduces WB incidence, the condition remained notably high despite this vascular response. This indicates that while hypoxia can alleviate some of the vascular stress, it is not sufficient to fully prevent WB. These findings highlight the need for further investigation into the molecular mechanisms driving WB development and suggest that early-life hypoxia may serve as a partial but not definitive mitigation strategy for WB in commercial broilers.

Session 68

Theatre 5

What are the levers to improve the rearing of males from layer strains?

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Improving performances led to specialised chicken breeds for either egg or meat production. While meat production uses both sexes, egg production only needs females resulting in culling one day old male chicks from layer strains ("brother roosters"). Several countries banned this practice for ethical and animal welfare reasons. Two solutions raised: in ovo-sexing and fattening brother roosters from layer or from dual-purpose strains. In Switzerland, Bio Suisse opted for fattening the males. Knowledge on performance and welfare of these males is scarce. Therefore, we evaluated one week before slaughter 20 animals/flock of (i) males (brother roosters or dual-purpose strains) on 19 farms and (ii) mixed-sexed chickens of slow-growing broiler strains on 7 farms and determined welfare scores and live weight. Management differed with regard to age at slaughter, housing and feeding system and between season. Dual-purpose males and broilers were first reared in a rearing house (starter feed) and then transferred to a mobile house (broiler feed). Systems for brother roosters were diverse: (a) pullet house (starter followed by pullet feed or pullet feed only), (b) rearing house (starter feed) then mobile house (broiler or pullet feed) or (c) broiler house (starter followed by broiler feed). We included these factors in our models to identify potential levers of improvement for performance and welfare. Final live weights differed mostly by genetic strain. Within the brother roosters, live weight was significantly higher with pullet feed from day 1 compared to starter feed followed by pullet or broiler feed. Welfare was very good in all herds as the worst score was 6 out of 54 (27 indicators from 0: excellent to 2: severe problems). We first performed a PCA and summarized the 27 welfare indicators into 2 variables. We then performed a linear mixed-effects model and highlighted that the genetic strain, the type of poultry house (mobile or fixed) and the season influence animal welfare. Sustainability goes beyond performances and animal welfare. Life cycle assessment is currently being established on production models including specialized layer strains and dual-purpose strains to assess the sustainability of these new systems.

Co-designing agroecological systems combining poultry and crops to enhance (eco)system services and identify genetic needs for poultry

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The agroecological transition requires a better coupling of animal and plant productions to take advantage of reciprocal benefits. However, poultry farming is often disconnected from crop production at both farm and regional levels. Additionally, current breeds and varieties may struggle to adapt to the variable and heterogeneous environments often found in agroecological systems. Furthermore, the agroecological transition requires a shift in research practices, including the active engagement of non-scientific stakeholders to ensure effective field implementation. In this context, this study aimed to co-design agroecological systems integrating poultry with crops or fruit trees to enhance ecosystem services. The goal was to refine breeding objectives and optimize poultry sector organization in terms of feeding, genetics, and economics. To achieve this, we conducted semi-structured interviews with farmers across France who are experimenting with innovative practices in more integrated systems. Initial findings show that poultry-crop systems offer multiple benefits, including diversification, food autonomy, and biological pest control. However, they also present challenges, particularly due to unsuitable regulations and equipment. While farmers' satisfaction with current poultry genetics varies, all farmers seek greater independence from dominant industry players. Achieving this goal necessitates collaboration between, breeders, farmers and local stakeholders at the regional level. Building on this observation, we applied the DKCP (Define-Knowledge-Concept-Proposal) method to facilitate knowledge sharing and explore innovative ideas during a codesign process. This phase involved a group of organic farmers in the Massif Central, a mid-altitude region in central France.

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Theatre 7

The Role of Mathematical Models in Designing Climate-Resilient Poultry Production Systems

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Climate change is exacerbating heat stress challenges in the poultry sector, threatening animal welfare and performance, and therefore economic sustainability. As global temperatures rise, operators must adopt innovative strategies to ensure resilience and efficiency. To address these challenges, mathematical modelling and computational simulations have emerged as powerful tools for adaptation. These tools offer a vast range of applications, from animal selection to slaughter, enabling targeted solutions across every stage of poultry production. First, the analysis of individual responses to perturbations using modelling will help to improve genetic selection of birds for better robustness and resilience to heat stress. The use of Dynamic Thermal Simulation (DTS) and Computational Fluid Dynamics (CFD) models will be also be useful to create climate-resilient housing systems. In poultry houses, heat transfers can be analysed with DTS models in order to optimise insulation or management, thus maintaining stable temperatures and reducing heat stress on birds. Similarly, CFD models simulate airflow, temperature, and gas concentrations for the optimization of ventilation and cooling systems to enhance bird comfort and energy efficiency. In farms, daily nutrient requirements of birds could be updated automatically with models for a real-time adjustment of feed composition. For example, they can help to optimise the energy supply to reduce metabolic heat production, while maintaining animal performance during heat waves. Finally, regarding animal transport, CFD simulations can be used to optimise vehicles design, airflow, and stocking density, ensuring conditions that minimise heat stress and maximise welfare. By considering these various applications, mathematical modelling provides a holistic framework for addressing heat stress and enhancing sustainability across the entire poultry sector.

Is brighter truly better? Optimizing light spectrum and intensity for turkey breeder management and production

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Light intensity is a key management tool in commercial turkey parent stock production, yet its effects can be paradoxical. High light intensities are used to maximize productivity and discourage floor-egg laying, but may also increase injurious pecking, energy expenditure, and feed intake. Red spectrum lighting has been shown to improve avian reproductive performance and alter the perceived light intensity and visual properties of the environment, potentially mitigating unwanted behaviours. This study compared hen activity and nest use under two light spectra, red (60% 620-700nm, 20% 500-550nm, 20% 440-490nm; R) or white (W) at two intensity levels, high (0.3W/m²; H) or low (0.1W/m²; L). A total of 402 female grandparent Hybrid Converter hens were placed in 24 pens within four rooms at 20 weeks of age (woa), with colour assigned by room (N=2), and maintained under L intensity. At photostimulation (30 woa), intensity was assigned by pen for a split-plot design (n=6). Body weights (BW) were measured biweekly and eggs were collected 12 times daily. Cameras recorded one full day per week at key points: baseline (24, 26 woa), entry to lay (32, 33 woa), peak production (35, 37 woa), mid-production (43, 46 woa), and late production (56, 60 woa). Footage was converted to black and white to blind observers. Hourly scan samples of mutually exclusive behaviours (active, inactive, nutritive, and inside the nest box) were taken during the photophase. Egg data and BW were analyzed with the GLIMMIX procedure and Tukey's post-hoc test in SAS. Scan sample data were analyzed descriptively. At baseline, hens had increased BW under R (P<0.05) and spent more time inactive (R: $\mu=62.4\pm9.26\%$; W: $\mu=56.8\pm9.56\%$). During lay, this colour effect dissipated, and hens under L had increased BW (P<0.001), but time spent active was similar. Per circadian data, nest use peaked seven hours after lights-on: one hour before peak egg collection. Interestingly, hens under L laid their eggs earlier in the day (P<0.01). While nutritive and pecking behaviours decreased with age, incidences were similar between treatments. Overall, light treatment shifted the oviposition cycle, which could have implications for commercial management. Although lighting had a small effect on activity, changes in BW suggest a biologically relevant response. Further analysis to assess the impact of lighting on melatonin profiles and injurious pecking are currently underway.

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Is it possible to shift meal distribution from the afternoon into the night for lactating sows in summer?

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Decreased feed intake and performance occur when lactating sows are exposed to hot conditions. The difficulties encountered in dissipating postprandial heat production (due to the digestive and metabolic use of the feed) are suspected to increase heat load, which could be alleviated by changing meal times. Since sows have a diurnal feeding behavior, this study was carried out to assess their ability to adapt to a change in meal distribution strategy and its impact on performance (litter growth rate (ADG), sow feed intake (FI), body weight (BW) and backfat thickness (BT) changes). From day 6 after farrowing to the day before weaning, all sows were fed three times per day: meal 1 was delivered between 7:00 and 8:00 AM, meal 2 between 1:00 and 2:00 PM, and meal 3 between 7:00 and 8:00 PM for group D (i.e., day) or between 1:00 and 2:00 AM for group N (i.e., night). Five batches of 24 Large White x Landrace sows were used. Within each batch, pairs (replicates) of two sows were formed based on their parity, BT and BW and then assigned to treatments. Litter ADG (group D: 3.13 vs. group N: 3.11 kg/d, P = 0.85) and sow BT (D: -4.9 vs. N: -4.5 mm, P = 0.34) and BW (D: -40 vs. N: -41 kg, P = 0.85) losses were similar for both groups. These results were consistent with a similar FI between day 6 and 25 (D: 5.65 vs. N: 5.61 kg/d, P = 0.72), including FI during meal 3 (D: 1.82 vs. N: 1.75 kg, P = 0.35). These results show that sows can adjust their daytime feeding behavior, consuming at night what they do not eat in the afternoon. This conclusion, obtained in a mild summer (from May to September 2024, Brittany, France), is a prerequisite for testing this lever under more extreme temperatures. This study is a part of the Fermadapt program funded by Région Bretagne.

Extra air velocity to prevent heat stress of sows in farrowing rooms

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With the average temperatures and the prevalence of tropical days during Belgian summers increasing throughout the last decades, there is a growing need for measures to prevent heat stress in pigs. Especially peripartum and lactating sows are highly susceptible to heat stress, mostly due to their high metabolic state. Some technological measures to reduce the heat load in farrowing barns are expensive or only applicable during the construction of new barns. We therefore tested the effect of extra axial fan ventilation in farrowing rooms as cost-effective measure to reduce heat stress of peripartum and lactating sows. Forty-nine peripartum sows were divided into 2 groups and 3 rounds and were included in a trial for 5 weeks, from 1 week before farrowing until weaning at 4 weeks of age. The control group was housed in farrowing rooms that followed the normal ventilation regime and the fan group was housed in rooms where two extra axial fans were installed at the air inlet. When the ambient temperature in the rooms exceeded 25°C, the extra fan was turned on, creating a higher air velocity throughout the rooms. All sows had free access to water and were fed two times a day with the same lactation feed. Each day, feed gifts and feed leftovers were recorded to calculate the feed consumption. Piglets were individually weighed at birth and at weaning. Respiration rate, rectal temperature and a heat stress score of the sows were registered on several hot days when the fan was operating and on control days. The extra fans ensured that the incoming air was efficiently mixed with the air already present in the room, resulting in a temperature drop up to 2°C on hot days at the level of the sow. The respiration rate of sows housed in the room with extra fans, decreased with 25% compared to the sows housed in the room without the fan. Rectal temperature and heat stress score were also lower in the fan group compared to the control group. Piglets of the fan group had a higher daily gain compared to the control group. Installing an extra fan in the farrowing barn alleviated heat stress in lactating sows and improved the performance of the piglets under the present conditions.

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Theatre 11

Impact of the water quality on performance and health in 598 French pig farms

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Drinking water is an essential resource on pig farms. Daily water consumption per growing animal represents an average of 10% of body weight, with considerable variability between individuals (Massabie, 2001). Sub-optimal physico-chemical or bacteriological quality has consequences for animal health (Brilland, 2015), which has an economic impact on farms. However, the direct consequences of poor physico-chemical or bacteriological quality on animal performance have been less studied. For the year 2023, technico-economical performance, technical performance of the Pass Cheptel software and carcass data at slaughterhouse were collected from 598 farms in western France and cross-referenced with the results of an annual compliance audit. The weaning to effective service interval is higher in the farms where the water is bacteriologically unsuitable ($p=0.059$). The percentage of live-born losses is smaller when the water comes from a borehole with bacteriological treatment ($p=0.069$). The number of piglets weaned in each litter is higher in farms where water comes from a borehole with bacteriological treatment compared to mains water without treatment (13.02 weaned piglets vs 12.43, $p<0.05$). Farms with bacteriologically optimized water have a better technical 8-115kg FCR (2.37 vs 2.52, $p<0.001$), an earlier standardized age at 115kg (174.45 days vs 195.14 days, $p<0.001$) and a more homogenous ADG ($p<0.05$). Farms with bacteriologically optimized water have fewer seizures for respiratory reasons at slaughter (0.41% vs 0.51%, $p<0.05$). The rate of joint seizures in farms with borehole water is lower in the presence of bacteriological treatment (1.73% vs 1.83%, $p<0.05$) or in the presence of physico-chemical treatment ($p=0.09$). These results show the importance of taking water into account at all stages of a pig's life in order to optimize farm performance, and encourage farmers to raise their awareness. In addition, the mechanisms linking water quality and sanitary seizures at slaughter should be explored more specifically, for example by looking for the pathogens responsible for seizures in drinking water.

Training pigs to excrete in a toilet area by manual rewarding; preliminary study

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Reducing fouled floor area in the pig pen can reduce ammonia emission and improve pen hygiene. The pig toilet can positively motivate pigs to excrete in a designated small area. To further develop an automated toilet system, the aim of this proof-of-principle study was to train pigs by rewarding them manually to use a designated corner of the pen indicated by a light as the toilet area, and to determine whether the designated area could be moved to another location. Fattening pigs in 2 pens (4 pigs/pen) were rewarded with apple pieces via a steel pipe when they excreted in the toilet. In phase 1, there were six 15-min training sessions per day with 1 hour interval, 5 training days/week, for a period of 16 days. In phase 2, the toilet location was switched to the lying area of the pigs for 2 weeks. In phase 3, the toilet location alternated between two areas (the corner trained in phase 1, and the corner near the feed trough) for 13 days. Excretion location and rewarding events were recorded during training. Descriptive analysis was done to summarize the results. A total of 1196 defaecations and 1175 urinations were recorded. In phase 1, 70.1% and 96.8% of excretions were in the toilet area for pen 1 and 2, respectively. For pen 1, excretion in the toilet significantly decreased as the toilet area got more crowded over training days. In phase 2, almost no excretion (5.1%) occurred in the toilet. In phase 3, pigs kept using the location previously trained in phase 1 for elimination, while there was no significant increase in excretion in the designated toilet area near the feed trough. Pigs would use a small, designated area for excretion and get a reward, however, increased activity may reduce the number of excretions in the designated area. The toilet area should be placed in a preferred place for pigs, away from other functional areas.

Farmers' perceptions of leg health issues and their risk factors among organic pigs

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Ensuring good leg health is a challenge faced by organic pig farmers. Previous studies have shown that organic pigs suffer from more leg problems than conventional pigs, despite using the same breeds. The aim of this study was to investigate Swedish organic farmers' perceptions of the current magnitude of various leg health issues, potential risk factors and differences across breeds. An online survey was sent to all Swedish organic pig farmers (N=47), exploring their perceptions of leg issues on their farm, their risk factors, and choices of breeds. Questions on leg issues included e.g. lameness, joint inflammation, and physical injuries. The survey was followed by interviews to refine individual perceptions of the most concerning risk factors and issue(s), their frequency and severity, and discuss their choice of breeds. Preliminary results from the survey (30% response rate) show that the majority of the farmers, regardless of the production type (piglet, finisher, or farrow-to-finish), classified most issues as rarely occurring. Joint inflammation seemed to be the most frequent issue in piglet farms, while stiffness (stiff movements) was the most common problem in finisher farms. Perceptions of severity varied across issues and respondents, but the same problems were generally perceived as more severe when affecting piglets than finishers (such as joint inflammation) regardless of frequency. Views on risk factors also varied, but in general stocking density and feed were perceived as having considerable effect, with the most affecting factors being outdoor activity and indoor environment. The results will provide valuable insights into the perceived leg health situation of organic pigs in Sweden and highlight areas for improvement. The obtained knowledge will be used to further improve leg health and ultimately animal welfare in organic pig herds.

Early Gut Immune Cells Colonization after Supplementation of Yeast Polysaccharides On Broiler Chicks

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The inclusion of yeast cell wall products in poultry diets has been shown to enhance immune responses, leading to improved disease resistance and overall health. While the innate immune system is functional at hatch, the adaptive immune system components are absent at that time. Bacterial colonization significantly influences the maturation of the gut-associated immune system. This study aimed to investigate the immunomodulatory effects of a specific yeast cell wall (OPTIWALL), focusing on its potential to improve gut maturity through various criteria: weight of immune organs (bursa of Fabricius and spleen), gut histomorphometry, and immune cell migration (lymphocytes) along the digestive tract. In the study, 169 one-day-old male ROSS 308 chicks were divided into 13 pens (6 control pens and 7 supplemented pens with 2 kg/tonne of feed of specific yeast polysaccharides). On day 15, one bird per pen was euthanized, and the caecum was collected for immunohistology to analyze immune cell colonization in the gut tissue. Lymphocytes (cytotoxic T lymphocytes expressing CD8 and T-helper cells expressing CD4) were observed in the lamina propria and epithelium as intra-epithelial lymphocytes. Lymphoid organs were weighed as well as the sacrificed birds. Further to growth performance improvement (ADG, FCR) in this study (Barbe et al., 2024), metrics related to intestinal health (bird maturity, immunity) improved with daily feed supplementation of the postbiotic. The bursa of Fabricius (BF) was heavier (+29%; $p=0.05$), and the BF to spleen weight ratio was higher too (+50%; $p=0.059$). Lymphocyte counts on day 15 were also increased in the caecal tonsils (+243%; $p=0.0002$). In conclusion, the specific yeast cell wall supplementation increased gut anatomical maturity and immunity, with higher immune cell concentrations in the treated group. Supplementation from day 1 with a natural microbial solution can potentially help reduce the negative consequences of inflammation that can easily be triggered in birds with poor immune status, leading to downgraded performance.

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Poster 15

Investigation of group housing concepts in the mating area with regard to sow welfare

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In 2021, the EU Commission announced plans to phase out sow fixation by 2027. To compare different implementation concepts for group housing in the mating area and to evaluate how sows respond to the modified conditions, we conducted a welfare assessment in two conventional sow housings: farm A (5 m²/sow mating area, $n=468$, 9 batches) vs. farm B (5 m²/sow mixing pen followed by 3.5 m²/sow mating area, $n=272$, 15 batches). Welfare was assessed at three time points (day 0, day 2, day 6/7) post-weaning, based on the Welfare Quality® assessment for pigs. Additionally, performance and fertility data as well as group characteristics and climate data were collected. Linear mixed model and generalized mixed model were used to compare housing concepts and to identify animal, management and environmental factors influencing sow welfare. In general, farm B showed a significantly higher increase in skin lesions during the first two days after weaning compared to farm A ($p < 0.001$). While farm A maintained a constant lesion level over the following period, the increase in lesions on farm B slowed down ($p < 0.001$). Parity ($p < 0.001$), number of weaned piglets per litter ($p < 0.05$), and insemination index ($p < 0.05$) significantly influenced increase in skin lesion. A comparison of other welfare indicators showed that sows on farm B had a significantly higher probability of stereotypies ($p < 0.001$) and hind leg swelling ($p < 0.01$). The results highlight the complexity of adapting housing systems for sows, with farm A showing more stable welfare outcomes despite both systems affecting skin lesions. This study is funded by the German Federal Ministry of Food and Agriculture (BMEL) and granted by the Federal Office for Agriculture and Food (BLE; grant number 28N305603).

Water consumption of different pig livestock systems in the French Auvergne-Rhône-Alpes region

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As the Auvergne-Rhône-Alpes region of France is at the forefront of climate change, which is increasing temperatures, especially in summer, the region must manage the major issue of water resources. Tensions around the water supply peak in summer, when precipitation is lowest and the needs of livestock are greatest. The Cerceau project aims to update knowledge on the water consumption of pig farms to optimize water resource management. For more than two years, water consumption was recorded on nine pig farms with several types of housing (e.g., fully slatted, litter, outdoor) representative of the diversity of pig production in the region. Temperatures were also recorded to relate them to water consumption. The data collected and analysed for the weaning and growing stages showed that water consumption (liters per animal per day) was similar to the french national benchmark used by the pig sector, regardless of the type of housing. However, when we used temperature to compare batches raised during “mild” and “hot” periods, higher consumption was observed during the “hot” period but depended on the type of housing. In conclusion, water consumption was similar to the french national benchmark, but the influence of temperature depended on the type of housing; thus, both criteria need to be used to manage water resources.

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Poster 17

Does a low crude protein content reduce the environmental footprint of pig feed?

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Swine feed crude protein (CP) content reduction is strategic to tailor amino acid requirements and reduce N excretion. It requires modifying feed ingredients and their environmental impact. We evaluated the effects of low CP content feeds for finishing pigs on 5 feed footprint attributes: P consumption, cumulative energy demand (non-renewable sources), climate change, acidification, eutrophication, and land competition based on lifecycle assessment (version 9 ECOALIM database). Scenarios assumed an average or no deforestation risk for imported soybean, application of balanced organic and synthetic fertilizers coupled to legume crop rotations for cereal cropping and sunflower imports. To achieve a 2% CP content reduction (14.5 to 12.5%), while maintaining dietary lysine and ideal protein supply, feedstuffs were adjusted. The incorporation of barley, wheat middlings and in-feed amino acids were increased. Minerals had minor increases. Soybean meal, wheat and sunflower meal were decreased by reducing CP. Maize and lard were similar across diets. Low CP feed increased phosphorus consumption (+5.9%), cumulative energy demand (+16.0%), climate change (+4.2%), acidification (+9.1%), and land competition (+2.7%), and decreased eutrophication impact (-3.1%) compared to standard feed. Trend results were steady when assuming a soybean meal source without deforestation risk, but eutrophication was more reduced (-5.4%) and climate change impact increased (+7.9%) when compared to standard feeds. Low CP pig diets reduce the nitrogen load but may have limited interest to mitigate carbon and phosphorus footprints in Southern Europe.

Effect of antioxidant supplementation on lactating sows exposed to mild heat stress conditions

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Due to their high metabolic rate, lactating sows are particularly susceptible to heat stress. Exposure to high temperatures increases the risk of oxidative stress, leading to cellular and tissue damage, which negatively impacts performance and health. Literature suggests that dietary supplementation with multiple antioxidant ingredients is more efficient than using a single one in alleviating the negative impacts of heat stress. As Europe had recently experienced increasingly hot summers, a study performed in the summer of 2023 compared the performance of lactating sows fed a diet including either a standard trace-mineral and vitamin premix (treatment C) or one enriched with additional antioxidants (treatment A). Accordingly, the premix A supplied (/kg feed) 245 mg of vitamin E (vs. 45 mg with premix C), 200 mg of vitamin C (vs. 0 mg with C), 1000 mg of betaine (vs. 0 mg with C), 25 mg of superoxide dismutase (vs. 0 mg with C), 0.35 mg of Se (0.20 mg from selenite and 0.15 mg from selenized-yeast vs. 0.25 mg from selenite with C). Other trace-minerals and vitamins were supplied at the same level in both premixes. Four batches of 24 Large White x Landrace sows were studied from July to October. Within each batch, pairs (replicates) of two sows were formed based on their parity, backfat thickness (BT) and body weight (BW) and then assigned to treatments. Data from 44 pairs of sows were collected. No significant effect of the treatment was observed on sows average feed intake (C: 5.61 vs. A: 5.38 kg/d, $P = 0.59$), BW change (C: -39 vs. A: -42 kg, $P = 0.27$) or BT change (C: -4.8 vs. A: -4.9 mm, $P = 0.66$), or litter ADG (C: 2.95 vs. A: 2.94 kg/d, $P = 0.86$). As hourly ambient temperature always remained above 25°C but rarely exceeded 30°C, hot conditions were likely too mild to greatly influence the oxidative stress status of sows thereby limiting the possibility of demonstrating a significant impact of antioxidants. This study is a part of the Fermadapt program funded by Région Bretagne.

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Poster 19

Impact of stocking density on animal welfare and performance in finishing pigs

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The critical role of stocking density in pig farming is well-documented, as it directly impacts feed intake, growth, and welfare in finishing pigs. In response to recent Spanish welfare standards (RD 159/2023) have mandated increased space allowances for pigs, aiming to better accommodate their size and essential behaviors. This study evaluated the effect of this increased spacing on the productive and welfare parameters in finishing pigs. A total of 283 finishing Duroc x Danbred pigs (72 ± 6.64 kg body weight (BW)), from 2 batches, were involved in a study of 40-day finishing period. Pigs were housed in pens with an available area of 4.45 m², balanced by sex and BW, and were fed ad libitum. Each pen was assigned to one of 3 stocking densities: low (L), medium (M) and high (H), with 0.86, 0.74 and 0.65 m²/pig, respectively. Individual BW and pen feed intake were recorded every 3 weeks, as well as slaughterhouse performance at the end of study; welfare was assessed, every 3 weeks as well, through behavioral observations, body dirtiness and wounds scoring based on Welfare Quality®. Fecal and saliva samples were collected from two randomly selected pigs per pen at the beginning and end of the study to assess cortisol levels using competitive EIA kits (Neogen® Corporation Europe, Ayr, UK). Data were analyzed using mixed-effects models with repeated measures (PROC MIXED of SAS®). Findings indicate significant improvements in feed intake, average daily gain, final BW, and carcass weight in the L group compared to M and H ($p < 0.05$). There was also a noticeable trend ($p = 0.07$) towards higher incidences of body lesions and dirtiness in the M and H groups compared to L. In conclusion, while transitioning from H to M density does not yield significant productivity or welfare benefits, providing a L-density environment markedly enhances both performance and welfare outcomes for finishing pigs. Key words: finishing pigs, stocking density, available space, cortisol, welfare, performance.

Influence of rearing systems on the liver transcriptome profile of krškopolje pigs: indoor vs. outdoor conditions

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Key words: local pig breed, RNA-sequencing, rearing system The Krškopolje pig is an autochthonous Slovenian breed, raised in various, mainly low-input production systems. To better understand, the impact of the rearing environment on this breed, this study aimed to characterize the liver transcriptome of Krškopolje pigs reared indoors (IND, n = 9) and outdoors (OUT, n = 10). At 331 days of age, the pigs were slaughtered, and liver samples were collected for RNA sequencing using the Illumina NovaSeq platform. On average, sequencing generated 45 million paired-end reads, with more than 88.4% uniquely mapped reads. Differential gene expression analysis identified 144 differentially expressed genes, including 85 upregulated and 59 downregulated in the OUT compared to the IND group. Genes upregulated in the OUT group were associated with neuronal development (e.g., UNC5D, PRTG, TENM4, BEND6), liver fibrosis via PFKFB3-mediated glycolysis (PFKFB3), hepatocyte regeneration and growth (FGF21, FGF14), and glucose metabolism (FGF21, RGS16). Additionally, the OUT group exhibited upregulation of GABRRQ, which encodes gamma-aminobutyric acid receptor linked to lipid metabolism; DIO2 associated with adaptive thermogenesis, and gene TRARG1, a transport regulator of GLUT4. Among the downregulated genes, NNMT gene previously linked to fatty acid oxidation and liver fibrosis was detected. This study provides the first insights into the genetic regulation of the liver in Krškopolje pigs raised in different production systems, contributing to a deeper understanding of the breed's adaptability and metabolic responses. Acknowledgements: Funding by Developmental Funding Pillar of Agricultural Institute of Slovenia, Slovenian Research and Innovation Agency (P4-0133, J4-3094 and Z4-60178) and project GeroNIMO (EU H2020 GA no. 101000236) are acknowledged.

Tracing Transcriptomic Changes in Chicken Embryonic Blood: Multi-Generational Effects of In Ovo Stimulation with Nutriepigenetic Factors

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Epigenetic modifications regulate gene expression and are influenced by environmental factors, shaping phenotypic and clinical outcomes. These changes can persist across generations, though their stability can vary by tissue. This study aims to assess the effects of prenatal stimulation with nutriepigenetic factors on the transcriptome of embryonic blood across generations. Since primordial germ cells (PGCs) circulate in embryonic blood before settling in the gonads, this tissue likely represents both somatic and germline lineages. We established a novel in ovo model over three generations of Green-legged partridge-like with an added assessment of F4 embryos. Synbiotic PoultryStar and choline were injected in ovo on the 12th day of egg incubation. F1 embryos were divided into control (0.9% NaCl), synbiotic (SYN, 2 mg PS), and synbiotic + choline (SYNCH, 2 mg PS + 0.25 mg choline). In F2 and F3, SYN and SYNCH were split into two subgroups each: A) solely injected in F1 (SYNs and SYNCHs); and B) repeatedly injected in every generation (SYNr and SYNCHr). Fertilized eggs from all groups laid by F2 and F3 hens were incubated until HH stages 14–16, at which point embryonic blood was collected from the dorsal aorta of embryos. Embryos were sexed using PCR-based sex determination, and blood samples were pooled by sex. RNA was isolated from male samples for RNA sequencing. Our preliminary results revealed effects of in ovo stimulations on embryonic blood transcriptome in F3. However, the effect diminished in F4 embryos. Notably, synbiotic treatment primarily influenced cellular homeostasis, metabolic activity, and stress response mechanisms. The addition of choline further enhanced regulatory effects on gene expression and membrane-associated functions. Funding: National Science Centre, Poland (2020/37/B/NZ9/00497). Bioinformatics analysis: Poznań Supercomputing and Networking Center resources.

Effect of medium-chain fatty acids in calf milk replacer on fecal microbiome development of dairy calves
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Neonatal calf diarrhea can have an impact on intestinal development and concomitant performance, with the Firmicutes to Bacteroidetes ratio (F/B) as a marker of gastro-intestinal microbiota dysbiosis. A complete random block design was used to evaluate the effect of Aromabiotic® Calf (AC), a mixture of medium-chain fatty acids (MCFA), on fecal microbiome in dairy calves at Blanca from the Pyrenees (Lleida, Spain). 74 newborn dairy calves were randomly assigned to 2 milk replacers (MR; Rumilac Comfort, Agrifirm; 150 g/l, 22% CP, 18% EE): 1) a control without MCFA (CON), or 2) containing 2.14 g MCFA/kg of MR (AC). Calves were individually housed until d21 and in groups of 3-4 until weaning at d70. Fecal scores (FS; 0-3) were determined and samples taken from 1 calf within each calf group on d14, 29 and 70 for targeted full-length (V1-V9) 16S rRNA gene amplicon sequencing on a GridION nanopore sequencer and bacterial taxonomic identification. The Shannon diversity index and Bray-Curtis dissimilarity matrix was used to assess α and β -diversity, resp. No differences in bacterial diversity were found between treatments. α -diversity increased ($P<0.001$) over time, while d14 and d29 samples clustered closely together on β -diversity ordinations but d70 clustered separately. Further, d70 samples clustered close to each other with minimal variation, while d29 samples showed the highest within-group variation in bacterial composition, indicating fecal microbiome was under full development at this age. Whereas Firmicutes were always the most abundant for both treatments at all time points, an increase was seen ($P<0.001$) in Bacteroidetes at d70 (CON=32%; AC=35%) versus d14 (CON=1.7%; AC=2.6%) and d29 (CON=4.6%; AC=9.0%). FS were lower for AC than CON ($P=0.047$) which were correlated with F/B ($R=0.52$; $P<0.001$) and α -diversity ($R=-0.55$; $P<0.001$). In conclusion, MCFA exerts a positive effect on maintaining intestinal homeostasis, especially when a calf's microbiome is under full development.

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Theatre 2

Impact of neonatal health status on serum and saliva immunoglobulins (IgG, IgA, and IgM) concentrations in of newborn calves

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Saliva sampling is a non-invasive and an easy method for assessing animal immune status. This study aimed to evaluate serum and saliva Ig (IgG, IgA, and IgM) concentrations during the first week of life in healthy (HC, n=11) and sick (SC, n=9) calves. Blood and saliva samples were collected from 20 dairy calves at birth and on d 1, 2, and 7 of life. The Ig and total protein concentrations in serum and saliva were evaluated with Linear Mixed Models including health status, time and the interaction between both as fixed effects. At birth, all Ig isotypes were present in saliva, with IgA present in higher concentrations in HC (31.12 [23.09, 41.93] $\mu\text{g/mL}$) and SC (44.65 [32.58, 61.19] $\mu\text{g/mL}$). Serum and saliva Ig concentrations increased after colostrum intake (d 1), although saliva IgG concentration declined on d 2, unlike serum IgG concentration. A tendency for lower saliva IgA and IgM concentrations was observed in SC (49.35 [36.01, 67.64] and 1.86 [0.97, 3.16] $\mu\text{g/mL}$, respectively) compared to HC (92.10 [69.26, 122.48] and 5.12 [3.37, 7.58] $\mu\text{g/mL}$, respectively) on d 1. Similarly, SC (0.52 $\pm$ 0.50%) showed a reduced proportion of IgA in saliva relatively to serum compared to HC (3.07 $\pm$ 0.50%) on d 1. In conclusion, saliva Ig concentrations during the first week of life were affected by the calf health status, being the calves from the SC group the ones showing lower saliva Ig concentrations.

Retrospective assessment of high-resolution weight data of calves in the first week of life

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Weight determination of calves is crucial in animal production and science for evaluating feeding strategies. Typically, calves are weighed weekly from birth until after weaning, with daily weight data rarely collected unless automated scales are used. This study aimed to analyse high-resolution weight data of neonatal calves during their first week after birth to predict postprandial weight loss curves. We monitored the body weight of 19 Brown Swiss calves during their first week of life to visualize growth curves and retrospectively analyse weight development. The calves were housed in calf hutches placed on animal scales, which continuously recorded their weight at ten-second intervals. They were fed colostrum and transition milk twice daily in unlimited volumes. Urine drained off the scales into containers, and feces was collected at the end of the week. The raw data was smoothed by calculating the median of ten neighboring values to eliminate outliers. Errors from feces accumulation were corrected using linear and quadratic interpolation. Weight development was analysed using polynomial and linear regressions for the whole week and each weight loss phase after feeding. The calves showed an average daily gain of over 1 kg/day. Weight trends exhibited a reoccurring, specific pattern, with the highest weights post-feeding and the lowest pre-feeding. Postprandial weight loss phases were best described by polynomial functions ($R^2 > 0.90$) when milk intake was high and by linear functions when low amounts of milk were consumed. Higher milk intake resulted in a higher rate of weight loss. This study successfully generated high-resolution growth curves for Brown Swiss calves' first week of life, demonstrating that weight loss phases can be modelled.

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Theatre 4

The impact of extended versus short duration colostrum feeding and milk replacer type on neonatal calves' performance, morbidity and mortality

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The importance of feeding colostrum to newborn calves is well known, but whether feeding colostrum past 24 hours impacts passive transfer of immunity, morbidity, growth and mortality is not well documented. Milk powder components vary, especially functional protein content. The effect of this is not fully known. One hundred Jersey heifer calves were enrolled (October 2021-July 2023) for a 2×2 factorial design study. Independent variables included colostrum feeding duration (1 or 4 days) and milk replacer type (two different levels of whey protein phospholipid concentrate (WPPC); isocaloric; 23.8% protein). We hypothesised feeding colostrum for 4 days would reduce failure of passive transfer (FPT), morbidity and mortality rate, and feeding higher levels of WPPC would reduce morbidity and mortality and improve growth. Standard husbandry procedures were carried out. Initial colostrum management was the same for all; 2.5 litres fed within 6 hours of birth, followed by 2.5 litres within 12 hours. Calves fed 1 day colostrum moved to milk powder from their 3rd feed. Calves fed 4 days colostrum received colostrum/transition milk for 3 more days, then moved to milk powder. Passive transfer of immunity was assessed by serum TP. Weights were obtained at birth, 1 month, 8 and 10 weeks. Treatment records were kept on farm. Lung pathology was identified by thoracic ultrasonography. Two tailed Fisher's exact tests, Independent t-tests and multivariable logistic regression modelling were used for data analysis. 4/50 (8%) calves that received 1 day colostrum died during the pre-weaning period; all calves from the 4 day group survived ($P=0.12$). 8/50 (16%) calves from the 1 day group had FPT; 1/50 (2%) calves in the 4 day group had FPT ($P=0.04$). Multivariable logistic regression showed calves in the 1 day group were 10.98 times more likely to have FPT than those in the 4 day group ($P=0.03$). Colostrum feeding duration and milk powder type had no significant impact on morbidity or growth. Milk powder type had no association with mortality rate or FPT.

Effect of tributyrin content in milk replacer on the need for drinking assistance, intake and growth in 2-week-old calves

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Milk replacers (MR) for calves contain alternative fat sources leading to lower butyric acid levels than found in milk fat. While tributyrin allows restoring C4:0 levels in MR, its bitter taste may affect palatability. This study evaluated the effect of inclusion of a spray-dried tributyrin containing fat concentrate on the level of drinking assistance, MR intake and growth in 2-wk-old calves. Forty-eight Holstein male calves (15.6 ± 1.81 ; $42.8 \text{ kg} \pm 2.68$) were brought from a collection centre and housed individually. Calves were blocked by BW at arrival and randomly assigned to one of five MR treatments: control MR (CON) and 4 levels of tributyrin (0.4%, 0.7%, 0.9% and 1%, as % product). MR was supplied at 7.0 L/d twice daily (150 g/L) in teat buckets until d 21 after arrival. Faecal consistency was evaluated daily using a 3-level scoring system from normal to watery faeces. BW was measured weekly. MR intake was recorded daily by weighing refusals 10 and 30 min after meals were offered. The drinking assistance need between 10 and 30 min was recorded using a 4-level scoring system, ranging from no help to high assistance. Abnormal fecal scores increased with increasing tributyrin level in wk 1 ($P < 0.01$), but not in wk 2-3. No effect of treatment was observed for weekly MR intake nor refusals. However, in the first 7 d, MR intake was higher in CON calves than in those fed in 0.4%, 0.7%, and 1% ($P = 0.03$). Consistently, calves fed 0.4%, 0.7%, and 1% required more drinking assistance than CON group at wk 1 ($P = 0.01$). In contrast, calves fed 0.9% did not differ from CON group for MR intakes nor drinking assistance. This resulted in a linear decrease of ADG in wk 1 ($P = 0.01$), whereas in wk 2 ($P = 0.03$), ADG linearly increased with the tributyrin dose. Similarly, BW was lower with increasing tributyrin dose in wk 1 ($P = 0.01$), but not in wk 2-3. These data suggest older calves cannot distinguish taste differences between 0.4% and 1% tributyrin in MR. MR refusals in wk 1 limited growth, but this effect disappeared by wk 2-3.

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Theatre 6

Implementation of a hygiene protocol for teat buckets in newborn individually housed dairy calves

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Improving milk feeding equipment hygiene by properly cleaning buckets and teats can reduce pathogen spread and enhance calf health. This study evaluated the efficiency of a standardized teat bucket cleaning scheme (SCS) versus farm-specific cleaning schemes (FCS) by analyzing microbial load of the bucket before feeding and assessing calf health. On ten dairy farms, 100 calves were divided into SCS ($n = 50$) and FCS ($n = 50$) groups. Calf health, calf welfare, and teat bucket hygiene were assessed three times within the first 15 days postpartum (0-3, 5-10, 11-15 days), totaling 300 samples. SCS buckets were cleaned daily before feeding using a standardized method, while FCS followed farm-specific routines. Additional factors analyzed included farmers' hygiene ratings, farm characteristics, and calf welfare indicators. Microbiological samples ($n = 300$) were tested for aerobic total viable count (TVC), total coliform count (TCC), *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), and 3rd-generation cephalosporin-resistant bacteria (CRB). After verifying the normal distribution assumption for all microbiological hygiene indicators, the effectiveness of the developed SCS was tested against FCS using an ANOVA on . Results showed significantly lower TVC in SCS buckets ($5.3 \pm 0.1 \log_{10} \text{ cfu/ml}$) compared to FCS ($5.8 \pm 0.1 \log_{10} \text{ cfu/ml}$). CC counts averaged $2.9 \pm 1.6 \log_{10} \text{ cfu/ml}$, ranging from 0.0 to 5.7 in SCS and 0.0 to 6.3 in FCS (mean $2.7 \pm 0.1 \log_{10} \text{ cfu/ml}$). All samples tested negative for MRSA, while CRB was detected in 1.9% (6/310) of samples, primarily from a farm which refrain from cleaning the buckets. Diarrhea incidence was lower in SCS calves compared to FCS calves (4.1% vs. 10.1%), with a 0.34 times reduced risk for diarrhea ($p < 0.05$), hinting at a positive impact of improved cleaning protocols on calf health.

Characterization of milk vein blood flow via Doppler ultrasonography from dry-off to calving and its association with colostrum composition and yield in multiparous Holstein cows

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Level of metabolic activity in the mammary gland is reflected in local blood flow (BF) during an established lactation; however, whether such a relationship exists during the dry period and its implications to colostrum production requires further investigation. Thus, the aim of this study was to characterize milk vein BF (MVBF), as a proxy of mammary BF, from dry-off to calving and to determine its association with colostrum yield and composition. Starting at d -58 ± 2.9 prior to calving, parity 2 (P2, n = 11) and 3 (P3, n = 10) Holstein cows were dried off and MVBF was measured by Doppler ultrasound of both milk veins at d -49, -35, -21, and -7 relative to expected calving, then every 4 d until actual calving, and at 2 h after colostrum collection. Colostrum composition was determined via mid-infrared spectroscopy and IgG by radial immunodiffusion. Data were analyzed using PROC GLIMMIX of SAS considering the fixed effects of parity and time and the random effect of cow. Total MVBF ranged from 139.6 to 173.7 ± 20.8 L/h from wk -8 to -2 (P > 0.52), then increased (P < 0.0001) to 206.7 ± 18.4 L/h at wk -1 and to 415.0 ± 35.7 L/h at calving. Both left and right MV area increased (P < 0.0001) from 1.5 ± 0.31 cm² at wk -8 to 2.0 ± 0.40 cm² at calving. Parity 3 cows had 24.9 and 29.5% greater left (P = 0.06) and right (P = 0.02) MVBF, respectively, compared with P2 cows. Colostrum IgG yield was correlated with MVBF at wk -1 (r = 0.49, P = 0.05) and d -2 (r = 0.85, P = 0.03). Colostrum yield was negatively correlated with wk -6 MVBF (r = -0.51; P = 0.08), but no further associations were observed. The results demonstrate that mammary BF during the dry period increases from P2 to P3 and suggests that BF – an indicator of mammary metabolic activity – in the week preceding calving may be an important determinant of colostrum IgG yield.

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Theatre 8

Correlations between fecal and urinary losses with metabolic parameters in neonatal calves with naturally occurring diarrhea

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Characterizing the correlations between fecal and urinary losses and metabolic parameters in diarrheic calves may expand our understanding of the physiology of diarrhea. These correlations were studied using data collected from 2 similar studies that evaluated the effectiveness of an oral rehydration solution (ORS). Sixty-six calves (14-21d old) were selected at a collection centre based on their BW (<55kg), blood base excess (BE), and visible signs of diarrhea and clinical dehydration. Selected calves were transported on the same day to the research facility. The next day (d1), calves were blocked in pairs based on their blood BE. Within each block, calves were randomly assigned to an ORS (osmolality=270mOsm/kg, Na+=80mM) or a placebo consisting of water with 5g/L of whey powder. Treatments were offered as 2L twice daily from d1-3. Calves were fed 2.5 L of milk replacer twice daily from d1-3. Fresh water was available ad libitum. Total faeces and urine were collected over 24h from 1200h on d1 to 1200h on d2, with blood samples taken on the morning of d2. Pearson's correlation was used for normally distributed variables, while Spearman's correlation was applied for non-normally distributed variables. Correlations were not affected by treatments. Fecal DM and fecal volume were negatively correlated (r=-0.70, P<0.01). Fecal water losses were 45.4 ± 37.91 mL/kg BW whereas fecal DM was 10.8 ± 5.22%. Fecal electrolytes (Na⁺, K⁺, Cl⁻) and fecal water losses were positively correlated (r>0.70, P<0.01). Fecal Na⁺ (r=-0.68, P<0.01) and serum urea (r=-0.54, P<0.01) were negatively correlated with urinary Na⁺. Urine osmolality was negatively correlated to urinary fluid losses (r=-0.68, P<0.01). Serum urea was positively correlated with urine osmolality (r = 0.57, P<0.01). Blood BE was negatively correlated with fecal water (r=-0.65, P<0.01) and fecal Na⁺ losses (r=-0.65, P<0.01). Diarrhea was characterized by metabolic imbalances and extensive water and mineral losses in faeces.

Effect of group housing on stress and behaviour of Holstein calves

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Group housing of dairy calves has been shown to enhance welfare and reduce stress compared to individual housing. Group-housed calves exhibit improved social behaviors and adaptability showing higher starter intake and weight gain, thus leading to better growth performance. Furthermore, social housing improves solid feed intake and weight gain before and after weaning. However, there is few information about stress parameters in calves housed in group. Therefore, the main objective of this work was to assess the differences in stress parameters and behaviour in 4-group housed (G) and individually housed (I) calves until weaning. No differences between groups were found for salivary cortisol, or the rest of the blood stress parameters [C-Reactive Protein (CRP), haptoglobin, serum amyloid A and IgM]. However, some behaviours differed significantly as following: (i) suckling empty and suckling object decreased in G, while (ii) eating, grooming, playing, ruminating, social (both positive and negative) and drinking increased in animals housed in groups. Conclusively, stress parameters in Holstein calves might not be affected by group housing, but positive behaviours were facilitated under these conditions.

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Theatre 10

Anticipate and mitigate thermal stress in calves faced with climate change

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The negative impacts of heat stress are well known in various livestock production sectors (pigs, cattle, sheep...), leading to reduced feed intake, growth, milk production, milk fat and protein content (TB and TP), fertility, and animal welfare. At the same time, it increases water consumption, somatic cell counts in milk, respiratory risks, and body temperature. Climate change increases these stress conditions, cattle being particularly sensitive: from 25°C, adaptation efforts are required; between 30 and 35°C, suffering sets in, and beyond 42°C, survival is at risk if the situation persists. The VOLCAN project (2024-2026) aims to model calves' metabolic responses to heat stress based on environmental conditions and thermal needs, and to assess two mitigation strategies: a nutritional approach and improved farm building management. As part of this initiative, two participatory workshops were conducted with veal industry professionals and farmers to evaluate their knowledge of heat stress, existing management practices, and the barriers and levers for implementing them. The impact of heat stress on both animals and farmers was emphasized. The key identified solutions include installing misting and cooling systems, though these practices are debated due to the humidity they generate. Few nutritional strategies were mentioned spontaneously, and some did not reach a consensus. Farmers also highlighted parasite management, particularly concerning flies, as a key issue affecting working conditions and animal welfare. The motivations and needs for implementing solutions vary, including the aim of improving the veal production's image, having access to reliable references, and financial support. The main barriers are economic constraints and feasibility concerns. Industry stakeholders showed strong interest and motivation in this debate, along with a demand for continued project updates.

Identification of differentially expressed biomarkers in blood and intestinal tissues from Holstein cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* and with multifocal lesions

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Bovine paratuberculosis (PTB) is a chronic granulomatous enteritis that causes significant economic losses to the dairy industry worldwide. Previous studies suggested that the formation of multifocal granulomas could constitute a mechanism of resilience to PTB by favoring the control of an aberrant pro-inflammatory immune response and preventing lesion progression. This study proposes the search of biomarkers in blood, jejunum, and jejunal lymph node samples from 11 cows with multifocal lesions and 4 cows without lesions in gut tissues using RNA sequencing. Each RNA library was prepared using 250 ng of RNA with the Illumina NEBNext Ultra Directional RNA library preparation kit and sequenced using the Illumina NovaSeq sequencer. Raw reads were filtered with FastQC and Trimmomatic and aligned with the *Bos taurus* reference genome (ARS_UCD1.3.113) using STAR. Annotation and read counting were performed with Rsubread, and normalization and differential expression analysis were carried out with DESeq2. Functional analysis was performed using the R ClusterProfiler package. The average raw reads were 33.38, 33.86, and 36.92 million in blood, jejunum and jejunal lymph nodes, respectively. In blood, 1,611 differentially expressed (DE) genes were identified in cows with multifocal lesions and associated with translation processes and viral infections. In jejunum, 7 DE genes were associated with immune cell migration. The glycoprotein 2 (GP2) gene was the most upregulated gene in the jejunum of animals with multifocal lesions versus controls (log2 fold change = 6.94). This gene encodes a protein that binds pathogens and plays an important role in the innate immune response. In lymph nodes, 12 DE genes were identified, associated with TOR regulation, a kinase involved in cellular responses to DNA damage and nutrient deprivation. The biomarkers identified could contribute to improving the available tools for detecting cattle resilient to PTB.

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Theatre 12

Effect of calf suckling duration on immunoglobulin G and oligosaccharide profiles in the early lactating period of Holstein cows

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The objective of this study was to determine if calf suckling affected IgG and OS concentrations and yield in the early lactating period. Multiparous (n = 8) and primiparous (n=4) Holstein cows and their calves were enrolled. All calves were provided their respective dam's colostrum at 8.5% BW, after which cow-calf pairs were assigned to one of two treatments: no contact (0d; n = 6), or delayed separation at 5d (n = 6) postpartum. Contact groups had continuous access to the dam except during scheduled milkings. Colostrum was collected within 2.2 ± 0.4 h postpartum, followed by twice-daily milking using a portable milker (milkings 2-11). Composite milk samples were obtained at each milking, and only machine-milked yields were recorded. Immunoglobulin G concentration was analyzed using radial immunodiffusion, and OS concentrations were measured using high-resolution mass spectrometry. Data were evaluated using PROC GLIMMIX in SAS, considering fixed effects of treatment, milking number, their interaction, the repeated effect of milking number and the random effect of pen. As expected, IgG concentration and yield were the highest (P < 0.0001) in colostrum, declined over time and were unaffected by treatment (P = 0.9). Similarly, total OS concentration and yield were greatest (P < 0.0001) in colostrum and declined gradually. Interestingly, the 5d group had higher (P = 0.03; 1046.8 ± 122.5 µg/mL) total OS concentrations, than the 0d group (731.2 ± 122.7 µg/mL), with no effect of treatment on OS yield (P = 0.8). These results highlight colostrum and transition milk's role in calf health, containing the highest IgG and OS concentrations and yields. While OS concentrations differed by treatment, yield was unaffected and likely due to unaccounted calf consumption. Future research is required to determine the influence of calf-suckling on milk composition, including OS concentration.

Bovine coronavirus and the occurrence of clinical and subclinical pneumonia in weaned calves

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Bovine coronavirus is a pneumoenteric virus whose epidemiology and role as a causative agent of bovine respiratory disease (BRD) in calves is poorly understood. One aim of our study was to determine the relationship between BCV presence in faeces and the nasopharynx and associations with clinical BRD and transthoracic ultrasound (TUS) scores. We studied 120 weaned Angus x Holstein calves from 10 source farms (SF) (3 cohorts of 40 calves). Calves were quarantined according to SF for 12-14 days and then randomized, within the strata of Farm and Sex, into one of four commingling pens. There were 10 calves per pen (5 x 3 m with solid walls). Faecal and nasopharyngeal swabs were taken on collection from the SF, then on Day 0 (day of commingling), 3, 7, and 21 post-commingling and subjected to quantitative PCR for BCV. TUS was performed at collection, Day 0, and Day 21 and scores greater than one classed as having subclinical BRD. Calves were scored daily for signs of clinical BRD using a modified Wisconsin system and scores greater than two were classed as having clinical BRD. Results from Cohort 1 (n = 40) were analysed using Fisher's Exact Test to determine the relationship between contemporaneous nasal and faecal BCV; nasal BCV and subclinical BRD; and, subclinical BRD and the occurrence of clinical BRD within 7 days of TUS scanning. Six (23), 4 (24) and 4 (20) calves were classed as having clinical (subclinical) BRD at collection, Day 0 and Day 21, respectively. Clinical BRD score was associated with subclinical BRD on collection ($P = 0.03$), but not at Day 0 or 21 ($P > 0.6$). Nine, 7 and 26 calves were PCR positive on either faecal or nasal, or faecal and nasal samples at collection, Day 0 and Day 21, respectively. Faecal and nasopharyngeal BCV tended to be contemporaneously shed at collection ($P = 0.13$) and Day 21 ($P = 0.06$), but not at Day 0 ($P > 0.99$). Nasal BCV presence was not associated with subclinical BRD at any time point ($P > 0.52$). Full results from all three cohorts will be presented and discussed.

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Theatre 14

Associations of neonatal hemoglobin level with growth performance in weaned holstein heifer calves

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Some evidence exists concerning the correlation between neonatal hemoglobin and average daily gain (ADG) and body weight (BW) at weaning in veal calves. This study aims at evaluating the effects of neonatal hemoglobin on performance, health, feed efficiency (FE) and feed conversion ratio (FCR) of weaned dairy heifer calves. A total of 45 newborn Holstein heifer calves were enrolled in the trial after the confirmation of a proper immunity passive transfer. Calves were divided in 2 groups based on the neonatal median Hb value (9.6 g/dl): high-Hb (HHb) and low-Hb (LHb). Calves were monitored every 3 days throughout the entire weaning period (63 d). Body condition score (BCS), BW, ADG, cumulative intake, FE, FCR, morphometric and healthy parameters were recorded at the same intervals. Blood and fecal samples were collected at 0, 15, 30, and 63 d and analyzed for blood count and metabolic profile, and fecal microbial load. Performance parameters were calculated for the entire period. Data were analyzed by ANOVA using SPSS software v.29. A Bonferroni post-hoc test was applied for pairwise comparisons. The HHb group showed higher BW, BCS, ADG ($P=0.04$), withers height, rump height and width compared to the LHb group, throughout trial. No differences were detected for FE, FCR, cumulative intake, or fecal microbial load. Higher red blood cell count (7.9×10^6 vs 9.2×10^6 ; 9.2×10^6 vs 1.1×10^7 and 9.5×10^6 vs $1 \times 10^7 \mu\text{L}$), hematocrit (23.1 vs 28.5; 23.6 vs 29.5 and 22.5 vs 25.3 %) was observed in HHb group at 0, 15 and 30 d ($p<0.05$). This group showed higher white blood cells at 15 and 30 (6.3 vs 7.9 and 5.9 vs $7.5 \text{ K} / \mu\text{L}$), while mean corpuscular volume (25.6 vs 27.2 fl) at 15d ($p<0.05$). The highest plasmatic glucose content at 30 d ($P=0.034$) and total cholesterol at 30 ($P=0.016$) and 63d ($P=0.012$), was observed in the HHb group. Regression analysis revealed a relationship between neonatal hemoglobin concentration and BW at weaning ($r^2=0.319$). Neonatal Hb concentration can be a tool for the early identification of the most performing animals.

Year-round supplementation of a *Bacillus*-based DFM to cow-calf herds: Maternal and offspring performance
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This study evaluated the impacts of year-round supplementation of a *Bacillus*-based direct-fed microbials (DFM) on productive performance of cow-calf pairs. On day 0 (94 days prepartum), 296 multiparous, Brangus beef cows were stratified by body weight (BW; 537 ± 56 kg) and body condition score (BCS; 5.46 ± 0.74 ; scale of 1 to 9) and were randomly allocated into 1 of 26 Paspalum notatum pastures (10 to 11 cows and 8 to 10 ha per pasture). Treatments were randomly assigned to pastures on day 0 (13 pastures per treatment) and consisted of free choice access to a trace mineral supplement added (BAC) or not (CON) with a DFM mixture containing *Bacillus subtilis* 810 and *B. licheniformis* 809 (Bovacillustm; Novonesis, Hørsholm, Denmark) from day 0 until day 330 (calf weaning). Cows calved from day 55 to 153. After birth, all calves had unrestricted access to their respective treatments assigned on day 0. Cows were placed with Brangus bulls from day 153 to 240. Pasture was considered the experimental unit. Non-binary and binary data were using the MIXED and GLIMMIX procedures of SAS, respectively. Effects of treatment $\times$ day tended ($P = 0.08$) to be detected for supplement DM intake, which was greater ($P \leq 0.03$) for BAC vs. CON cow-calf pairs on days 42 to 48, 56 to 62, 168 to 174, and 266 to 272. Cow BCS change from day 150 to 240 and cow BCS on day 240 were greater ($P < 0.01$) for BAC vs. CON cows. Effects of treatment were not detected ($P \geq 0.13$) for any cow reproductive data and for calf plasma concentrations of haptoglobin and cortisol at birth. Calf ADG from day 150 to 240 was greater ($P = 0.02$) for BAC vs. CON calves, whereas ADG from birth to day 330 tended ($P = 0.06$) to be greater for BAC vs. CON calves. Calf BW on day 330 was greater ($P = 0.03$) for BAC vs. CON calves. Thus, year-round supplementation of a *Bacillus*-based DFM did not impact cow reproductive success, but increased calf preweaning growth performance compared to non-supplemented cohorts.

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Poster 16

Effect of medium-chain fatty acids in calf milk replacer on gut and lung health of dairy calves

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Diarrhea and respiratory issues are still omnipresent in neonatal calves. Adapting the milk replacer (MR) is the most effective way to support health and productive development as soon as possible after birth. A complete random block design was used to evaluate the effect of Aromabiotic® Calf (AC), a mixture of medium-chain fatty acids (MCFA), on growth, gut and lung health in dairy calves at Blanca from the Pyrenees (Lleida, Spain). 74 newborn dairy calves were randomly assigned to 2 treatments: 1) a control without MCFA (CON, $n=38$), or 2) receiving 2.14 g MCFA/kg of MR (AC, $n=36$). Calves were individually housed until d21, in groups of 3-4 until weaning at d70, and further followed post-weaning until d120. Animals only received MCFA during the pre-weaning period via the MR (Rumilac Comfort, Agrifirm; 150 g/l, 22% CP, 18% EE). Starter concentrate (88.9% DM, 17.5% CP, 14.5% NDF) and straw were offered ad libitum. Individual body weights (BW) were measured weekly, whereas fecal scores (0-3) were determined daily. Lactulose (0.4 g/kg BW) and mannitol (0.12 g/kg BW) were given via MR to 12 calves/treatment on d14 and 28 to assess intestinal permeability using serum samples taken 2 h after the morning feeding. To assess lung health, quick thoracic ultrasound (qTUS, score 0-5) was performed by a veterinarian on d7, 14, 28, 56, 70, 90, and 120. No differences were observed between treatments for MR and feed intake nor daily gains. The proportion of animals with fecal score >0 between birth and d120 was lower ($P=0.016$) for AC (0.054) than CON (0.076) calves, which was most notable as calves on AC recovered faster from a typical diarrhea peak at weeks 2-3. Lactulose-mannitol ratios were lower ($P=0.006$) for AC (0.12) than CON (0.15) calves, indicating a lower permeability and positive effect on gut health in the first weeks of life. Moreover, a relationship ($P=0.018$) between treatment and qTUS was observed, indicating calves on AC were less likely to develop lung pneumonia (score >1). In conclusion, MCFA positively influence gut and lung health when given via MR of neonatal calves.

Effects of supplemental calcium gluconate on the rumen and duodenum biome of Bonsmara steers

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Prebiotics are indigestible or only partially digestible feed additives that assist the host by promoting the growth of bacteria in the gastrointestinal tract (GIT). Prebiotics have been utilized as functional feed additives in animal nutrition due to their ability to stabilize gastrointestinal microbiota and modulate gastrointestinal activities, thereby providing significant benefits to the host animal. The prebiotics, calcium gluconate, has been shown to stimulate the growth of beneficial microbiota in the hindgut of ruminants and can improve gut function and integrity, which can lead to increased butyrate production. Butyrate is a short-chain fatty acid that can improve gut health. In a fully randomized design, 20 Bonsmara steers (treatment and control, $n = 10$) were fed a 14-day starter diet for adaptation, a grower diet for 72 days and a finisher diet. During the grower phase, 0.7 kg of calcium gluconate was added to the treatment diet daily. Steers were fed twice daily. All 20 steers were slaughtered and the offal used for sample collection and measurements. Targeted 16S metagenomic sequencing showed no significant differences in the rumen biome however the duodenum showed a significant decrease ($p < 0.05$) in relative abundance of *Turicibacter* spp and *Romboutsia* spp both genera which have been shown to be negatively correlated to butyric acid production. An average increase in treatments samples for the genera *Syntrophococcus* spp, *Treponema* spp, *Prevotella* spp and *Ruminococcus* spp was also observed in the duodenum, all genera capable of producing butyric acid through fermentation. A significant difference ($p < 0.05$) and an average increase in butyric acid production was also observed in the rumen and duodenum, respectively of treatment groups. Butyric acid serves as an essential energy source for the development of the rumen and overall gut health. It also supports intestinal integrity, enhances nutrient absorption, and plays a key role in improving immune function and feed efficiency.

Session 69

Poster 18

Microbiome and resistome in diarrheic and non-diarrheic calves shedding *Cryptosporidium parvum*L. Bachmann¹, N. Lechleiter², J. Wedemeyer², T. Homeier-Bachmann²¹ University of Applied Science, Neubrandenburg, Faculty of Agriculture and Food Science, Brodaer Str. 2, 17033 Neubrandenburg, Germany, ² Friedrich-Loeffler Institute, Institute of Epidemiology, Südufer 10, 17493 Greifswald, Germany

One of the leading pathogens causing diarrhea in newborn calves is the protozoan *Cryptosporidium parvum* (C. parvum). Both impaired fecal consistency and infection with C. parvum lead to a shift of microbial community (Gomez et al., 2017; Ichikawa-Seki et al., 2019). Moreover, a change in resistome was found in calves with diarrhea of unknown reason (Zhang et al., 2025). Based on the studies, it cannot be verified whether the altered stool consistency or the infection with C. parvum is responsible for the changes in microbiome and resistome. Therefore, the aim of the study was to gain a comprehensive insight into the microbiome and resistome of calves shedding C. parvum with and without diarrhea. On a dairy farm diarrheic and non-diarrheic calves were tested with an on-farm test (Fassisi BoDia®) for the most important pathogens causing diarrhea (rota and corona virus, E. coli K99 and C. parvum). Calves were categorized in three groups consisting of five calves, respectively: (1) diarrheic calves shedding C. parvum, (2) non-diarrheic calves shedding C. parvum and (3) non-diarrheic calves without any detection of C. parvum. Fecal samples of the calves were subjected to metagenomic sequencing. Metagenome sequencing data were then analyzed using kranken 2, CC-Metagen and AMR++. Alpha and β -diversity were tested for statistical differences by ANOVA or PERMANOVA, respectively. Infection with C. parvum caused a shift in microbiome in calves irrespective if they had diarrhea or not. Calves shedding C. parvum had higher counts of Fusobacteriaceae. Above all, F. mortiferum was increased. Alpha diversity indices were not but β -diversity was significantly different among groups. Percentages of antibiotic resistance genes were quite similar in all calves. According to our data, the infection with C. parvum is responsible for the shift in microbiome in diarrheic calves and not the change in fecal consistency. Moreover, infection with C. parvum does not result in a shift of resistome which might be different in diarrheic calves infected with other pathogens.

Influence of artificial nipple characteristics on milk intake behavior and quantity in suckling calves

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The animal welfare livestock farming ordinance (TierSchNutzV) requires farmers to provide calves at least twice daily with milk, ensuring natural suckling behavior. If the suckling wish fails to be adequately satisfied, calves may start cross-suckling or suckling on objects. In infant bottle feeding nipple hole increases with age, while farmers report cutting bigger nipple holes for first feeding after birth. A wide variety of artificial nipples are available for calf feeding. A literature search revealed that hardly any study investigated the current diversity of artificial nipples on the milk intake behavior of suckling calves. This study aimed to analyze the milk intake behavior of calves at different ages. Twelve different artificial nipples, varying in hardness, shape, length, and cross-diameter, were tested on 11 Brown Swiss calves in 132 video-recorded feeding sessions at the dairy farm Veitshof of the Technical University of Munich. The behavioral observations of calves during each feeding session were assessed using a standardized behavioral protocol, including drinking duration, breaks, suckling frequency, or pushes. Statistical analysis included descriptive statistics and Spearman rank correlations. These were performed to evaluate relationships between observed behaviors and artificial nipple characteristics. Initial results indicate a strong age and cross-diameter-related influence on the observed behaviors. The length of the artificial nipple has hardly any influence on drinking behavior. To promote well-being and prevent unwanted suckling behavior, the choice of artificial nipples plays a crucial role. Individual differences among calves should also be considered when selecting the appropriate artificial nipple to meet their specific needs.

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Poster 20

Tetracycline use on dairy farms influences the presence of tetracycline resistance in commensal E.coli isolated from pre-weaned calves

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Over a one-year period, data on antimicrobial use (AMU) on 51 dairy farms in Austria were collated. In the same year, all farms were sampled with respect to the presence of antimicrobial resistance among commensal E.coli isolated from cows, pre-weaned calves (<6 weeks) and older calves (>6 weeks). AMU was quantified in Defined Daily Doses (DDDvet) per cow and year, according to the values published by the European Medicines Agency (EMA 2016). Total AMU (excluding dry cow therapy) ranged from 0.028 to 6.910 DDDvet/cow/year (mean 2.504; median 2.580) (Werner et al. 2023). AMU for calves made up <5 % of the total. After cephalosporins and penicillins, tetracyclines were the most frequently used antimicrobials, with a mean 0.275 (median of 0.084) DDDvet/cow/year. On each farm, faecal samples were collected from a maximum of five calves in each age group and pooled for laboratory analysis. Pre-weaned calves were most frequently determined to harbour resistant E.coli. Resistance to tetracyclines among commensal E.coli isolated from this age group was detected on 45% of farms. For the statistical analysis, the farms were divided into two groups based on the median DDDvet/cow/year for tetracycline use. The “low tetracycline” group of 25 farms ranged from 13 farms which did not use tetracycline in the study year (0 DDDvet) to a maximum of 0.076 DDDvet/cow/year. The “high tetracycline” group of 26 farms ranged from 0.084 to 1.764 DDDvet/cow/year. Among the low use group, tetracycline resistance was detected in 28% (7/25) of farms from pre-weaned calf samples; in the high use group this proportion rose to 62% (16/26) of farms. The Chi-square test confirmed a statistically significant difference between groups ($\chi^2 = 5.7901$, $df=1$, $p<0.05$). References: EMA. 2016. Defined Daily Doses for Animals (DDDvet) and Defined Course Doses for Animals (DCDvet). European Medicines Agency, London, UK. Werner, T. et al. 2023. Antimicrobial Resistance and Its Relationship with Antimicrobial Use on Austrian Dairy Farms. Front. Vet. Sci 10:1225826.

The impact of extended versus short duration colostrum feeding and milk replacer type on maiden heifer fertility performance

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The importance of feeding colostrum to newborn calves is well known, but whether feeding colostrum past 24 hours impacts future fertility performance is not known. Milk powder components vary, especially functional protein content. Whether feeding higher levels of functional proteins can influence fertility performance is not fully known. One hundred Jersey heifer calves were enrolled (October 2021-July 2023) for a 2×2 factorial design study. Independent variables included colostrum feeding duration (1 or 4 days) and milk replacer type (two different levels of whey protein phospholipid concentrate (WPPC); isocaloric; 23.8% protein). We hypothesised colostrum feeding duration would not influence fertility, but that feeding higher levels of WPPC would positively impact maiden heifer first service conception rate (CR). Standard husbandry procedures were carried out. Initial colostrum management was the same for all; 2.5 litres fed within 6 hours of birth, followed by 2.5 litres within 12 hours. Calves fed 1 day colostrum transitioned to milk powder from their 3rd feed. Calves fed 4 days colostrum received colostrum/transition milk for 3 more days, then moved to milk powder. Reproductive data was kept on farm, including service dates, service type (artificial insemination (AI) or natural), service breed, AI straw type (conventional or sexed), outcome of service (pregnancy determined by ultrasonography). Only heifers served by AI were included in the analysis. Two tailed Fisher's exact tests, Independent t-tests and multivariable logistic regression modelling were used for data analysis. CR to first service was 58% for those fed standard milk powder, and 80.6% for those fed high WPPC milk powder (P=0.006). Colostrum feeding duration alone had no association with first service CR (1 day=70.9%; 4 day=55.2%; P=0.22). 1 day colostrum 4 days colostrum Standard powder 68.8% 31.8% High WPPC powder 73.3% 87.5% Table 1: First service CR for the 4 study groups The combination of 4 day colostrum and standard powder showed a significant reduction in first service CR (P=0.002).

Session 70

Theatre 1

Histological and molecular signature of in utero hyperthermia in the mammary fat pad of newborn heifers

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Early-life mammary development requires a crosstalk between the mammary fat pad (mFP) and parenchyma, with the mFP providing structural support and paracrine signaling. Our objective was to characterize the microstructure and transcriptomic signature of mFP exposed to in utero hyperthermia. During a subtropical summer (Florida, USA; THI > 79), pregnant Holstein cows were heat stressed (shade) or cooled (shade, fans, water soakers) in the last 54 ± 5 d of gestation, generating in utero heat stressed (IUHS) or cooled (IUCL) heifers which were euthanized (n = 6/group) at birth. Dissected mFP from the udder was fixed in NBF or placed in RNAlater and frozen. Tissue for histological analysis was processed, stained with Masson's Trichrome, imaged, and analyzed using the ATAT plugin in ImageJ. Adipocyte size data were analyzed in SAS using a mixed model. Total RNA was extracted, assessed for quality, and sequenced using the Illumina NovaSeq 6000 platform. Differentially expressed genes (DEG) were identified using fold change > ±1.5 and 5% false discovery rate. The mFP of IUHS heifers exhibited greater numbers of small adipocytes (200-400 µm²; P < 0.05; 400-600 µm², P = 0.09; 600-800 µm², P = 0.07) and fewer large adipocytes (1600-1800 µm², P = 0.05; 2000-2200 µm², P = 0.06, and 5000-5200 µm², P = 0.07) than the mFP of IUCL heifers. The transcriptomic analysis revealed 122 DEG between IUHS and IUCL. Upregulated DEG in IUHS were related to increased apoptosis (IP6K2), decreased transcription (MIER2), inhibition of mTORC1 (AKAP13), and inhibition of estrogen signaling (PITX1). Downregulated DEG in IUHS were related to tight junctions (CLDN1), amino acid transport (SLC7A8), fatty acid synthesis (ACACA), and estrogen signaling (TRIM24, TDRD3). These results demonstrate that in utero heat stress can alter the adipocyte population within the mFP and affect signaling and growth, which may impact future glandular development.

Heat load at birth has a carryover effect on Holstein heifers' age at first calving

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Heat stress (HS) occurs when cows do not manage dissipate excess body heat, leading to behavioral changes, elevated heart and breath rate, and reduced milk production in lactating cows. HS also impacts fertility, causing issues with estrus detection, oocyte quality, implantation, embryo development, and fetal development, particularly in the final weeks of pregnancy. Specifically, HS in utero during the last three weeks of gestation results in lighter calves at birth, which may affect survival in the first days of life, growth and performance as heifers. This study aimed to detect whether HS affects age at first calving (AFC) in Holstein heifers born in summer. The AFC in 750 Holstein heifers born between Jul. and Aug. from 2012 to 2015 and reared in 125 farms of the Po Valley in Italy, were identified. Each farm was located nearby, a weather station (12 in total in this study), that measured temperature and humidity on hour basis which were used to calculate the temperature-humidity index (THI). An analysis of variance was performed to investigate the differences between two extreme situations: 'cold group' (332 heifers with THI ≤ 68 on the birth date) and 'hot group' (468 heifers with THI ≥ 75 on the birth date). The fixed effects were the two THI group and the year of birth of the heifers, while the random effect was the farm. The least square means ($P < 0.05$) showed that heifers born under high HS conditions reached AFC two weeks later (830 d) than those in the 'cold group' (816 d). Although the least square means demonstrated that HS at birth causes a delay in AFC still it should be considered that cows born in summer tend to be inseminated approximately 15-18 months later, i.e. during winter (no HS), in the optimal fertility period. This, coupled with the fact that heifers recover, explains why HS at birth of future heifers has just a small impact on the AFC. Heifers born during hot periods would be inseminated in autumn when photoperiod is optimal and THI levels in the Po Valley is rather below the critical threshold.

Session 70

Theatre 3

The negative effect of heat load on the intercalving length in Italian Holstein cows

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In the era of climate change, the frequency of heatwaves is rising, becoming a chronic stressor for cattle. High-producing dairy cows struggle to dissipate heat due to their high metabolic activity, putting them at significant risk of heat stress (HS), which negatively impacts fertility by impairing heat detection, oocyte and embryo development, implantation, and the uterine environment. In this study, we evaluated if intercalving length (IC, d) was affected by season at calving (S-c) and season at insemination (S-i). Data referred to 66,686 lactations of 11,585 Holstein cows in 162 Italian commercial farms in the period 2012-2019. IC was calculated as a difference between two consecutive calving dates from 1st up to 4th calving. The minimum value was set at 280 d and the maximum at the 99th percentile, i.e. 723 (1st to 2nd calving), 705 (2nd to 3rd calving), and 693 d (3rd to 4th calving). Two analyses of variance were carried out separately: one with S-c as fixed effect and one with S-i. Other fixed effects were parity and herd-year, while cow (repeated data) and residual were the random terms. Although we had no information regarding farm-specific THI and heat mitigation strategies, weather stations located in the studied area revealed that THI peaked in Jul. and that days with THI > 70 (threshold conventionally considered as dangerous for cows) were mostly frequent in Jul. and Aug. Least square means revealed that, when conception occurred in summer, IC (419 d) was 21 d longer compared to spring (398 d) and, in any case, significantly higher ($P < 0.001$) than autumn and winter too. Least square means of S-i confirmed the pattern: IC was the greatest (419 d; $P < 0.001$) in spring-calving cows, which are those inseminated in summer. Regardless of the parity, HS had a significant impact on the cow's reproductive sphere, with delay in conception causing direct and indirect losses and being an evident cost for the farmer.

Associations Between Changes in Body Condition Score During the Transition Period and the Milk Fatty Acid Profile

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Milk fatty acid (FA) composition is a key indicator of dairy cow metabolic status, nutritional management, and milk quality. This study aimed to assess the variation in milk FA profiles and composition during the transition period, and their association with body condition score (BCS) changes. Being part of an ongoing experiment, to date, 448 milk samples were collected from 112 Holstein cows at a commercial farm at 4 time points: T0 (60 days before the onset of the dry period), and at T2, T3, and T4, corresponding to 5, 30, and 60 days postpartum, respectively. BCS changes (Δ BCS) were calculated as the absolute differences between T2, T3, T4 relative to T0, and classified in 4 classes: Δ BCS $\leq$ -1.25, $-1.25 < \Delta$ BCS $\leq$ -0.75, $-0.75 < \Delta$ BCS $\leq$ -0.5, and $-0.5 < \Delta$ BCS $\leq$ 0.5. Milk FA, determined by Gas Chromatography and Near-Infrared Spectroscopy, were grouped into SFA, UFA, MUFA, PUFA, de novo, mixed, and preformed. A two-way ANOVA analysis used time points and Δ BCS as fixed effects and their interaction to evaluate the influence on milk FA and composition parameters. Milk fat content decreased from T0 to T3, while fat-to-protein ratio peaked at the onset of lactation (T2). Similarly, at T2, the FA profile showed reduced de novo FA, compensated by increased preformed FA (g/100g). Overall, cows experiencing greater BCS loss (Δ BCS $\leq$ -1.25), consistently displayed higher proportions of unsaturated and preformed FA, coupled with reduced de novo synthesis. These alterations were linked to reduced protein and casein levels at T2 and a rise in acetone at T2 and T3, indicating a metabolic alteration in cows undergoing substantial BCS loss. In conclusion, BCS changes are associated with a specific metabolic shift in milk FA composition, underscoring the milk FA profile as a robust indicator of dairy cows' metabolic health. Funding: 2024DAFNAE1SID-PROGETTI-00006 Real-time monitoring of rumen pH: a tool to address subacute ruminal acidosis and enhance understanding of fermentation processes (RTM-SARA)

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Effect of feed restriction on dynamics of feed intake, milk yield and energy balance in dairy cows

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Due to climate change, prolonged forage shortages may become more frequent, potentially leading to underfeeding in dairy cows. Limited knowledge exists on how milk yield and energy balance evolve during and after the underfeeding phase. This study aimed to assess the impact of 2 feed restriction durations (4 and 6 weeks) on the changes in feed intake, milk yield, and energy balance in dairy cows. A total of 42 cows were first fed ad libitum for 3 weeks and then divided into 3 groups of 14 cows. Two groups were underfed by 5 kg of dry matter (DM) per day for 4 or 6 weeks (R4 and R6). After the restriction phase, cows were again fed ad libitum for 5 weeks. The control group was fed ad libitum throughout the trial. Analysis of variance was used to assess the effects of feed restriction by including parity in the statistical model. Performance data from the first two weeks were included as a covariate, with a distinction made between primiparous and multiparous cows. During the restriction phase, energy-corrected milk (ECM) yield dropped by 4.61 and 4.62 kg ($P < 0.001$) and energy balance dropped by 2.22 and 1.92 UFL ($P < 0.001$) in the R4 and R6 groups, respectively, compared to the control group. During the refeeding phase, both DMI and ECM yield increased progressively. Three weeks after the refeeding, previously restricted cows exhibited higher DMI than the control group (+1.15 kg DM for R4, $P < 0.001$; +0.99 kg DM for R6, $P = 0.107$), while ECM yield remained similar across all groups. The energy balance during refeeding phase was higher in the restricted cows compared to the control group (+1.20 for R4, $P < 0.01$, and +1.02 UFL for R6 group, $P < 0.05$), suggesting a greater rate of body reserve reconstitution. Compared to the R4 group, a longer restriction in the R6 group did not result in a significant difference in the evolution of feed intake, milk yield, or energy balance during the refeeding phase. In conclusion, despite the negative effects of feed restriction, dairy cows demonstrated an ability to adjust feed intake and efficiently restore body reserves during the refeeding phase. These results could contribute to the development of a dynamic prediction model for the intake capacity of dairy cows under different feeding conditions.

Impact of various LPS doses on dry matter intake, milk yield and rectal temperature in lactating Holstein cows

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Lipopolysaccharide (LPS), an endotoxin produced by most gram-negative bacteria like *E. coli*, is widely used to study the immune response in cows. However, there is no consensus on the optimal LPS dose, complicating the comparison between scientific studies. This study evaluated various intravenous LPS doses (0.08, 0.09, 0.2, 0.375, 0.5 µg/kg BW of LPS *E. coli* O55:B5) from separate studies ($n=12, 29, 6, 6, 6$, respectively) in lactating Holstein cows and their effects on dry matter intake (DMI), milk yield (MY), and rectal temperature (RT). DMI and MY were recorded daily for 2 days, and RT was measured in the first 6 hours post-LPS bolus. Baseline values were obtained pre-LPS administration. Responses were assessed via the cumulative area under the curve (AUC) for DMI, MY, RT, and via slope calculations for DMI and MY. Each variable was analyzed as absolute values and percentages. One-way ANOVA was used to compare LPS doses, with significance declared at $p<0.05$, and the Tukey test was used as post-hoc. LPS dose had a non-linear effect on absolute DMI and MY. The 0.375 µg/kg dose caused the most severe ($p<0.01$) DMI reduction (75.2%) compared to 0.09 µg/kg (32.5%). Further, a difference ($p<0.001$) in the magnitude of MY reduction was observed among the 0.375 µg/kg (83.3%) and two lower LPS doses (41.4 and 45.1% for 0.08 and 0.09 µg/kg of LPS, respectively). Other LPS doses had middle values. No differences ($p>0.05$) in MY slope were observed among LPS doses, either in kg or percentage. However, the rate of DMI reduction was increased ($p<0.05$) in the 0.375 vs. 0.09 µg/kg LPS dose (-17.27 vs. -8.79 kg/d, respectively). As a percentage, the slope for DMI was steeper ($p<0.01$) in the 0.375 µg/kg (-69.1%) relative to the 0.08 µg/kg (-38.7%) and 0.09 µg/kg (-29.6%) LPS doses. LPS doses did not affect RT AUC, with all curves following a similar trend ($R^2=0.908$, $p<0.05$) in a quadratic polynomial equation. Overall, these preliminary results suggest that the 0.375 µg/kg LPS dose has the most negative impact on cows, significantly reducing MY and DMI. Granted by AGRITECH-spoke 5

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Effects of incorporating tributyrin and tricaproin in milk replacer on plasma metabolome and lipoprotein profiles of calves

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This study examined the impact of milk fat composition and the addition of tributyrin (TB) and tricaproin (TC) to a milk replacer (MR) on the plasma metabolome and lipoprotein profiles of dairy calves. 45 male dairy calves were assigned to one of three MR treatments ($n = 15/\text{group}$): (1) a control MR (CON) containing vegetable fats (palm and coconut oils), (3) an MR (TRI) containing the same vegetable fats as CON with TB (source of C4) and TC (source of C6) added, and (3) an MR with milk fat (MF). C4 and C6 are natural milk fat components that are not present in standard MR; therefore, the addition of TB and TC to MR could better mimic the fatty acid profile of milk. The MF treatment served as a biological reference for milk fat composition. All MR were isoenergetic with 36% lactose, 27% fat, and 24% protein (DM basis). Calves were housed individually and received MR (13.5% solids) via nipple buckets twice daily. Calves had ad libitum access to water and chopped straw, but no starter feed was fed. On d 35, plasma samples were analyzed using targeted metabolomics (MxP Quant 500 kit). Statistical analysis was performed using MetaboAnalyst 6. Principal Component Analysis and Partial Least Squares Discriminant Analysis revealed distinct metabolic profiles between MF-fed calves and those fed CON or TRI. Volcano plot analysis showed that 39 metabolites increased, and 37 metabolites decreased in MF-fed vs. CON-fed calves. Increased metabolites included 6 CE, 18 TG, 11 PC, 3 lysophosphatidylcholines (LysoPC), and SM OH C16:1. Decreased metabolites included 18 TG, 7 PC, 2 Cer, 3 HexCer, 2 LysoPC, 2 SM, CE 18:2, FA 18:2, and taurocholic acid. A similar pattern emerged when comparing MF and TRI, with 45 metabolites increased and 58 decreased in MF-fed calves. Plasma total cholesterol, HDL, and LDL levels were higher in calves fed CON and TRI than in those fed MF. Total TG and VLDL cholesterol levels remained unchanged. Tributyrin and TC in MR had no effect on plasma metabolome and lipoprotein profiles. In contrast, differences between MF and vegetable fats led to marked differences in lipid metabolism and cholesterol regulation in pre-weaned calves.

Rumen-protected grape extract supports milk production in high-producing dairy cows in early lactation under heat stress conditions

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With the intensification of global warming come negative consequences for animal production, the most visible one being heat stress, leading to physiological stress and negative impacts on health and production. Combined with other intrinsic stressors such as the transition period in peri-parturient cows, it can be detrimental to milk production. The aim of the present study was to evaluate the potential for a standardised rumen-protected grape extract (RPGE, Nor-Grape® BP-O, Nor-Feed, France) to alleviate the burden of heat stress in dairy cows at the start of lactation. 30 high-producing Prim-Holstein cows from a commercial dairy farm in Western France were selected, and allocated, in the same barn, to a control group (CTL, n=15) or a supplemented group (BPO, n=15, receiving an extra 500mg RPGE/head/day through an automatic dispenser located at the milking robot), over a summer period of 66 days and lasted from lactation L1 to L40 for every cow enrolled. Cows' milk production was recorded daily using the data from the two milking robots of the farm. Daily THI value was calculated based on 2 dataloggers placed at cows' level in the barn. Average milk output after 20 days of lactation and after 40 days of lactation, as well as their fat and protein content were compared between groups using Welch t-test. Overall, BPO cows' milk output tended to be higher than CTL ones, both on L20 (+3.8kg, p=0.096) and on L40 (+3.9kg, p=0.097). No difference was observed in terms of fat or protein content of the milk between groups at both time-points of the study. The intensity of heat stress was overall moderate, with only 13 days of 'moderate heat stress' (72<THI<78) and 4 days of 'intense heat stress' (THI>78). The milk production of BPO cows was significantly higher than CTL ones during moderate heat stress days (+4.6kg, p<0.001) and tended to also be higher during intense heat stress days (+3.8kg, p=0.059). The results show that efficient dietary intervention with RPGE to support dairy cows in their early lactation helps them face heat stress challenges and results in higher milk output when heat stress intensifies, thus benefiting for the farm productivity, even in challenging conditions.

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Metabolic and oxidative stress responses to dietary changes in plasma and milk of Holstein cows in mid to late lactation

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The aim of this study was to investigate the effects of dietary energy transitions on metabolic and oxidative stress responses in plasma and milk of Holstein cows during mid to late lactation. A total of 48 German Holstein cows (178.2 ± 23.5 days in milk) were initially assigned to two feeding groups: high energy (HE; 45% concentrate, n = 24) and low energy (LE; 25% concentrate, n = 24). From week 1, the diets were changed, with the cows that initially fed HE being switched to LE (20% concentrate) and the cows that initially fed LE being switched to HE (50% concentrate). The experimental feeding continued until week 7, with samplings at weeks 0, 4 and 6. The groups were termed HE-LE (high to low energy) and LE-HE (low to high energy), depending on the feed switch. Plasma and skim milk samples were analysed for total protein (TP), oxidative stress markers (derivatives of reactive oxygen metabolites (dROM), ferric reducing ability of plasma (FRAP), oxidative stress index (OSi = dROM/FRAP), thiobarbituric acid reactive substances (TBARS) and advanced oxidation protein products (AOPP), leptin, lactoferrin (Lf) and haptoglobin (Hp). Data were analysed using the MIXED procedure in SAS with parity, treatment, time, and their interaction as fixed effects and cow as a random effect. In skim milk, TP, TBARS, and Lf levels showed significant interactions between treatment and time with changes in the LE-HE group. FRAP was not significant for treatment, time and their interaction. In plasma, no significant effects of treatment, time or their interaction on Hp, Lf or OSi were observed. However, time-dependent effects (P ≤ 0.01) were observed for leptin, TP, AOPP, TBARS, FRAP and dROM. Concentrations of FRAP, AOPP and dROM peaked at week 4 before declining at week 6, while leptin and TBARS concentrations decreased in the HE-LE group. The results suggest that the dietary change affected the oxidative status and, as indicated by lactoferrin, the inflammatory status, primarily through time-dependent effects.

Impact of heat stress-derived small extracellular vesicles on bovine immune cell function

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Rising global temperatures negatively impact immune function in dairy cattle, increasing their susceptibility to infections. Heat stress (HS) disrupts physiological balance, leading to impaired immune responses. Small extracellular vesicles (sEVs) play a crucial role in intercellular communication and may mediate immune responses under HS conditions. This study investigated the immunomodulatory effects of plasma-derived sEVs from Holstein and Brown Swiss cows under thermal comfort (TC, THI < 68) and heat stress (HS, THI_{max} = 84, THI_{min} = 69). sEVs were isolated from the plasma of both breeds, and immune cells, including monocytes and polymorphonuclear leukocytes (PMNs), were co-cultured with TC- and HS-derived sEVs. The results demonstrated that HS-sEVs significantly reduced PMN chemotaxis, inhibited phagocytosis, and decreased ROS production in Holstein cows, while Brown Swiss cows showed no significant changes. Monocyte chemotaxis and phagocytosis were also impaired by HS-sEVs in both breeds, indicating a broader immunosuppressive effect. These findings suggest that Holstein cows are more immunologically vulnerable to heat stress than Brown Swiss cows, with sEVs contributing to immune dysfunction and potentially increasing infection risk. The study highlights the importance of developing breed-specific strategies to enhance resilience in dairy cattle, focusing on maintaining immune function and improving overall health under heat stress conditions.

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Development of a protocol for the Purification and Characterization of *S. uberis*-derived Extracellular Vesicles associated with bovine mastitis

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Mastitis caused by *Streptococcus uberis* presents a significant challenge in dairy farming. Traditionally considered an environmental pathogen, recent studies have indicated its potential for contagious transmission. Bacterial extracellular vesicles (BEVs) are essential for intercellular communication and may play a role in pathogenesis and gene transference. This study aimed to establish a standard protocol for the purification of BEVs starting from *S. uberis* ATCC 19436 strain using an available protocol for Animal Extracellular Vesicle. *S. uberis* was cultured on blood agar, Edward medium, and directly from ATCC stocks in Brain Heart Infusion (BHI). Following 24 hours of incubation at 37°C, cultures were transferred to BHI for enrichment by adding 5 ml of culture into 140 ml of fresh BHI and incubating for 24 and 48 hours. After incubation, serial centrifugations of 1000, 2000, and 3000 g for 15 min at 4°C were performed on bacterial debris. The supernatant was filtered through a 0.22 µm membrane and ultracentrifuged at 100,000 × g for 1 hour at 4°C. BEVs were isolated using Size Exclusion Chromatography (SEC) with two different protocols: A 'quick collection' protocol, where 5 fractions were collected, and an 'extended' protocol, where 24 fractions were collected, following the manufacturer's instructions. Protein quantification was conducted using the Pierce BCA Protein Assay Kit according to the manufacturer's instructions. This approach enables standardized BEV purification, providing a framework for further functional and proteomic analyses. The peak protein concentrations were in fraction 5 for the "short" protocol and beyond 15 in the "extended" protocol, indicating a different BEV pattern. NanoSight analysis showed higher BEV concentration and size in the mentioned fractions. Further study will characterize the molecular cargoes of *S. uberis*-isolated BEVs to elucidate their role in host immune modulation.

Urinary biomarkers as indicators of heat stress in dairy cows

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One of the most current relevant raising issues for dairy cattle farmers is heat stress, especially in the temperate area. Heat stress occurs in response to high temperature and humidity, prompting animals to trigger metabolic changes to maintain homeothermy. Several metabolites have been identified as potential biomarkers of heat stress in plasma or milk, but only a few suggestions are available for urine. The aim of this study was to investigate the variations of some urinary metabolic biomarkers associated with the occurrence of heat stress as measured by the temperature humidity index (THI). For this purpose, between April and November 2023, urine samples were collected from 1,077 Holstein cows in 16 herds of northern Italy. Farms were visited multiple times and at each visit different cows were randomly sampled, so that each cow was sampled once. Throughout the entire sampling period, temperature and humidity values were recorded in each farm every 15 minutes and THI was calculated as: $THI = (1.8 \times AT) - (1.0 - RH)(AT - 14.3) + 32$, where AT is the daily average temperature and RH the relative humidity. Urine samples were analysed using the reference methods to determine pH, specific gravity, and concentrations of urea, protein, creatinine, allantoin, and uric acid. Then, the protein-to-creatinine ratio was calculated. A mixed linear model was used to compare thermal comfort vs discomfort. Fixed effects were THI class (<68; 68-71; 72-79; and 80-89), parity, and stage of lactation, and random effects were herd and the residual. The urinary traits were influenced by THI, except for proteins. In particular, when cows were at high risk of heat stress (exposed to $THI \geq 68$), creatinine and allantoin concentration in urine increased, while uric acid and protein-to-creatinine ratio decreased. Urine can be a valuable matrix for monitoring heat stress in dairy cows as sampling procedure is non-invasive. This study received support from the project Cool-Cow 2023 of the Breeders Association of Veneto Region (ARAV, Vicenza, Italy).

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Theatre 1

Mapping, manipulating and mining the microbiota of industrially produced insects: Where do we stand and where do we need to go? Lessons from the black soldier fly

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The black soldier fly (BSF) has garnered significant attention for its potential in sustainable bioconversion of organic waste. To fulfill this potential, consistent, safe, and economically viable industrial rearing methods are key, and their development requires basic scientific knowledge. As in other livestock, the gastrointestinal tract of insects harbors a complex and dynamic community of microorganisms, and alterations in these communities can be linked to changes in performance and health of insects. A thorough understanding of the (gut) microbiota from industrially produced insects is thus needed to standardize rearing methods, improve waste degradation, maximize biomass output, and meet hygienic standards. This presentation takes stock of what we know about the insect microbiota, starting from my own research on BSF. BSF harbors a dynamic microbiota that responds to environmental conditions (diet, temperature, location, gut region, etc.). This creates opportunities to implement targeted microbiome engineering; yet, past attempts to engineer the BSF microbiome have yielded inconsistent results. The fact that some key microbial genera persist under nearly all conditions, indicates community resistance which could explain the results inconsistency. Further investigation is warranted to unravel the mechanisms governing the microbial community structure and define the function of microbes and/or functional properties of the community that enhance insect performance and sustainability. Implementing recent advances in protocols to use axenic larvae and/or -omics technologies (metagenomics, transcriptomics, metabolomics, etc.) will help address these research gaps. This in-depth mapping will not only improve our ability to engineer the microbiome to boost insect production. It also allows the mining for microbes with interesting properties, like strong antimicrobial activity or high enzymatic activity. It's clear that there is still a long way to go to reach a mechanistic understanding of the roles that insect microbiota play in insect production, but we should not shy away from the challenge, as it holds great promise as shown in other livestock.

Nutritional Modulation in Black Soldier Fly Larvae: Insights into Diet-Insect-Microbe Interactions

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The black soldier fly (*Hermetia illucens*, BSF) is recognized globally for its potential in sustainable waste-to-nutrient conversion. While BSF larvae thrive on various organic substrates, the interplay between dietary macro-nutrient composition, host physiology, and microbial communities is underexplored. This study examines BSF larval responses to controlled dietary protein-to-carbohydrate (P:C) ratios, focused on nutritional optimization and gut microbiota's role in nutrient assimilation. Three formulated diets were examined: carbohydrate-biased (1P:5C), balanced (1P:1C), and protein-biased (5P:1C). BSF larvae showed optimal growth and survival on the balanced diet, indicating equal protein and carbohydrate provisioning enhances performance. Larvae provided the carbohydrate-biased diet had delayed development and reduced weight, while those fed the protein-biased diet faced higher mortality. Insect transcriptomic analyses revealed significant shifts in gene expression, particularly in pathways linked to digestion, metabolism, and immune function. Notably, genes involved in protein catabolism and biosynthesis were upregulated in protein-biased conditions, while carbohydrate-biased diets increased immune-related responses, suggesting trade-offs in nutrient utilization and potentially the provisioning of proliferating microbes for nutrients. Microbial metatranscriptomic analysis revealed diet-dependent changes in microbial diversity and function. The carbohydrate-biased diet promoted higher gut microbial diversity, with increased expression of carbohydrate metabolism genes, whereas the protein-biased diet did not significantly alter microbial composition. Correlations between microbial gene expression and host immunity highlight a potential role for microbial communities in modulating host digestive efficiency. These findings underscore the need for precision with diet formulations in order to optimize insect nutrition, while minimizing costs.

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Theatre 3

Optimizing Rearing Substrates for Black Soldier Fly Larvae: Hydration and Macronutrient Effects

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As the global population grows, the demand for sustainable protein sources is increasing. Black soldier fly (BSF) larvae offer a promising solution by converting organic waste into high-quality protein for animal feed. This study optimizes BSF larval growth by examining the effects of hydration and feed macronutrient composition on bioconversion efficiency. The ideal dry matter content was first assessed, comparing the standard 30% dry matter (DM) with substrates hydrated to their maximum water holding capacity (WHC). The WHC indicates the amount of water required to fully hydrate the substrate without any free water remaining. It was assessed by saturating the substrate with excess water, centrifuging to separate phases, and removing unbound water by decantation; the remaining mass reflects the amount of water retained within the substrate matrix. The WHC approach significantly improved larval survival rates ($p = 0.0006$) and was adopted for further experiments. Using artificial substrates composed of chicken starter mash (CSM), sunflower oil, wheat starch, casein powder, and cellulose powder, fractional factorial designs were employed to evaluate macronutrient effects on bioconversion efficiency by varying carbohydrate (CHC), fat, and protein contents, revealing significant main, interaction, and quadratic effects. A predictive model indicated optimal larval growth at 23.48% protein, 20.64% carbohydrates, and 1.91% fat (DM basis). Simulations showed that lower fat and carbohydrate levels required higher protein content to maximize efficiency, with optimal crude protein ranging between 15.23% and 25.50%.

Black Soldier Fly (*Hermetia illucens* L.) Larvae Growth Performance on Composted and Fermented High-Fibrous Residual Streams

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The study explored the potential of high-fibrous residual streams, specifically clippings from reed, nature straw, ditch grass, and Japanese knotweed, as substrates for black soldier fly larvae (BSFL) growth. These residual streams, often underutilized in circular agriculture, were processed via ensiling followed by composting with broiler chicken manure or ammonium sulphate. Ammonium sulphate, serving as an effective nitrogen source, eliminated the need for broiler chicken manure in the composting process. The goal was to enhance the sustainability of these biomasses by converting them into valuable substrates for insect rearing, contributing to sustainable insect protein production and supporting the transition to a circular economy. The processed residual streams (10 g each) were mixed with chicken feed (20 g) and water (70 g), and tested in triplicate with 200 five-day-old BSFL per box over a 6-day growth period. In the control treatment, 10 g of sawdust replaced the residual stream. Each treatment had a starting substrate weight of 100 g, with 0.5 g of substrate per larva. The experiment was conducted at 39°C to mimic a reduced temperature environment, as opposed to the standard 27°C, due to the small experimental setup. Key performance indicators were measured, including larval survival, weight gain, feed conversion ratio, and substrate reduction. The results indicated that the processed residual streams were suitable as structural agents in BSFL substrates, with performance data comparable to the control treatment. Notably, larval weight was significantly higher under knotweed (6.01 mg DM) and reed (5.80 mg DM) composted with ammonium sulphate compared to those composted with chicken manure (5.02 mg DM and 4.89 mg DM, respectively). In conclusion, the study demonstrates that high-fibrous residual streams, particularly after composting or ensiling, can effectively serve as structural ingredients in substrates for BSFL cultivation, offering a sustainable, circular approach to biomass valorisation in insect protein production.

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Theatre 5

BSF under the magnet: ¹H/²H-NMR as tool to measure lipid synthesis in black soldier fly larvae

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Black soldier fly (*Hermetia illucens*) larvae are widely used to upcycle biowaste into insect biomass for animal feed. Yet, their lipid metabolism, particularly de novo lipogenesis, remains underexplored. This study applied a proton/deuterium Nuclear Magnetic Resonance spectroscopy (¹H/²H-NMR) approach to quantify lipid synthesis using deuterated water (²H₂O) as a stable isotope tracer. First, a body water enrichment trial determined that larvae fed a substrate containing 10% ²H₂O reached approximately 5% body water ²H-enrichment within a 24 h period. A subsequent feeding trial assessed how macroalgae (*Gracilaria vermiculophylla*) inclusion influences lipid synthesis. Larvae were reared on a control diet consisting of chick feed or diets where 25% or 50% of the feed was replaced with macroalgae. After lipid extraction, NMR spectroscopy allowed the quantification of ²H incorporation into triacylglycerol-bound fatty acids. Fractional synthesis rates (FSR) were calculated relative to body water ²H-enrichment and assessed using the Kruskal–Wallis test. Lipogenesis was increased in larvae fed the control diet, significantly reduced in the 50% macroalgae group, and intermediate in the 25% group. These findings reinforce BSF as a valuable model for modulating lipid metabolism and consequent applications of insect oils, while demonstrating the dietary influence on lipid synthesis and establishing NMR as a robust tool for studying insect lipogenesis.

Nitrogen and amino acid deposition in *Tenebrio molitor* larvae fed barley-based substrates supplemented with lysine and isoleucine

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The effects of lysine (Lys) and isoleucine (Ile) supplementation in a barley-based substrate (10.4% crude protein, CP) on N and amino acid (AA) deposition in *Tenebrio molitor* larvae were evaluated. Thirty plastic trays, each containing 60 larvae (37.2 ± 1.40 mg/larva), were assigned to five experimental treatments: barley as the sole substrate; a mixture of 85% barley and 15% soybean meal (BS; 15.8% CP); or barley supplemented with Lys (L), Ile (I) or a combination of Lys and Ile (LI). Each tray contained 15 g of the initial substrate, and synthetic AA were provided in excess, diluted in agar cubes, and supplied twice per week as a water source. The experiment was conducted in two consecutive 21-day periods, with three trays per treatment per period. At the end of each period, larvae from each tray were collected, weighed and analyzed for dry matter (DM) content. Additionally, larvae from each treatment within each period were pooled and analyzed for total N and complete AA profile. Data were analyzed by ANOVA, and treatment means were compared using the Tukey's test. Larval weight (on DM basis) averaged 42.0, 44.7, 44.9, 42.7 and 43.4 mg for barley, BS, L, I and LI, respectively ($P = 0.255$). Total contents (mg/larva) in N and true protein (sum of all analyzed AA) were significantly higher in larvae fed BS than in those fed barley alone, with larvae fed L, LI and I showing intermediate values ($P < 0.001$). Furthermore, the contents in key AA (Lys, Met, Cys, Thr, Trp, Val and Ile) were higher in BS-fed larvae than in those fed L and LI, with the lowest values observed in larvae fed I or barley alone ($P < 0.01$). In conclusion, barley supplementation with Lys improved larval AA content, although levels remained lower than those observed in larvae fed BS, suggesting the presence of additional limiting AA for optimal growth of *T. molitor* larvae. Conversely, Ile supplementation had no apparent effect on overall AA deposition, and the combination of Lys and Ile did not enhance AA production beyond that achieved with Lys alone.

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Growth performance and composition of *Tenebrio molitor* larvae fed on barley substrate supplemented with lysine and isoleucine as limiting amino acids

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This study aimed to evaluate the potential benefits of supplementation with a combination of lysine (Lys) and isoleucine (Ile), in barley-based substrates. Thirty-six trays, each containing 15 g of substrate and 60 larvae (35.5 ± 0.76 mg) were assigned to 3 experimental treatments: barley alone (10.4% crude protein, CP); barley supplemented with Lys plus Ile (LI), or a 85:15 mixture of barley and soybean meal (BS, 15.8% CP). Synthetic amino acids (AA) were supplied in excess diluted in 2.5% agar cubes, that were provided twice a week as water source. Four consecutive 21-d experimental periods (three trays per treatment per period) were conducted under controlled environmental conditions. Larval growth and substrate intake per tray were recorded weekly. At the end of the experimental period, larvae from each tray were collected and analysed for dry matter, ether extract (EE) and CP contents. Data were analyzed by ANOVA, and treatment means were compared using the Tukey's test. Mortality averaged 9.1% and was not affected by treatment. Final larval weight and total biomass production were higher ($P < 0.001$) in BS compared to the other treatments. Substrate intake did not differ among treatments ($P = 0.333$), and consequently, the feed conversion ratio was improved in larvae fed BS respect to those fed the other treatments. Fat deposition per larvae was lower in those given BS than in those receiving barley alone or LI ($P = 0.011$), whereas CP deposition was higher in BS-fed larvae compared to those fed barley, with LI showing intermediate values ($P < 0.001$). The greater growth observed in larvae fed BS confirms that the lower AA content of barley is a limiting factor for larval growth. However, supplementation of barley with a combination of Lys and Ile improved larval CP deposition respect to barley alone, although results remained below those obtained with BS. These results suggest that Lys and Ile content of barley is likely below larval requirements, although the response to soybean meal supplementation indicates that other AA may also be limiting factors for maximizing larval growth.

Nutritional recommendations to optimise growth and survival of young yellow mealworms

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A fundamental stage in the production of *Tenebrio molitor* larvae is the initial period following hatching, during which the survival and development of the larvae are pivotal in determining the overall efficacy of the process. The objective of this study was to ascertain the optimal levels of digestible energy (DE) and protein (DP) in diets to optimise the growth and survival of young yellow mealworms. Nine diets were evaluated, varying in DE content (on average, Low (L): 2235, Medium (M): 2461 and High (H): 2706 kcal DE/kg dry matter (DM)) and DP (on average, L: 75, M: 90 and H: 105 g DP/kg DM). The diets comprised 39.6% carrot and 60.4% dry diet. Diets were identified with two letters, the first being the DE and the second the DP. At the beginning of the trial, 24 g of eggs were incorporated into each of the 72 trays (10 x 16 x 7 cm; 8 replicates per diet) within a climatic chamber (28°C and 60% relative humidity). The most effective diet was subjected to a scaling test in comparison to a commercial diet composed of wheat bran and carrots in the same proportion. The number of larvae at 28 days was 29% higher in the H compared to the L diet in DE ($P < 0.05$), not being affected by the DP level. The highest survival was achieved with HM diet. Diets with H level of DP showed a higher average daily gain of larvae (+14% compared to L and M diets; $P < 0.05$), being unaffected by the DE level. The conversion rate was improved with M and H compared to L diet in DE (+29%; $P < 0.05$), being unaffected by PD level. Final larvae production per tray at 28 days was greater with the diets with higher DE (H) compared to L and M (+26 and +20%, respectively; $P < 0.05$), not being affected by DP level. However, the HM diet optimised the overall production per tray, with a 40% higher total weight of larvae compared to the LL diet ($P < 0.05$). The scaling test under commercial conditions confirmed the enhanced productivity with the HM diet compared to a standard one. During the early stages of life, it is recommended 2700 kcal of DE and 90 g of PD per kg of DM to ensure optimal survival and growth of yellow mealworms.

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Optimizing Yellow Mealworm Diets: The Viability of Composted and Ensiled Organic Residual Streams

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Large quantities of organic residual streams (RS) remain underutilised due to their low nutrient composition and high fibre content, leading to significant waste. Composting is a promising pre-treatment strategy to enhance their nutritional value, making them suitable diets for yellow mealworms (YMW). This study evaluates the impact of incorporating composted and ensiled RS into YMW wheat-bran-based diet, with RS constituting 20% of the dry ingredients. RS tested were nature straw (NS), ditch grass (DG), reed and Japanese knotweed (JKW). The experiment was conducted in triplicate under controlled conditions. The key performance indicators analysed were harvest time (d), cumulative mass (g DM) and mass gain (g DM/d), feed conversion ratio (FCR) and rejection rate (RR). Results showed that the harvest time was significantly reduced with ensiled DG (22.2 d), composted DG (21.9 d) and composted JKW (22.8 d) compared to the control. Cumulative mass was significantly higher in composted NS (173.8 g) compared to ensiled NS (157.7 g). The latest had significantly lower mass gain per day compared to control, ensiled reed, and ensiled JKW. In contrast, the highest larval mass gain was achieved with composted NS, DG and JKW (6.4 g/d each). Although FCR (DM/DM) showed no significant differences, numerically, composted DG had the lowest value (5.5), while ensiled reed had the highest (6.3). The RR varied by the RS type. Larvae reared on composted NS had the lowest RR (56%), followed by ensiled NS and composted DG, whereas ensiled reed and ensiled JKW had the highest RR (62%). These findings suggest that composted NS, composted DG, composted JKW and ensiled DG are promising feed alternatives that can accelerate development without negatively impacting biomass accumulation. Further research is needed to determine whether observed benefits are due to water retention properties, nutrient composition, or other physicochemical factors. Additionally, year-round availability and composting costs should be assessed for practical implementation.

Evaluating Dairy Waste as a Feed Source for *Tenebrio molitor*, *Alphitobius diaperinus* and *Hermetia illucens*: A Sustainable Approach to Waste Valorization

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Dairy waste presents a significant environmental challenge due to its high organic load, which, if left untreated, can lead to eutrophication and pollution of natural ecosystems. This study investigates the potential of three insect species—*Tenebrio molitor* (yellow mealworm), *Alphitobius diaperinus* (lesser mealworm), and *Hermetia illucens* (black soldier fly larvae)—to utilize dairy waste as a feed substrate, thereby transforming it into high-value biomass. Various dairy byproducts, including expired cow milk, plant milk, kefir, yogurt, and dairy processing residues (lactose and whey derivatives), were used in species-specific diets. For mealworms, these wastes were combined with agar and provided as wet feeds, while for *H. illucens*, they were mixed directly with wheat bran. Our findings show that all three insect species displayed positive growth responses, with larvae fed on dairy waste achieving high survival rates and competitive growth rates compared to conventional feeds. Additionally, proximate analysis of the harvested larvae of *H. illucens* confirmed the potential of dairy waste to produce high-quality insect biomass, with protein content ranging from 31% to 40% and lipid levels from 11% to 31%, depending on the diet. These results highlight the feasibility of integrating dairy waste into insect farming systems, offering a circular economy solution for waste valorization. The research work was partially supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 11297) and the funding programme “MEDICUS” of the University of Patras.

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Antioxidant activities and total polyphenolic content of mealworm growth on herb containing substrates

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Mealworms (MW, *Tenebrio molitor*) can be reared on diets such as grain by-products or grain-based former foodstuffs, resulting in the production of high-quality biomass. These diets might also contain several herbs. The high levels of polyphenols in these herbs have the potential to negatively affect the growth of the insects given the elevated presence of polyphenolic compounds therein. Therefore, the objective of this project is to assess the impact of herb inclusion on the total polyphenolic content (TPC) and antioxidant activity in the larvae. The MWs were raised on a control diet consisting solely of wheat bran, as well as on wheat bran supplemented with four different herbs: namely, basil, oregano, rosemary, and thyme. The herbs were incorporated at two concentrations, 0.125% and 2% on a dry matter (DM) basis. All experiments were carried out in darkness at 27°C with 60% relative humidity for a period of five weeks. There were no significant differences in growth performance among the groups. However, the inclusion of herbs did have a minor detrimental effect on larval composition. The elevated TPC levels in the herbs negatively correlated with the TPC levels in larva. In contrast, the antioxidant activity of the larvae (as measured by FRAP and ABTS) was enhanced by the presence of herbs and their respective inclusion levels. Therefore, it can be concluded that the inclusion of herbs at elevated levels had an impact on the larval composition and antioxidant profile. Further research is required to investigate these effects more extensively.

Evaluation of balanced diets with Greek agricultural by-products for growth and development of *Tenebrio molitor* larvae

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Agricultural by-products represent an underutilized resource as they may serve as nutrient-rich substrates for insect rearing, thereby mitigating the environmental impact. Insects can metabolize the nutrients in by-products, converting them into high-value nutrient source. The objective of this study was to assess the suitability of seven locally produced agricultural by-products derived from Greece as feed for the larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). The by-products derived from the production of triticale, oat, barley, lupine, hemp, rice bran, and cotton. For the bioassay, plastic vials were used, with 50 early-stage larvae placed in each vial. The study examined the larval development across four isoproteinic diet groups: i.e., 17% protein, 20% protein, 23% protein, and 25% protein. The larvae were kept under controlled conditions with adequate moisture, and their weight and survival were recorded biweekly until the first pupation. Additionally, three feed conversion indices—Specific Growth Rate (SGR), Efficiency of Conversion of Ingested Food (ECI), and Feed Conversion Ratio (FCR)—were calculated, alongside an analysis of larval body composition, including protein, fatty acid content, dry matter, and ash percentage. The data were submitted to ANOVA ($P < 0.05$) and analyzed with SPSS 26.0. The results indicated that triticale, lupine, and hempseed by-products consist suitable nutrient sources for the successful development of *T. molitor* larvae. The majority of the diets on which larvae performed good belong to the group with 17% protein. This research underscores the potential of local agricultural by-products as sustainable feed substrates, contributing to waste valorization and promoting circular economy principles. This research is supported by the EU-PRIMA program project ADVAGROMED (Prima 2021 – Section 2)

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Poster 13

Effects of feed composition and environmental factors on performance parameters in *Tenebrio molitor* – a meta-analysis

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Introduction: The aim of this study was to evaluate the impact of feed composition and environmental factors on the body composition and performance of *T. molitor* larvae using a meta-analytical approach. **Material and methods:** A systematic literature search resulted in a total of 21 studies comprising 144 individual treatments reporting at least one outcome parameter (body composition, performance) along with feed composition and environmental data. Data were grouped in diets containing yeast (yes: $n = 27$, no: $n = 117$) and treatments including additional moisture sources, e.g. vegetables (yes: $n = 97$, no: $n = 47$). Descriptive statistics were computed and the effects of dietary crude protein (CP), fat, temperature, humidity, yeast supplementation, and additional moisture sources on larval body composition and performance parameters were analysed with a multilevel mixed-effects meta-regression model (metafor package in R version 4.3.1). **Results:** Overall, dietary fat, CP, and crude fibre were 6.5 ± 4.71 , 19.1 ± 12.4 , and $15.3 \pm 12.08\%$ DM. Temperature was $25.6 \pm 3.24^\circ\text{C}$. Humidity was $60.5 \pm 10.08\%$. Larval fat, CP, and fibre were 26.7 ± 13.87 , 47.9 ± 9.89 , and $9.0 \pm 7.17\%$ DM. Increasing dietary CP significantly increased larval CP content ($\beta = 1.36$, $p < 0.01$) while decreasing larval fat ($\beta = -1.55$, $p < 0.01$) and crude fibre content ($\beta = -0.63$, $p < 0.01$). Yeast supplementation significantly reduced body fat content ($\beta = -1.04$, $p < 0.01$) while increasing body CP ($\beta = 1.09$, $p < 0.01$). Providing an additional moisture source positively affected larval CP content ($\beta = 1.09$, $p < 0.01$). Increasing temperature reduced larval crude fibre content ($\beta = -5.98$, $p < 0.01$). Higher humidity levels significantly increased larval CP ($\beta = 0.67$, $p = 0.04$). **Conclusion:** Dietary composition and environmental factors significantly influence the body composition of *T. molitor* larvae, highlighting the importance of optimizing feed composition and environmental conditions to improve mealworm production efficiency and nutritional quality.

Growth curves: Fundamentals for determining the optimal time of harvest of *Hermetia illucens*Y. G. Hernandez Castañeda¹, C. Pincet¹, M. Tejeda^{1,2}, M. Bolard^{1,2}¹ FlyGenetics AD, Saedinenie Str. 299, 1151 Lozen Village, Sofia, Bulgaria, ² Nasekomo EAD, Saedinenie Str. 299, 1151 Lozen Village, Sofia, Bulgaria

The optimization of harvest timing is a critical factor in ensuring the efficiency and sustainability of large-scale insect breeding systems, particularly for protein production. In this study, we aimed to identify the optimal harvest point for larvae of the FlyGenetics® line by analyzing growth patterns and key nutritional parameters. Multiple batches of larvae were monitored at various ages, up to pupation, to capture the full spectrum of developmental stages using the FlyGenetics® Diet Base chicken feed. To ensure robustness and accuracy, samples were analyzed in three independent specialized laboratories, focusing on critical nutritional components such as protein, fat, water and ash content. These data were used to reconstruct the larval weight growth curve, providing a comprehensive understanding of growth dynamics. Growth patterns were better described by a logistic growth model, with the Weibull growth function selected for its ability to accurately describe the nonlinear progression of larval development. The model revealed distinct phases of growth, allowing for the identification of the optimal harvest point that maximizes biomass yield and nutritional quality. The results demonstrate that the current methodology employed in protein production, which aligns with the peak of larval growth and nutrient accumulation, delivers the highest performance efficiency. This study highlights the importance of integrating precise growth monitoring, advanced analytical techniques, and mathematical modeling to optimize industrial insect production and selective breeding processes. The findings provide valuable insights for scaling production while maintaining quality and sustainability, offering a framework for data-driven decision-making in the rapidly growing field of insect-based protein production.

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Poster 15

Evaluation of pollen availability and quality during a two-year monitoring of a sub-urban area apiary

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Pollen is vital for honeybees (*Apis mellifera* L.) (HB) colony health, varying with environment and seasons, but its nutritional impact remains poorly understood. In this study, curbicular bee pollen (CBP) was collected from seven *A. m. ligustica* Spin. colonies during the same season in 2016 and 2019 to assess variations in availability and quality in terms of nutritional composition due to years-to-years and seasonal variations in the PLANT-B apiary of the UNITUS experimental farm. From May to August CBP was monthly harvested by means of commercial pollen traps activated for 24h. The CBP samples were weighed to evaluate the dry matter (DM) importation per hive (g DM/24h), stored frozen, and then analyzed to determine the DM%, the ash (ASH%DM), the crude protein CP (% DM) and the fibrous (as aNDFom %DM) contents. Separate factorial ANOVA using the year (Y), the month (M) and their interaction as fixed factors were carried out for the above-mentioned variables. Differences between means were tested through the Tuckey post-hoc test. The average DM importation was lower in the 2019 (21.37±25.87 gDM) respect to 2016 (34.83±33.22 gDM) ($p<0.05$). The year had effect on DM, CP, and fiber content of CBP, with higher amount of DM (90.0±3.3% vs. 75.2±27.5%; $p<0.05$) and fibrous fraction (19.5±7.5%DM vs. 2.1±1.7%DM, $p<0.05$) in 2016 compared to 2019, but the CP content showed the opposite pattern (20.4±4.4%DM vs 22.8±9.1%DM, in 2016 and 2019 respectively; $p<0.05$). As expected, the sampling month had effect on all the compositional variables, with a common decreasing trend toward the end of the sampling period for the DM importation but also of the CP and ASH content of CBP. The DM and fiber content of CBP changed over time with no clear pattern. However, significant Y×M interactions were recorded for all the CBP compositional traits investigated. Results show that year-by-year and seasonal variation affects the availability and quality of pollen harvested by the bees, though for the same location, year by season interaction play a role in the pollen availability and nutritional value.

Supplementary nutrition and energy content in honey bees: A comparative study of feeding approaches
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The honey bee (*Apis mellifera* L.) represents a unique holometabolous insect, characterized by its complex ontogeny. This complexity implies that a straightforward correlation among age, body mass, heat production, and energy expenditure is not anticipated, making honey bees a compelling subject for calorimetric investigations. The objective of this study was to assess the impact of supplementary nutrition—specifically protein or pollen candies, and sugar candies enriched with spirulina—on the growth and development of honey bee colonies using calorimetric methods. Following the final honey harvest in August 2024, we established 36 experimental colonies in a controlled apiary setting. The colonies were divided into four distinct feeding groups: Group 1 receiving a sugar syrup (1:1 sugar to water), Group 2 fed with commercial protein cake, Group 3 included 9 colonies given pollen candy, and Group 4 fed with 2% spirulina and sugar candy. The sampling of adult honey bees was before feeding, on day 22 and finally on day 43. Pupae and larvae (6-7 ml/sample) were sampled at the beginning and at the end of the experiment. Samples were dried in an incubator (adult bees) or lyophilized (developmental stages) for calorimetry. We quantified GE of the samples using calorimeter (IKA C6000 global standard, Staufen, Germany). Initial findings for adult bees indicated notable differences between sampling events ($p < 0.0001$). The average GE displayed an upward trend across the sampling periods, rising from 10.246 ± 0.289 MJ/kg in the first round to 10.747 ± 0.254 MJ/kg in the third, with the second round reflecting intermediate values (10.374 ± 0.347 MJ/kg). Specific analysis by group revealed diverse GE patterns, with Group 1 (sugar syrup) exhibiting the highest energy levels in the third sampling round (10.827 ± 0.190 MJ/kg), while the other groups showed more modest fluctuations. These results highlight temporal variations in energy content, emphasizing significant differences across both sampling periods and feeding groups.

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Poster 17

Effects of varying dietary gross energy levels on growth performance and nutrient deposition in house crickets

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Crickets represent an environmentally sustainable protein alternative to conventional animal sources. This study aimed to evaluate the effect of various dietary energy levels on feed intake, growth, and body composition in house crickets (*Acheta domesticus* L., Orthoptera: Gryllidae) during 45 d of development. The study was divided into two phases (7 to 20 and 21 to 45 d of age), with crickets randomly allocated to five dietary treatments, each with six replicates (4,500 crickets/container) using a completely randomized design. Gross energy (GE) levels in the diets ranged from 3,819 to 4,265 kcal GE/kg in phase 1 and from 3,978 to 4,405 kcal GE/kg in phase 2. Data were analyzed using one-way ANOVA in SAS software. Tukey's test was employed for multiple comparisons among dietary treatments. In addition, broken-line regression analysis was applied to estimate the dietary GE level required to maintain a constant feed conversion ratio (FCR). Results indicated that higher dietary energy led to a linear decline in feed intake while promoting increased body mass. Protein retention increased from 72.1% to 85.5% as GE increased from 3,819 to 4,265 kcal/kg. Similarly, in phase 2, protein retention increased from 53.3% to 59.3% as GE increased from 3,978 to 4,379 kcal/kg. The FCR also improved with increasing energy concentration. Broken-line analysis revealed the optimal FCR at 4,158 and 4,382 kcal GE/kg feed for house crickets from 7 to 20 and 21 to 45 d after hatching, respectively. These findings confirm the relevance of energy density in achieving optimal growth performance and provide valuable guidance for diet formulation. However, the diets in this study were based on GE rather than metabolizable energy (ME). Future studies are needed to establish ME values specific to crickets for precise dietary recommendations.

Knowing how to coexist: a voyage of discovery on the movement of animals in an anthropized world
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Wildlife is exposed to a variety of anthropogenic threats, either indirect, such as climate change, or direct, such as legal or illegal hunting, non-lethal disturbance from outdoor activities, habitat fragmentation or loss. These threats can have important effects on wildlife behavior, eventually affecting individual fitness, population dynamics and species distribution. At the same time, changes in socio-economic habits (e.g. the abandonment of rural areas) combined with the protected status of some species (e.g. the wolf *Canis lupus*) can determine important changes in ecosystem function, offering the room for the restoration of important top-down population regulatory mechanisms (e.g., predation). In this complex context, the study of animal movement, namely movement ecology, combined with community ecology, is crucial to improve our understanding of the consequences of the above-mentioned threats and ecosystemic changes on wildlife behavior and occurrence. In this talk, I will first present some insights from global-scale multi-species and multi-population analyses that show the complexity of interactions between terrestrial mammals and human activities, and in particular how different species and populations can locally adapt their behavior to cope with human footprint. I will then narrow the focus of the talk to the Alpine context by investigating how space use behavior and activity patterns of mountain ungulates are affected by anthropogenic threats and the return of top predators, using different study systems. I will conclude by discussing how these findings, ensemble, can orient wildlife management and outdoor practitioners towards biodiversity values.

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Theatre 2

New perspectives on large herbivores in northern Europe

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The European continent have during a long historical perspective been dominated by the domesticated herbivores. However during the last decades wild populations of large herbivores have increased in numbers and there are now vital populations of e.g. deer, mouflon sheep and wild boars and other herbivores in many regions that until recently had only fragmented populations or few animals of wild large herbivores. The increased populations of wild herbivores are a result of reduced livestock production, altered farming practices and novel game and conservation management. The changes in the wild populations have altered the vegetation in the landscapes but have also induced conflicts of interests between farmers, hunters and public demands for a more diverse and authentic fauna. There are also large interest in introducing extinct species such as aurochs-like cattle, wild horses and European bison to suitable habitats in order to recreate ancient landscape with high values of biodiversity. Despite this relative clear development of reduction in livestock and increase of wild herbivores in the Nordic Countries, the general public, policy makers and parts of the scientific communities are to some extent unaware of the ongoing shift of animal populations and its consequences for farming, forestry and rural life. With this in background it is a need to present the contemporary changes in populations of important herbivores, their effects on ecosystem and distribution. Based on most recent state of the art estimates of populations, we explore the opportunities and challenges that emerge from different scenarios of altering the populations of wild herbivores in the Nordic ecosystems. We try to highlight challenges and opportunities in the perspectives of the farming communities and the interests from the general public and environmental organisations. The presentation will also include suggestions of new perspectives on the use of game meat, as a sustainable source of protein with a high demand from the concerned modern consumer.

Human–carnivore conflict in North–Eastern Italy: Is coexistence really achievable?

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Extensive livestock practices provide essential ecosystem services in mountainous areas and represent a significant cultural heritage for local communities. While large carnivores contribute to ecosystem stability, they also pose a threat to grazing activities, often leading to herder dissatisfaction. To assess carnivore impact in North–Eastern Italy, from June to October 2023, we monitored 58 of 162 Alpine summer farms in Friuli Venezia Giulia through structured interviews. Data included farm characteristics, livestock bred, adopted grazing systems, and herder perceptions towards carnivores and related damages. Spatial analysis in QGIS examined grassland fragmentation, elevation, and slope, while Bayesian GLMs in R assessed statistical patterns. Sheep and goats were the most predated species, likely due to their smaller size and weaker anti–predatory behaviours. Grazing system had no significant effect, possibly because free–ranging was mainly used for cattle, the least affected category. Similarly, grassland features showed no significant effect, likely because herders deliberately avoided fragmented, high–elevation, and steep–slope grasslands to minimize predation risks. Herders were dissatisfied with compensation schemes that excluded indirect losses (e.g., stress on animals, reduced foraging time, abortions). Moreover, regardless of predation experience, herders perceived coexistence with carnivores as unachievable in the long run. The findings achieved revealed the presence of a conflict–ridden situation in the study area. With large carnivores recolonizing North–Eastern Italy, continuous monitoring is therefore essential to mitigate impacts on livestock farming and prevent biodiversity loss or the abandonment of extensive grazing in mountainous territories.

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Theatre 4

Wild ungulates and livestock coexistence in mountain ecosystems: a case study from the Italian Alps

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In this study, we examined whether the presence and density of livestock grazing at high elevations during the summer influences the activity patterns and occurrence of two wild ungulates, roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*). We relied on data collected from 77 camera traps deployed in two close-by areas in the Italian Alps in July–August 2022 and 2023, following a random systematic design. We computed the diel activity patterns for livestock, roe and red deer, and we estimated the overlap between the obtained curves. Then, we applied Generalized Linear Mixed Models to model the occurrence of red and roe deer in function of livestock presence, density and proximity to an alpine herdsman's hut, while controlling for other potential sources of disturbance such as human outdoor activities. Roe and red deer were primarily active at dawn and dusk, while livestock was mostly diurnal, with moderate to high overlap between livestock and the wild ungulates (54.8% for red deer and 63.1% for roe deer). The occurrence of red and roe deer was marginally limited by livestock density, as well as by static (proximity to roads) and dynamic (recreational activity) proxies of human disturbance, while the proximity to the huts did not affect the occurrence of wild ungulates. Our results do not support any important effect of the presence of livestock on the spatial behavior and activity patterns of roe and red deer in the central part of the summer. We discuss future directions of investigation that can provide further insights into our understanding of the coexistence of wildlife and livestock in an Alpine landscape.

Characterisation of the risks to agro-pastoral farming systems linked to the interface with wildlife

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Mountain agro-pastoral systems evolve within dynamic ecosystems where interactions between livestock, wildlife and human activities give rise to multiple risks. In order to gain a better understanding of these interactions and the associated risks, this study proposes a conceptual framework for this complex interface, highlighting the challenges and opportunities it represents. This work is based on case studies from France and Italy, and on a bibliographic analysis of the main publications dealing with the risks and interactions associated with the wildlife-livestock interface. The analysis focuses on three areas: (1) characterisation of the risks associated with the wildlife-livestock interface, including predation, pathogen transmission, competition for resources, climate change and socio-economic tensions; (2) assessment of the direct and indirect effects of these risks on breeders and other local stakeholders; (3) identification of adaptation strategies to promote sustainable coexistence. The results highlight the complexity of these interactions and the diversity of adaptive responses implemented by the various components of this dynamic interface. Predation and the transmission of pathogens are major factors of vulnerability, exacerbated by climate change and socio-economic constraints. Management of this interface is based on a combination of strategies whose effectiveness varies according to the context. However, coexistence is not based exclusively on technical solutions: it requires adapted and evolving territorial governance, based on the sharing of resources, the recognition of multiple uses, the adaptation of management practices and rules in a context of persistent tensions. Faced with the growing challenges associated with biodiversity conservation and the sustainability of livestock farming practices, this study looks at the conditions for a balanced coexistence between livestock farming and wildlife. Identifying blocking factors and effective levers for action thus appears to be a central issue in mitigating the risks linked to this interface and guaranteeing the sustainability of mountain agro-pastoral systems in the context of global change.

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Theatre 6

Factors affecting livestock-wild ungulates co-occurrence in extensive silvo-pastoral system

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This study, conducted within the AGRITECH project funded by the NextGeneration EU (grant number J83C22000830005), examined co-occurrence of cattle (*Bos taurus*), red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), and roe deer (*Capreolus capreolus*) in a 2,330-ha fenced silvo-pastoral farm in central Italy (Lazio region). Camera traps were employed to assess species occupancy and spatiotemporal overlap in a Mediterranean silvo-pastoral system during winter (January). Cattle and red deer exhibited local overlap, while in other areas they showed complementary spatial distribution. To investigate species-specific attractive and repulsive factors, Kendall's Tau (Statistica 10, StatSoft) was applied to assess correlations between relative abundance and environmental/anthropogenic variables, setting the significance threshold at $p < 0.05$. Relative abundance was determined for each species using the Trapping Rate ($TR = \text{events } n^\circ / \text{camera days effort}$) at each camera station. For distance-based explanatory variables (e.g., Euclidean distance from fences, edges, and watering troughs), a significant negative Z score indicates a preference for proximity. Roe deer prefer heterogeneous environments with high edge density ($Z = 2.606$, $p = 0.009$) and proximity to sparse vegetation ($Z = -2.072$, $p = 0.038$), but avoids fences ($Z = 2.267$, $p = 0.023$). Red deer is strongly associated with cattle ($Z = 2.670$, $p = 0.007$) principally near the fences ($Z = -2.131$, $p = 0.033$). Wild boar also overlaps with cattle ($Z = 2.093$, $p = 0.036$), but mainly in areas with low human presence, far from landing sites ($Z = 3.097$, $p = 0.002$), gates ($Z = 3.170$, $p = 0.001$) and feeding stations ($Z = 3.59078$, $p = 0.0003$). They also avoid cattle watering troughs ($Z = 5.305$, $p < 0.001$), likely due to poor accessibility, preferring more easily accessible natural waterholes. Continuous passive monitoring through camera trapping allowed us to quantify livestock and wild ungulate co-occurrence, highlighting its potential as an effective tool for adaptive management. This approach can help balance ecological sustainability with the health and coexistence of domestic and wild animals.

Vulnerability to Wildlife Predators in Extensive Livestock Farming Systems Across Europe

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For much of their shared history, pastoralists and large wild predators shared space in a tense yet accepted and economically viable relationship. In the 20th century, large carnivores were drastically reduced or exterminated across most of Europe. This allowed traditional pastoral systems to evolve into modern extensive livestock systems, adopting contemporary production methods and new demands for work and life quality—often in predator-free landscapes. Since the late 20th century, these areas have seen a marked recovery of large carnivores. This has brought direct and indirect impacts on livestock systems, fueling polarized social conflicts over livestock practices and carnivore management. Interactions between livestock and wildlife vary greatly across Europe's landscapes, from benign to highly damaging, reflecting differing vulnerability levels among livestock systems. Farming practices believed to mitigate them remain poorly documented in comparative European studies. Likewise, livestock production research rarely considers constraints from carnivore return. To address these gaps, this invited talk presents preliminary results from structured, on-farm farmer surveys in 12 European countries. These cover a broad range of extensive livestock systems—including cattle, sheep, and reindeer—and focus on interactions with large predators such as wolves, bears, and lynxes. The surveys explore technical and operational characteristics—farm size and structure, grazing patterns, shepherding, husbandry cycles, types of products, economic aspects, and protection measures—and how these relate to direct losses from predation and broader indirect impacts.

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Theatre 8

Assessing socio-ecological dynamics: shifts in human attitudes and wolf management alongside recolonisation

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The recolonisation of human-dominated landscapes by wolves is increasingly challenging livestock farmers and fuelling ongoing public debates with diverging human attitudes. Switzerland legalised the proactive shooting of wolf packs, marking a significant policy shift with potential impacts on the Alpine wolf population. This study aims to analyse changes in human attitudes and in the spatial distribution of wolves integrating data from nationwide surveys conducted in 2015 and 2023. Using Structural Equation Modelling (SEM), we identified that positive attitude towards wolves was influenced by perceived benefits of their presence, while negative attitude was associated with indirect experiences of livestock loss and fear. Acceptance of wolves correlated with support for livestock protection measures and reliance on experts/science information, whereas negative attitude was linked to support for lethal management and reliance on social circles for information. A spatial modelling approach showed that socio-economic disparities between lowland and mountain areas were stronger predictors of human attitudes than proximity to wolves, depredation incidents, or livestock abundance. Potential human-wolf conflict areas were predicted by comparing habitat suitability for the wolf, human attitude, actual wolf occurrence, livestock abundance, depredation intensity, and existing livestock protection measures. This identified current and future human-wolf conflict hotspots and provided recommendations for adaptive management strategies. Our findings emphasise the need for a nuanced approach to wolf management that integrates local conflict characteristics, public perception, and effective communication to address the complex socio-ecological dynamics of human-wolf conflicts.

Dog predation on sheep flocks in wolf-free mountains: a synthesis based on surveys carried out in France
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Within the framework of the joint works carried out with a view to creating an inventory of canine predation sites in several wolf-free mountains throughout France, the CERPAM, the pastoralism department of CRA Languedoc Roussillon and VetAgro Sup conducted a study based on surveys carried out in 11 territories attached to 12 French départements. These surveys were conducted between 1999 and 2013 over multi-annual periods chosen before wolves arrived into the territories in order to obtain a database concerning the damage on flocks of sheep attributable to dogs. 307 farming, summer pasture and transhumance units were surveyed, making an average of 24 per territory. The study examines the category of male and female sheep over 4 months old put out to graze. Two indicators were prioritized: the annual frequency of attack and the predation rate. These attacks primarily occur by day and the dogs are seen in 86% of cases. The annual average frequency of dog attacks is 0.22 (minimum 0.10 and maximum 0.47 per territory), representing an average of one attack per farm every 5 years. On average, every dog attack results in the loss of 6.3 animals (from 1.6 to 22.9 depending on territory). The average predation rate is 0.29%, (from 0.06% to 0.60%), representing 1 animal per year for a flock of 330 animals. Sheep density has no significant effect on the predation rate in sheep farming areas with more than 10 sheep/km². The predation rate would nevertheless appear to increase where sheep density is very low or human density is very high.

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Theatre 10

Farmers, Wildlife, and Tourism: Navigating Bio-Cultural Frictions in Alpine Protected Areas of Piedmont, Italy
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In this contribution, we will present some anthropological reflections based on the opinions of farmers, gathered through interviews conducted by the University of Turin's team as part of the 2022 PRIN project WilDebate: Co-existences, Bio-cultural Frictions and Pastoralism in Protected Areas, in four protected areas of the Piedmont Region (northwestern Italy): Alpi Marittime, Alpi Cozie, Val Sesia, and Val d'Ossola. We will highlight some of the challenges they face in their work, often carried out in high-tourism areas, related to the complex coexistence of farmers, domestic and wild animals, and visitors. This situation requires them to adopt new livestock management practices due to the inability to continue farming "as we were used to" following the return of wild animals. The perceived issues varied across the different areas, ranging from pathogen spillover; the competition with deer and wild boars on alpine pastures; the presence of wolves that, beside the direct impact represented by livestock predation, also forces the adoption of guardian dogs. These, in turn, can pose "a greater problem than the predation itself" due to the danger they may present to tourists—whose purchasing of the farmers' products is crucial for the profitability of their activities. This will allow us to reflect more broadly on the relationship farmers have with their place of residence, the impact of so-called "ecosystem services" provided by livestock farming, and their absence in areas experiencing abandonment. The relationship with the wild that emerged from these interviews also forces us to reconsider the distance between the more romanticized view of Nature and the Mountain held by some visitors, the anthropic history of these alpine landscapes, and the ecosystemic role played by farmers, who remind us that "behind the clean mountain, there is someone".

Sensor technology and public engagement ideas to help bridge the Urban / Rural divide over livestock-wolf conflicts in Colorado

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Throughout the world, an urban/rural divide exists around predator conflict management and reintroductions and/or range expansions. During the last few years in Colorado, tensions between stockgrowers and much of the general public have grown very intense—for the first time ever in the U.S., Colorado voters approved a measure to reintroduce wolves, effectively pitting urban and rural audiences firmly against each other. The first round of wolf introductions last year did not go well for ranchers and their animals (cattle, sheep, dogs). This presentation will describe a new internet-based effort in Colorado to help bridge this urban/rural divide, stimulating public engagement by utilizing the following strategies: Using short video interviews from many producers, build urban appreciation for the many direct and indirect impacts livestock producers experience with predators such as wolves. The indirect impacts to producers are highly significant, and are not compensated by state funds, nor are they understood by the general public. Increase awareness of the strong connections between privately owned wild lands (large farms and ranches), and the reasons many people live in Colorado (habitat, open space and scenic values, wildlife, hunting/fishing, ecosystem services, maintaining the state's agricultural heritage, etc.). Channel newfound public appreciation and empathy for producers into very specific actions people can take to help producers (e.g., “adopting” livestock guardian dogs, sending needed equipment to Range Riders, funding new sensor/collar IOT technologies that alert producers to a predator attack in the making, creating an Innovation Idea Incubator to facilitate producer-led technology testing and ideation. Wild Range has begun testing and implementation in Colorado, and will soon expand to all other wolf states in the US.

External biosecurity in outdoor pig farms: Gaps in fencing and wildlife control

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Outdoor pig farming is increasingly favoured by consumers due to its perceived benefits for animal welfare. However, ensuring adequate biosecurity remains a major challenge, particularly in preventing disease transmission between livestock and wildlife. This study aimed to evaluate key external biosecurity measures, with a focus on fencing, in 40 outdoor pig farms in France. Data were collected from free-range pig farms housing at least 30 animals. The presence, type, maintenance, and functionality of fencing were assessed, along with potential exposure to wildlife and contact, based on farmer interviews. Overall, 59% of farms did not comply with national biosecurity regulations designed to prevent contact with wild boar. Non-compliance varied by production stage: 54% of farms with outdoor farrowing, 29% with outdoor mating and gestation, 45% with outdoor quarantine, and 25% with outdoor finishing units. Electric fence integrity was deficient in 25% of mating and gestation areas and 19% of farrowing areas. Proper vegetation management around electric fences was conducted in 69% and 55% of farrowing and mating/gestation areas, respectively. Wild boar observations were reported near 75% of farms, with 43% of farms reporting sightings more than twice a year. Direct contact between wild boar and pigs occurred on 20% of farms, leading to pig-boar hybrids in two cases. Other frequently observed wildlife included foxes, hares, badgers, roe deer, rabbits, martens, and crows. While 70% of farms employed fencing or trapping to mitigate fox intrusion, 5% implemented protective measures against crows. These findings underscore the need to strengthen external biosecurity measures in outdoor pig farms to mitigate the risk of disease transmission from wildlife, particularly African swine fever.

Effect of breed and colostrum quantity fed at birth on carcass traits, meat chemical composition and fatty acid profile in Holstein and Holstein-Angus steers

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This study investigated the effect of breed and colostrum quantity fed at birth on carcass traits, meat chemical composition, and fatty acid (FA) profile in Holstein (n = 20) and Holstein-Angus (n = 20) steers. At birth, calves were assigned to either a low (n = 10/breed) or a high (n = 10/breed) quantity of colostrum replacer, providing 2.5 g of IgG/kg of birth body weight (BW) and 5.0 g of IgG/kg of birth BW, respectively. Calves were then fed milk replacer up to 15% of birth BW and weaned at 9 wks of age. At 12 wks of age, calves were moved to a feedlot and remained there until slaughter at 56 wks. At slaughter, BW, and hot carcass weight (HCW) were recorded, and ribeye area (REA; at the 12th rib) and subcutaneous fat thickness (SFT) were measured. Carcasses were graded for marbling 72 hr postmortem between the 12th and 13th ribs by the Canadian Beef Grading Agency. Longissimus thoracis rib portions anterior to the 12th and 13th ribs were collected, vacuum-packed, and aged for 14 d before being frozen for meat chemical composition and FA profile analysis. Analysis of variance was conducted using colostrum quantity and breed as fixed effects. At the same slaughter age, crossbred steers had higher BW, SFT, HCW, dressing percentage, REA, and carcass yield than Holsteins ($P < 0.05$). Collagen and fat content were higher in the meat of crossbred steers, while water content was lower compared to Holsteins ($P < 0.05$). Among Holstein, those fed low colostrum at birth had the lowest fat and highest water content ($P < 0.05$). Crossbred steers had the highest percentage of saturated FA in meat ($P < 0.05$), whereas Holstein meat contained more polyunsaturated FA, conjugated linoleic acids, and omega-3 and omega-6 FA ($P < 0.05$). Overall, crossbred steers exhibited greater carcass yield and quality than Holstein when slaughtered at the same age, while colostrum quantity had only minimal effects on final carcass traits. Effect of breed on meat FA profile requires further analysis to understand the impact on shelf-life conservation and flavor of the meat.

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Theatre 2

Understanding the key management strategies to improve health of calves in the veal husbandry

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In the Netherlands, dairy beef calves are usually transported via a collection centre before entering a veal farm, and their management strategy used at the farm of origin is unknown. The aim of the project 'Progressie met Programmakalf' was to improve the link between the dairy and the veal husbandry in order to improve calf health, performance and lower antimicrobial use. We investigated the effects of surpassing the collection centre on health and performance of veal calves. Two batches of calves (N= 1,882 in total) were assigned to two treatments and were transported to four Dutch veal farms: one group was transported via a collection centre, and another was directly transported from the dairy to the veal farm. Treatment groups were assigned to specific compartments in the farm, separated both physically and hygienically. Body weights were obtained upon arrival at the veal farm and blood samples (N=300) were collected and analysed for biomarkers of health (haemoglobin, total protein, cholesterol, β -hydroxybutyrate, urea, and gamma-glutamyl transferase). In addition, carcass weight, average daily gain (ADG) and the use of medication were recorded in individual calves. All measures were analysed with linear mixed models including the fixed effects of treatment, sex and breed of calves and their interactions, the random effects of batch and compartment, and body weight upon arrival as a covariate. Blood biomarkers were analysed in association to other performance data. A survey on management practices of calves at the dairy and veal farms was carried out and the data were analysed in relation to health, growth and carcass weights of calves. Results showed no difference in carcass weights and ADG between the treatment groups, but carcass weights were significantly lower for calves at least once medicated individually at the veal farm (146 vs. 154 kg). Results from the use of medicines, blood biomarkers, and the survey will be presented. (Belongs to the project: 'Progressie met Programmakalf')

The effect of origin on calves' health at the rearing farms

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A total of 78 unweaned dairy beef calves (46.2 ± 0.99 kg of BW) were used to evaluate the effect of origin on calves' health after arrival at the rearing farm. Calves were randomly allocated to 6 pens of 13 calves/pen and distributed based on origin: calves collected from several farms and transported to the rearing farm after 8 hours of transport (C; $n = 39$) and calves from a single proximity farm, transported for 1 hour to the rearing farm (P; $n = 39$). At arrival, blood samples were collected to analyze gamma-glutamyl transferase (GGT) as an indicator of maternal immunity and complete blood cell count. Lung ultrasonography was performed at arrival, 7 and 28 days after arrival to the rearing farm, and was scored based on a 4-point scale to evaluate the incidence of pneumonia. Data were analyzed with mixed models and a Chi-square test. At arrival C calves had lower ($P = 0.03$) serum GGT concentrations compared with P calves (63.5 ± 9.46 and 93.0 ± 9.58 IU/L, respectively), indicating that calves from a single proximity farm had a greater amount of colostrum intake after birth. At arrival, concentrations of white blood cells and neutrophils were greater for C calves, and lymphocyte concentrations were lower for P calves, probably due to the stress of the longer transport duration. At arrival, C calves tended ($P = 0.10$) to have a higher incidence of pneumonia than P calves. Moreover, these differences were exacerbated after arrival giving pneumonia incidences of 51.3% and 43.6% for C calves, and 7.7% and 7.7% for P calves, on day 7 and 28, respectively. Finally, the correlation ($P = 0.05$) observed between the concentration of GGT at arrival and the incidence of pneumonia on day 28 indicated that there were benefits of maternal immunity on disease protection. Results from this study demonstrate that, in unweaned dairy beef calves, animal origin and its relationship with maternal immunity impact the incidence of respiratory disease.

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Theatre 4

The effect of varying transport and feed withdrawal durations on the physiological status of unweaned calves

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Long distance transport and associated feed deprivation are major welfare concerns for calves moved off the dairy farm. This observational study aimed to determine how variations in transport and fasting durations impacted the blood physiology of 115 calves in three transport groups; IRE [$n = 20$, shorter transport (29h, within Ireland) and shorter feed deprivation (11h)], INT [$n = 65$; longer transport (79h, Ireland to Netherlands) and longer feed deprivation (28h + 25h)], and NLD [$n = 30$; shorter transport (28h), within Netherlands) and longer feed deprivation (>18h)]. INT and NLD calves arrived at the same location. Each calf was blood sampled on arrival, 1-week and 3-weeks post arrival. Generalised linear mixed models were used to assess the effect of transport group and timepoint on various blood variables. Despite differing transport durations, both INT and NLD calves exhibited glucose, beta-hydroxy-butyrate, non-esterified-fatty-acids and sodium levels outside of reference ranges, which differed from normal levels in IRE calves ($p < 0.05$). Lactate and potassium were above reference ranges for INT calves, and higher than in IRE and NLD calves on arrival ($p < 0.05$). One- and three-weeks post arrival, differences between groups were minimal and not clearly associated with either transport duration or feed withdrawal during transport. In conclusion, changes in the physiological status appeared to relate more to the feed withdrawal duration than to the transport duration, except for lactate and potassium, which related to muscle fatigue. Most variables showed signs of recovery at 1-week and 3-weeks post arrival. Minimizing the duration of feed deprivation during transport should be a key consideration for the dairy industry to reduce the impact of transport on calf welfare.

Effects of transport and early administration of antibiotics on health of veal calves

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Transport via a collection centre and early-life administration of group antibiotics are two major challenges faced by calves in the veal sector. This study aimed to investigate the effects of these factors on the fecal microbiome, inflammation and general health of veal calves. The study had a 2×2 factorial design with factors ‘origin of calves’ (transport via a collection centre (CC) or direct transport from dairy farms (DF)), and ‘early-life administration of group antibiotics’ (AB or no AB). A total of 89 bull and 5 heifer calves of average 46.4 ± 5.1 kg body weight (BW) at arrival were included in the study. Calves were transported in two batches, were studied for a period of 8 weeks, and subsequently euthanized. Animals were housed in groups of three calves/pen divided over six rooms. Treatment groups DF_AB and CC_AB received oral antibiotics (oxytetracycline and aspirin) via the milk replacer. On days 21 and 45 of the experiment fecal samples and broncho alveolar lavage fluid (BALF) were collected to investigate the microbiome and lung immunity. Blood samples were collected on day 1, 7, 21, 35, 45 and 51, BW was recorded upon arrival, and on days 28 and 49, and clinical observations were conducted twice a week. During post-mortem examinations weights of lungs and heart were recorded and lungs were scored for abnormalities. Treatment group CC_noAB had the lowest number of species in the fecal microbiome measured on day 21 ($\Delta = -170.2$; $P < 0.01$), the lowest Shannon Index on day 45 ($\Delta = -0.2$; $P < 0.01$), the lowest % of alveolar macrophages ($\Delta = -26.1\%$ on average; $P < 0.01$), most clinical problems ($\Delta = 9.2\%$ for fever and $\Delta = 13.1\%$ for loose or liquid manure on average; $P < 0.05$) and lowest BW during the trial ($\Delta = -4.4$ kg on average on day 49; $P < 0.01$). Results showed that transportation via a CC without group administration of antibiotics negatively affected health of veal calves.

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Theatre 6

Modelling antigenic drift of bovine coronavirus in beef-on-dairy calves during a controlled commingling event

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Bovine respiratory disease (BRD) is a leading cause of calf morbidity and mortality worldwide. Bovine coronavirus (BCV) is a well-studied enteric pathogen which has also been implicated as a causative agent of BRD in calves. However, epidemiology of this virus is poorly understood, and there is no available data on BCV antigenic variants circulating in the UK. The aim of this study was to investigate transmission of respiratory BCV during a controlled commingling event. Commingling events, such as mixing animals at auction markets or feedlots, are well-known to increase risk of disease transmission, and are a major risk factor for the development of BRD. The study was conducted on 120 weaned Angus x Holstein calves from 10 source farms (SFs) (3 cohorts of 40 calves). Following quarantine at the research facility, calves were randomised into four commingling groups: none, low-level, moderate, and intensive. Serum and nasopharyngeal swabs were collected on the start of quarantine, the day of mixing, and then 3, 7 and 21-days post mixing. Furthermore, calves were scored daily for signs of clinical BRD using a modified Wisconsin system. Serum samples were subjected to anti-BCV IgG ELISA. Swabs were subjected to PCR for BCV, and positive samples were sequenced. Based on preliminary results from the first two cohorts, all calves were sero-positive for BCV on SF collection, and their antibody levels remained high during the study period. Nonetheless, BCV prevalence increased significantly with mixing duration. 68 BCV isolates were sequenced, and 16 genetic variants were assigned into 6 genogroups. Isolates clustered within SFs during quarantine period, but not on subsequent study days. Furthermore, isolates from two genogroups were found on one of the SFs, and several calves were infected with isolates belonging to different genogroups at collection and D21 of the study. Altogether, this suggests lack of sterilising immunity to BCV and continuous circulation of the virus in populations, despite lack of clinical disease. Analysis of the final cohort is currently ongoing, including additional environmental sampling, which should help to further disentangle the relationship between BCV and BRD.

Milk feeding plan and type of milk fed at the dairy farm is linked to entry weight and performance of veal calves

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The aim of the project ‘Progressie met Programmakalf’ was to improve the link between the dairy and the veal husbandry in order to improve calf health, performance and lower antimicrobial use. Because feeding choices in the first weeks of life can influence calf performance at later stages, part of this project was a prospective cohort study to examine the correlation of type of milk fed to calves (cow’s milk or milk replacer (MR)) and feeding plan (restricted or ad libitum) on body weight of veal calves at the collection center (entry weight) and performance (carcass gain) during fattening. The dataset contained data of over 80,000 veal calves slaughtered from July 2021 to December 2024 from over 500 dairy farms. These dairy farmers were interviewed on calf management practices. The carcass gain of every veal calf was predicted via a model using sex, breed, entry weight and fattening days. The realized performance was compared to the prediction and corrected for veal farmer management. The result of this calculation is the residual growth which was used as parameter to evaluate fattening performance. Analyses of the data showed that ad libitum feeding of milk is more common in farms using cow’s milk than in those using milk replacer (Chi-square; $P=0.025$). Regarding calf related parameters, entry weight was higher of calves from farms feeding cow’s milk compared to MR (general linear model (GLM), $P<0.05$). Moreover, ad libitum feeding was associated with higher entry weights compared to restricted feeding (GLM, $P<0.05$). Veal calf performance, measured as residual growth, was higher with restricted feeding compared to ad libitum feeding (GLM, $P<0.05$). Overall, restricted feeding plans and use of MR were related to better growth at the veal farm, potentially yielding 4 kg more carcass weight per animal. This may be attributed to a closer match of calf management between dairy and veal farm, resulting in less environmental stressors for these calves. The results show potential to use veal calf integration and dairy farm data to optimize feeding management for dairy beef calves both on dairy farms and veal farms which has a positive effect on calf lifetime performance. (Belongs to the project: ‘Progressie met Programmakalf’)

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Theatre 8

Partial replacement of skim milk powder in calf milk replacer with enzyme treated soybean meal improves nitrogen utilization

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The objective of the present study was to evaluate the effect of replacing skim milk powder (SMP) in calf milk replacer (CMR) with vegetable protein sources on calf rearing performance. A total of 56 female Holstein calves were used in the study from birth until 135 days of age, with weaning after 75 days. The calves were allocated to one of four dietary treatments: 1) control, containing 50% SMP, 2) HP-100, containing 12% HP-100 (enzymatically treated SBM from Hamlet Protein), 3) wheat gluten, containing 8.65% hydrolyzed wheat gluten, and 4) SBM containing 13.5% soybean meal (SBM). Each vegetable protein source replaced 44% of the total amount of SMP, corresponding to 22% in the total formula. The CMRs were formulated to be isoenergetic (17% fat and 20 MJ GE, as-is) and isonitrogenous (22.5% CP, as is). Throughout the preweaning period, calves received two daily meals of CMR, 4 L each with a concentration of 160 g of CMR per L. Solid starter feed was provided ad libitum throughout the experimental period. Calves were weighed at birth and in weeks 5, 8, 11, 15, and 20. Fecal samples were collected by grab sampling at weeks 1, 5, and 10 and analyzed for dry matter (DM) and nitrogen (N) contents. Statistical analysis of growth performance was performed using a mixed model in SAS (version 9.4). Fecal data were analyzed as repeated measurements, including the effect of week. Calves fed CMR with HP-100 had similar preweaning ADG compared with calves fed the control CMR. Calves fed CMR with wheat gluten and SBM had lower ADG than both the control and HP-100 groups ($P=0.02$). No effect of CMR type on post-weaning ADG was observed ($P=0.76$). Fecal N was lower for calves fed CMR with HP-100 (5.1%) compared to calves fed the control (6.3%), wheat gluten (6.7%), and SBM (6.1%; $P<0.001$). Fecal DM tended to be higher in calves fed CMR with HP-100 ($P=0.06$). In conclusion, calves fed CMR with HP-100 performed equally well as calves fed CMR consisting entirely of SMP. Moreover, calves fed CMR with HP-100 had lower N content in feces, indicating better N utilization.

Skim milk-based or whey-based milk replacer for calves from 4 weeks of age

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The research question was if a rosé veal producer buying unweaning calves at 4-weeks of age could profit from using a whey-based (W) or if a more expensive skim milk-based (S) milk replacer (MR) was more beneficial for calves at this age. Thus, two different MRs, SMR and WMR, with same crude protein (23%) and crude fat (19%) contents, and produced by the same manufacturer (Denkavit Nederlands b.v.) were compared, and milk intake, drinking pattern and growth rate of calves were record. Calves (n=32) were purchased at 2-weeks of age from 5 herds, at least 2 calves of same sex and breed combination from each herd. Calves were housed in 4 straw-bedded pens (8 calves per pen each equipped with two drinking stations (4 calves assigned to each)). Most calves were fed cow's milk or SMR before arrival to the research farm. SMR was fed to all calves for the first 4 days after which 16 calves were transitioned to WMR over 24 h. Thus, all calves were on their treatments from 3-weeks of age. Concentrates, hay and water were always available. Cases of pneumonia + navel inflammation + diarrhea at arrival were 10 in both treatment groups, and the sick calves received medication. Daily MR feeding level was 7.6 L from 4 to 6 weeks, 5.0 L from 6 to 8 weeks, and 2.5 L from 8 to 10 weeks (all with 13.6% DM. Two VARIO Smart feeders (Förster-Technik GmbH) delivered the SMR and WMR to each of the 4 pens. Mean milk intake (4.8 L/d) and visits with intake (9/d) were not affected by MR type but visits without milk intake was higher for WMR in the first weeks, and mean drinking time was higher for SMR compared to WMR (41 vs 27 min/d). ADG for wk 2-10 (910 g/d) and for wk 10-12 (1,345 g/d), and BW at d 70 (95.5 kg) was not affected. In conclusion, based on performance and these two MRs, calves purchased at 4 weeks of age can be fed WMR and perform equally well as if fed SMR.

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Theatre 10

Predicting feed composition eaten by beef cattle using rumen metagenomic data

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The composition of the feed eaten by the animal is important to understanding feed efficiency, a key trait in livestock. It also provides relevant environmental information to consider when fitting prediction models for many traits. However, this information is often unavailable or inaccurate. With sequencing costs declining, metagenomics data are increasingly accessible. This study explores the ability of rumen microbiome composition—microbial genes (MG) and genera (MT)—to infer beef cattle intake of diet components like starch, crude protein, detergent fiber (neutral/acid), and global/metabolizable energy. This study utilised daily dry matter intake (DMI), average daily gain (ADG), methane production (MP), feed efficiency traits (FCR and RFI), and metagenomic data from rumen fluid samples of 142 calves fed a forage-based (n=70) or concentrate-based (n=72) diet. The chemical composition of the feed was determined using standard chemical techniques, and the total daily intake of each component was computed. The relative abundances of 3632 MG and 1137 MT across 43 phyla were characterized and compositionally transformed. Random forest (RF) and stepwise logistic regression (LR) models were optimized to classify diet type (forage/concentrate) and predict feed component intake based on microbiome data. Data were split into training and testing sets for model evaluation. Animals were classified by diet type with an AUC of 0.86±0.05 using the log-ratio of Verrucomicrobia/Chlorobi phyla in LR, and 0.90±0.05 using MG and MT abundances in RF. Predicted diet, together with MG and MT were used to predict feed component intake using RF. All feed component intake were predicted with accuracies between 0.77 and 0.83. We also tested the benefit of including feed component intake as a covariate in linear models aimed at predicting different traits. Improvements were observed in the accuracy of prediction of DMI and ADG, with a marked trend for MP. However, no improvements were found for FCR or RFI. These results were consistent for both phenotypic and genetic models.

Use of sunflower (oil and/or seeds) as dietary fat in fattening dairy beef bulls fed high-concentrate diets: performance and enteric CH₄ emissions

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The aim of this study was to evaluate the use of sunflower (oil and/or seeds) as a dietary fat source in dairy beef bulls fed high-concentrate diets on performance and enteric CH₄ emissions. Eighty-six bulls (281 ± 3.7 kg BW, and 190 ± 1.2 days of age) were group-housed in 6 pens and fed concentrate and straw separately, both ad libitum. Concentrate offered to bulls differed in dietary fat source: 1) SO (n=27), 3.5% sunflower oil; 2) SO-SS (n=30), 2.5% sunflower oil and 2.6% sunflower seeds; and 3) SS (n=29), 9.5% sunflower seeds. The study lasted 168 days. Individual feed intake was recorded daily. Bulls were weighed every 2 weeks. Enteric emissions were recorded daily during the first 42 days of the study with a CH₄ and CO₂ sniffer's methodology installed in each concentrate feeder per pen. Daily CH₄ production (L/day) was estimated based on Madsen et al. (2010). Carcass quality was assessed at the slaughterhouse. Data were analyzed using a mixed effects model. Body weight was higher (P<0.01) in SS than in SO between days 70 and 126. Concentrate intake was lower (P<0.01) in SO-SS and SS than in SO during the first month. Time by treatment interactions (P<0.01) with no clear pattern were observed for average daily gain, feed efficiency, daily CH₄ production, and daily CH₄ production expressed by BW. However, in the first 14 days, daily CH₄ production expressed by concentrate intake was greater (P=0.02) in SS than in SO (35.2 vs. 25.6 ± 4.15 L CH₄/kg). Carcass quality was similar among treatments but dressing percentage tended (P=0.07) to be greater in SO-SS than in SO (55.2 vs. 53.9 ± 0.55%). The type of sunflower source (oil and/or seeds) in fattening dairy beef bulls fed high-concentrate diets did not have relevant effects on performance; however, due to reduced concentrate intake with consumption of the seeds diets during the first days, the daily CH₄ production expressed by concentrate intake decreased with consumption of the oil diet at the beginning of the study.

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Theatre 12

Farming system is the main driver of enteric methanogenesis in grass-based veal calves

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Developing grass-based veal production from male dairy calves could be a low-input solution for raising such animals, particularly in organic systems. However, the impact of grazing on enteric methane emissions of young animals is not well assessed, yet. The study included three genotypes – Brown Swiss, Limousin × Brown Swiss, and Swiss Fleckvieh – raised in four distinct farming systems: a hay-based indoor system and three pasture-based systems, representing intensive, permanent, and alpine grasslands. While all grazing calves received comparable amounts of maize and alfalfa pellets, and a limited soy-free concentrate, the indoor control group followed a slightly different feeding regimen. Hay was provided ad libitum in all systems. Each genotype × system interaction comprised four male calves, raised together from day of life 90 to 180 in the respective systems. Rumen fluid was sampled by intubation after 11 weeks. In vitro gas production (ml/200 mg DM), absolute methane emissions (mg), and methane yield (mg/ml) were analysed after 24h of incubation at 38°C in a batch system. The time between sampling of the rumen fluid and start of the test run in vitro was different due to physical distances between sites and laboratory. It was included as a correction variable in the model. Analysis revealed a significant impact of the farming system on methanogenesis, whereas genotype effects were marginal and never significant. Total gas production volume was highest in the intensive grassland system and lowest in the alpine system (46.2 ml and 44.6 ml, respectively). Methane production was lowest in the indoor and intensive grassland systems, whereas the permanent and alpine grassland systems produced the highest levels—showing increases of 16% and 8%, respectively, in methane production (mg) compared to the indoor system. These results show relative differences between systems and genotypes. Scaling to emission values per unit of product is not possible, though. Further research on dietary strategies, economic feasibility, and land use efficiency is necessary to assess the sustainability of grass-based veal production.

Increasing slaughter weights of crossbred heifers for higher self-sufficiency of beef increases environmental impacts

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The supply of domestic beef has decreased in Finland in recent years. To compensate the decline, and keep the level of self-sufficiency, one solution is to increase slaughter weights. With the current slaughter weight of bulls, it is inefficient to increase it further as feed conversion declines due to decreased growth rate and increased dry matter (DM) intake. Thus, the greatest potential for increased beef supply exists for heifers raised for beef. Additionally, as the demand for food increases, it is increasingly important to use feeds not fit for human consumption. This favors grass silage (GS)-based feeding. A feeding experiment was conducted using 84 crossbred heifers (Holstein or Nordic Red × Blonde d'Aquitaine) which were fed total mixed rations. The carbon footprint (CF) of treatments were assessed with Life Cycle Assessment (LCA), to compare environmental performance of kg produced beef (functional unit, FU). System boundary was set from cradle to gate focusing on period of the experiment. The two experimental diets were: GS alone (GSA) and GS supplemented with barley (285 g/kg DM; GSB). On both experimental diets the heifers were slaughtered at three different carcass weights (CW): low 300, medium 325 and high 350 kg. Including barley in the diet increased energy intake, resulting in improved growth rate and feed conversion compared to GSA. GSB heifers had a higher dressing proportion and carcass conformation compared to GSA. GSB animals were also fatter compared to GSA heifers. Increased CW and increased growing period also increased CF per FU. This was due to increased methane emission from enteric fermentation and increased need for feed production. Although a reasonable growth rate was achieved with GSA, it was significantly improved with a moderate level of concentrate. This also improved environmental efficiency. The highest CW in GSB had lower CF per FU than the highest or medium CW in GSA. When the target is to increase the beef supply, inclusion of concentrate in the diet is justified.

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Theatre 14

Profitability and greenhouse gas emissions of dairy-beef steers of divergent beef genetic merit in pasture-based systems

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Pasture-based dairy-beef production systems offer the opportunity for profitable and environmentally efficient beef production, and is integral part of the beef supply chain in Ireland. However, the beef genetic merit of dairy-origin animals in Ireland has been in decline. The objective of this study was to utilize the Grange Dairy Beef Systems Model (GDBSM), a simulation model of a dairy beef farm, to investigate the economic and environmental performance of three dairy-beef genotypes differing in beef genetic merit, and managed on three pasture-based feeding treatments. Experimental data from a three-year farm systems study that assessed the phenotypic performance of each genotype was used to parameterize the model. The three genotypes modelled were: 1) steers produced from high beef merit Angus sires (HA); 2) steers produced from low beef merit Angus sires (LA); and 3) steers produced from Holstein-Friesian sires (HF). Each genotype was evaluated across one of three feed treatments: 1) conventional (CONV), pasture only during first and second grazing season; 2) medium concentrate (MC), concentrate supplementation during the first grazing season, pasture-only in second grazing season and 3) high concentrate (HC), concentrate supplementation during the first and second grazing season; amounting to nine modelling scenarios. Results from the model showed that HA steers were the most profitable genotype across all three feed treatments, with an average net margin per animal and per hectare of €424 and €1362, respectively. In comparison average net margin per animal and hectare were €337 and €1126 for LA, and €188 and €659, for HF steers, respectively. Greenhouse gas emissions per kg product were lower for both Angus steer groups within HC compared to CONV and MC, due to their reduced slaughter age when fed concentrates at pasture. However, similar emissions reductions were not seen in HF, as slaughter age was not reduced within HC. In conclusion, this study demonstrates both the financial and environmental benefits of using high beef genetic merit sires on the dairy herd.

Effect of breed and colostrum quantity fed at birth on growth, nutrients digestibility and serum profile of Holstein and Holstein-Angus steers from birth to slaughter

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This study investigated the effect of breed and colostrum quantity fed at birth on lifetime growth, nutrient digestibility, and serum profile in Holstein (n = 20) and Holstein-Angus (n = 20) steers. At birth, calves were assigned to either a low (n = 10/breed) or a high (n = 10/breed) quantity of colostrum replacer, providing 2.5 g of IgG/kg of birth body weight (BW) and 5.0 g of IgG/kg of birth BW, respectively. Thereafter, calves were fed milk replacer up to 15% of birth BW and weaned at 9 wks of age. Calves were moved to a feedlot at 12 wks of age and remained there until slaughter which occurred at 56 wks. Milk and solid feed intake were recorded daily, while blood samples and BW measurements were collected monthly. Apparent total-tract digestibility (ATTD) was estimated using an internal marker and fecal spot sample collection on wks 28 and 55. Analysis of variance was conducted using colostrum quantity and breed as fixed effects, with time as a repeated measure. Calves fed low colostrum had reduced solid feed intake during the first 12 wks (P = 0.05). Crossbred calves had greater intake during the growing (i.e., until wk 32) and finishing periods (P < 0.05), leading to increased growth rate (P < 0.01). On wk 55, Holstein had greater NDF and ADF ATTD (P < 0.05). Calves fed high colostrum at birth had increased total protein (TP), globulin, and non-esterified fatty acids (NEFA), but lower glucose levels in serum (P < 0.05) at 24 hours of life. However, these differences did not persist over time. Crossbred calves had higher urea, glucose, and beta-hydroxybutyrate (BHB) during the growing period (P < 0.05) and higher cholesterol and BHB (P < 0.05), with a tendency for increased NEFA (P = 0.07) during the finishing period. Creatinine was consistently higher in crossbred calves and in calves fed low colostrum at birth (P < 0.05). Overall, crossbred steers fully expressed their growth potential after 12 wks of age. Protein and energy metabolism deviated throughout time between breeds as illustrated by differences in lipid and protein serum biomarkers.

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Poster 16

Effect of pasture type on bio-economic dairy-beef heifer system performance

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Enhancing diversity by adding legumes or herbs to perennial ryegrass (PRG; *Lolium perenne*) swards offers the benefit of improved nutritive value and reduced inorganic nitrogen (N) fertiliser to maintain dry matter (DM) production. This study investigated how different pasture types affect the slaughter performance of early-maturing dairy-beef heifers. Three pasture treatments were evaluated: PRG receiving 150 kg N ha⁻¹; CLOVER (comprising of PRG, *Trifolium repens* and *Trifolium pratense*) receiving 75 kg N ha⁻¹, and multispecies (MSS) comprising of PRG, red clover, white clover, plantain (*Plantago lanceolata*), and chicory (*Cichorium intybus*) also receiving 75 kg N ha⁻¹. The sire breeds were Hereford and Angus and all progeny were from Holstein-Friesian dams. A higher proportion of heifers in the CLOVER (0.86) and MSS (0.75) treatments were drafted by the end of the second grazing season from a pasture-only diet compared to the PRG group (0.68), with remaining animals requiring a housed period on a diet of forage and concentrate. Additionally, CLOVER and MSS treatments produced significantly heavier carcasses (250 kg versus 243 kg for PRG; P < 0.05) due to enhanced lifetime growth performance that allowed them to reach a target fatness (fat score of 8 to 9). Carcass conformation remained consistent across treatments, averaging 5.1 on a 15-point scale (P > 0.05). The Grange Dairy Beef Systems Model (GDBSM) was used to investigate the financial performance of heifers consuming the three pasture types. Heifers consuming the MSS and CLOVER pastures were more profitable, and can generate an additional €100 to €150 net margin/ha over the PRG treatment (€1050, €1097, and €950/ha, respectively), due to improved animal performance and lower input costs. In conclusion, despite the light carcass weights achieved, this study highlights that dairy-beef heifer systems can achieve a high carcass output/ha, and that incorporating clover and herbs into pastures can increase profitability, as observed in the CLOVER and MSS treatments.

Effects of an intake promoter based on cow's milk aromas on concentrate consumption, growth, and health condition in rearing Holstein dairy beef calves from marketing

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Weanex® Calves (HTBA) is an intake promoter containing volatile compounds based on cow's milk designed to improve concentrate intake. Nutritional strategies aimed at promoting concentrate intake are particularly important for calves undergoing marketing and transportation, as these processes can lead to feed restriction and anorexia, impacting negatively performance and health. An experiment was conducted to investigate the effects on intake, performance, and health in Holstein calves adding Weanex® Calves at a 0.04% inclusion level in milk replacer (MR) and concentrate. Forty-eight male Holstein dairy beef calves (56 ± 4.2 kg of body weight and 27 ± 5.4 days of age) were assigned to 4 treatments following a 2×2 factorial design according to the intake promoter supplementation in MR or/and concentrate. All calves received 2 L of MR, at a concentration of 125 g/L as fed, twice daily for the first 21 days, and once a day MR feeding the last 7 days. Animals had ad libitum access to mashed concentrate and water throughout the 80 days of experimental period. Consumption of MR was registered daily, and concentrate intake and body weight were recorded weekly. To assess the general health status of calves, fecal consistency 4-point scale, respiratory score based on University of Wisconsin-Madison, and medical treatments were recorded weekly. Calves that received supplemented concentrate tended ($P = 0.08$) to have a greater feed consumption (2.42 vs. 2.17 ± 0.071 kg/day), growth (0.94 vs. 0.84 ± 0.040 kg/day), and body weight (131.3 vs. 122.7 ± 3.38 kg) in comparison with non-supplemented calves over the rearing period. Calves fed with supplemented MR and concentrate had lower ($P < 0.05$) diarrheic feces proportion than the other treatments during pre-weaning period (29 vs. 37%). The results showed that adding Weanex® Calves at 0.04% increased concentrate consumption and average daily gain in rearing dairy beef calves from marketing, being a promising nutritional strategy to encourage concentrate consumption and improve performance. Key words: concentrate consumption, dairy beef calves, intake promoter, milk replacer, performance.

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Poster 18

Assessment of hydrolyzed yeast as an alternative protein source in milk replacers: Effects on calf health, growth and carcass quality

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Protein is crucial in milk replacers (MR) for optimizing animal health, performance, and production costs. With rising milk protein costs, research has shifted towards alternative protein sources in MR. This experiment aimed to assess the impact of an alternative protein source on calf growth, development, and health. A total of 44 Holstein male calves (21 days at arrival) were randomly assigned to two treatments: a control MR (50% skimmed milk powder, 18% whey protein) or a modified MR (3% skimmed milk powder replaced by hydrolyzed yeast, Yelaprosecure; YelaP). This regimen was maintained for the first 63 days with two MR meals daily and ad libitum access to solid feed and water. Individual milk refusal and solid feed intake were monitored using automatic feeders throughout the experimental, growing, and finishing phases, which lasted a total of 162 days. Individual body weights, feed consumption, health records, and carcass characteristics were also gathered. Total solid and liquid feed intakes were similar between groups during the study, confirming the MR's palatability was similar between groups. The overall health status was consistent, with comparable white blood cell counts, and a low overall morbidity rate of 4%. Regarding average daily gain, no significant differences were found. Nevertheless, calves receiving YelaP showed numerically better growth after the experimental phase (949 vs. 914 g/day, $p = 0.29$) and by the end of the rearing period (1406 vs. 1335 g/day, $p = 0.16$). At slaughter, the carcass weights were similar (151 kg for YelaP vs. 145 kg for Control, $p = 0.84$) without compromising meat color and displaying more consistent and numerically higher conformation scores, indicating improved overall carcass quality. Incorporating Yelaprosecure in milk replacer at the beginning of the rearing phase for young calves may effectively substitute a high-quality milk protein source, achieving similar performance outcomes without compromising animal health and carcass quality.

Can sunflower (oil, seeds or by-products) reduce CH₄ emissions when substituting palm oil on dairy beef calves fed high-concentrate diets? In vitro screening

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The aim was to evaluate the rumen in vitro CH₄ production of fattening dairy beef concentrate when replacing partially or entirely palm oil by sunflower (oil, seeds or by-products). Treatments were 9 different combinations of palm and sunflower oil with sunflower cake or seeds as the concentrate fat source: 1) PO, 3.5% palm oil; 2) SO, 3.5% sunflower oil; 3) PO-SC, 2.5% palm oil and 16% sunflower cake; 4) SO-SC, 2.5% sunflower oil and 16% sunflower cake; 5) PO-SSL, 2.5% palm oil and 2.6% sunflower seeds; 6) SO-SSL, 2.5% sunflower oil and 2.6% sunflower seeds; 7) PO-SSM, 1.5% palm oil and 5.2% sunflower seeds; 8) SO-SSM, 1.5% sunflower oil and 5.2% sunflower seeds; and 9) SSH, 9.5% sunflower seeds. Samples were grinded at 1 mm and used in an in vitro digestibility assay. The rumen liquid was obtained at the slaughterhouse. Two incubation sets were performed: two sampling points lasted 24 and 48 hours to calculate DM digestibility; a third sampling point lasted 12 days to monitor CH₄ production. Data were analyzed using a mixed effects model. No statistical differences were found in CH₄ net volume per g degraded DM. However, a time by treatment interaction ($P=0.05$) was observed in the area under the curve (AUC) of CH₄ net volume per g degraded DM; while no differences were observed at 24 and 48 hours, treatments were different ($P<0.01$) after 12 days (44.7_{cd}, 95.0_a, 70.3_{abc}, 92.2_a, 42.8_d, 48.5_{bcd}, 64.0_{bcd}, 57.3_{bcd}, 72.2_{ab} ± 13.75 AUC; PO, SO, PO-SC, SO-SC, PO-SSL, SO-SSL, PO-SSM, SO-SSM, SSH, respectively). Fattening dairy beef concentrates in which palm oil is substituted partially or entirely by sunflower (oil, seeds or by-products) did not reduce in vitro rumen CH₄ emissions. However, concentrates based on palm or sunflower oil combined with 2.6 or 5.2% sunflower seeds kept in vitro rumen CH₄ emissions similar to that of concentrate with only palm oil.

Session 73

Poster 20

Which physical form of starter (mash vs. pelleted) is more recommended to promote the intake and optimize performance in rearing Holstein dairy beef calves?

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This study was designed to determine whether physical presentation of starter concentrate could influence consumption, growth, and blood metabolites in rearing Holstein dairy beef calves from marketing. Forty-eight male calves (54 ± 6.7 kg of body weight and 30 ± 9.5 days of age) were used in a 2 x 2 factorial design (n = 12 replicates/treatment) to evaluate the effect of flavor additive and starter presentation (mash vs. pellet). The present abstract only reports results of the starter presentation factor. The study lasted 28 days pre- and 50 days post-weaning. All calves were fed 4 L of milk replacer daily, at 12.5% of concentration, for first 21 days (2 feedings/day) and 2 L/day last week (1 feeding/day). In addition, they had ad libitum access to concentrate, straw and water. Milk replacer consumption was recorded daily, and starter intake and body weight weekly. Periodically, general health status of calves was assessed through recording fecal consistency and using multicriteria scoring system to evaluate respiratory clinical signs. Blood samples were collected on days 28 and 50 of study to analyze metabolic status of calves. During pre-weaning period, calves fed pelleted starter exhibited higher ($P < 0.05$) milk replacer consumption, better growth, and improved feed efficiency in comparison with mash form. However, no differences were observed in weaning body weight and concentrate intake. Otherwise, calves receiving pelleted starter tended ($P = 0.08$) to have higher starter intake, better growth, and enhanced feed efficiency during post-weaning phase. In addition, calves fed mash starter tended ($P = 0.07$) to have a higher incidence of diarrhea and a higher proportion of calves with fever during the first week than those fed pellets. Nevertheless, no differences were observed in respiratory diseases and antibiotic treatments, and metabolic parameters as well. In conclusion, the pelleted physical form of starter improved intake and performance in pre- and post-weaning period in comparison with mash, being the starter presentation recommended for rearing Holstein dairy beef calves. Key words: dairy beef calves, performance, physical form, starter intake.

Effect of pellet diameter on ruminal fermentation, animal behavior, and performance in fattening Holstein bulls fed with high-concentrate diets

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The objective of this research was to evaluate the effect of pellet size on ruminal fermentation, animal behavior, and performance in fattening dairy beef cattle. The hypotheses were that increasing pellet diameter may enable 1) to slow down concentrate consumption and thus increase chewing and saliva production, 2) to reduce the rate of fermentation in the rumen and passage out of the rumen, and 3) to promote a healthier digestive environment and thus improve feed efficiency. The present abstract only reports results from the growing phase, as the study is ongoing. Forty-eight Holstein bulls (179 ± 19.5 kg of body weight and 156 ± 24.1 days of age) were pair allocated in 24 pens following a completely randomized design ($n = 8$ replicates/treatment) to investigate the effects of pellet diameter (3.5, 8.0 and 16.0 mm) during 112 days of the growing period (351 ± 28.9 kg of body weight and 268 ± 24.1 days of age). Bulls had ad libitum access to concentrate, straw and water, and concentrate was formulated with the same ingredients and proportions. Weekly, feed consumption per pen was recorded and fortnightly, individual BW, bloat and fecal assessment were registered. At 56 and 112 days of study, about 120 mL of rumen fluid was collected from each bull using a stomach tube. After measuring pH, 4 mL of rumen content was kept for analyzing volatile fatty acids concentration. Animal behavior was assessed in each pen at 56 and 112 days of study. Increasing the pellet diameter tended ($P = 0.09$) to reduce the concentrate intake; however, no differences were observed among treatments in the other performance parameters. Rumen pH from bulls fed large pellets was numerically higher than those fed small pellets. No impact of pellet diameter was observed on animal behavior and for prevalence of digestive disorders. Results suggest that increasing the pellet diameter from 3.5 to 8 or 16 mm may enable an improvement in feed efficiency without impairing animal welfare and digestive status. Key words: beef cattle, behavior, pellet diameter, performance, ruminal fermentation.

Effects of L-carnosine on heat stress-induced oxidative damage, proliferation, and myogenic differentiation in bovine skeletal muscle-derived cells

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Heat stress is a significant environmental factor that negatively impacts skeletal muscle development in livestock, leading to impaired myogenic differentiation and increased oxidative stress. This study aimed to evaluate the effects of L-carnosine on heat stress-induced oxidative damage, myogenic differentiation, and proliferation in bovine skeletal muscle-derived cells (BSMCs). BSMCs were cultured for six days under differentiation conditions, with heat stress (41°C) applied from days 2 to 4 (48 h), followed by recovery at 37°C. The experimental groups included a control group (CON, 37°C), a heat-stressed group (HC, 41°C), and heat-stressed groups treated with L-carnosine (HT1-HT4, 41°C) at concentrations of 0.01, 0.1, 1, and 10 mM. Cell proliferation was measured after 48 hours of heat exposure using the CCK-8 (Cell Counting Kit-8) assay, and gene expression of myogenic markers, heat shock proteins, and antioxidant enzymes was analyzed using qPCR. The results showed that HSPB1 expression significantly increased in HC compared to CON ($p < 0.01$). L-carnosine-treated groups showed a decreasing trend in HSPB1 expression compared to HC. Myf6 expression significantly increased in all heat-exposed groups ($p < 0.05$). Lower L-carnosine doses (0.01, 0.1 mM) maintained Myf6 expression, while higher doses (1, 10 mM) showed a declining trend. Oxidative stress markers exhibited a dose-dependent response to L-carnosine. GPX1 and GPX4 expression increased in HC ($p < 0.01$). While a trend toward a decrease was observed at 0.1 mM, expression levels increased at 1 and 10 mM, indicating dose-dependent modulation of antioxidant enzymes by L-carnosine. Cell proliferation significantly decreased in HC compared to CON ($p < 0.01$). However, L-carnosine at 0.01 and 0.1 mM restored proliferation to CON levels, while 1 and 10 mM significantly increased proliferation compared to CON ($p < 0.01$). These findings suggest L-carnosine as a potential nutritional strategy to mitigate heat stress-induced muscle damage and improve muscle development in cattle.

Opportunities and challenges in automated objective detection of farm animal behavior

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The gold standard of analysis in ethology has been manual coding of behavior which is extremely time intensive and wrought with subjective undertones. Over ten years ago, we (and others) started to explore alternatives to the classic methodologies used for the study of behavior. Wearable 3-dimensional accelerometers held great promise for behavioral coding given the hypothesis that specific behaviors would generate specific identifiable force patterns. Our focus on swine breeding females provided a variety of challenges. Home-made devices were inexpensive to implement, but lacked sufficient housing, attachment capacity and battery life to be a robust solution in the barn. Several commercial accelerometers also were available, but most were developed for cattle and restricted access to the raw data – a necessary step for validation and application to a new species. Attachment of the sensor on the leg gave us great information about locomotion whereas on the body of the sow was ideal for posture. Both locations though yielded unwanted force generation as the sensor provided enrichment for other curious animals to manipulate. We found locating the sensor in the ear resulted in a secure and protected attachment site. However, the ear tag sensors were not without challenges as the ear is only loosely mechanically coupled to the body of the animal and forces generated there may not always represent whole animal behavior. They also were difficult for battery replacement and utilized a single, center attachment in the ear that allowed rotational freedom of the sensor complicating data analysis. Another challenge was that once we had data, we lacked a programming environment to reliably align the force traces with the video as the two data streams have almost a 1000X difference in time bases. Finding a commercial partner (AgriGates Inc.) with similar goals proved to be an important step and has greatly accelerated progress. Improvements have been achieved in sensors functionality and attachment as well as data analysis. The current goal is to use simultaneously coded behavior and sensor data to train machine learning models to identify behaviors based on sensor data alone. These advances promise to reduce coding time and expedite progress in the study of farm animal behavior.

Session 74

Theatre 2

Computer vision based monitoring of fattening pigs: how do pigs move?

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Until now, monitoring of fattening pigs has been focused primarily on production parameters such as feed and water intake and weight. However, camera monitoring using computer vision based approaches can offer new opportunities to continuously monitor both production parameters and animal behaviour. Specifically, monitoring the animal behaviour can enable insight into animal welfare issues such as tail biting, improve the knowledge of fattening pigs social structure in a pen or can even assist in modelling pig growth. The goal of this research is to explore the application of computer vision for behaviour monitoring of pigs. Specifically we investigate the most suitable “features” of movement that can be derived from the video data to assess the movement of fattening pigs. In this study, 72 pigs (1 compartment, 6 pens) were continuously monitored for 11 days (5 days before and 6 days after a mixing event) by an angled top view camera (1 camera per pen). The resulting videos were analyzed using modular computer vision deep learning models for pig detection (M3det), posture estimation, segmentation and keypoint detection (M3KPS), pig tracking (M3track), and pig identification based on large visual ear tags (M3id). Individual tracking data resulting from the computer vision analysis was filtered to remove outliers and reduce the dataset size by ignoring subsequent detections without movement. Methods to extract relevant movement features from the tracking data were explored and applied to the entire monitoring period to evaluate the effect of the mixing event

A computer vision approach to monitor animal-animal interactions in group-housed pigs

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Pig behavioral understanding is a fundamental research field in Computational Ethology, Precision Livestock Farming, and Digital Phenotyping. A key focus lies in leveraging computer vision to quantify both individual and interactive behaviors, enhancing genetic selection in breeding programs, improving animal health and welfare assessment. Understanding animal-object, animal-animal interactions in densely housed environments remains a complex and unresolved challenge. It seeks to address three essential questions in untrimmed, long-duration image sequences: WHAT — identifying types of behavioral events, WHEN — determining their start and end times, and WHO — identifying which animals are involved in each event. In this study, we aim to develop a spatiotemporal behavioral localization and categorization method to analyze multi-categorical animal-object, animal-animal interactions in long-untrimmed video. The proposed method adopts a two-stage pipeline: interaction proposal generation followed by interaction classification. An initial experiment demonstrates that the proposed method can accurately and efficiently locate and categorize five interactive behavior categories: fighting, tail biting, replacement at feeder, feeding and drinking.

Advancing automated detection of social interactions in gestating sows through image analysis

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Monitoring social interactions in group-housed gestating sows is essential for assessing welfare, understanding social structures, and detecting disruptions like excessive aggression. Traditional manual observation is resource-intensive, prompting the exploration of automated methods. This study evaluates the feasibility of using automatic image analysis software to detect and classify sows' social interactions based on posture and key point data. Two pens of 19 and 20 sows were video-recorded during two gestations on days 30 and 103, from 00:00 to 02:00. From these recordings, 120 positive interactions, 120 negative interactions, and 120 non-interaction moments were selected. For each moment, two sows from 10 images were manually annotated for posture (standing, sitting, lying), and key points (snout, neck, tail). In interaction moments, the valence (positive vs. negative) was also annotated. The 10 images were categorized into three phases: "before" the interaction (4 images), "during" (1 image at first contact, 1 image at the end of contact), and "after" (4 images). Using key points, variables like relative distances, movement speeds, and individual distances traveled were calculated. A decision tree analysis achieved 88% accuracy in detecting interaction onset, characterized by spatial proximity, key points convergence, and at least one standing sow. Interaction valence was determined with 61 to 80% accuracy, depending on data phase, with negative interactions involving faster movements and fleeing behaviors. Finally, detecting relative orientations (snout-snout, snout-neck, snout-tail) during interactions showed variable accuracy (67 to 74%), with snout-tail orientation being the most reliable. Increasing the number of observations could enhance model performance. Overall, this study demonstrates that automated image analysis can effectively identify and classify social interactions in sows, paving the way for continuous welfare monitoring through key point and posture data analysis.

Multi-block analyses reveal that standing activity on the day of farrowing and newborn aggression are main factors contributing to litter survival and growth in primiparous sows

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We used a crossbreeding design between the Meishan and Large White breeds to cover a maximum of the variability existing in maternal populations. Our objectives were to explore the relationships between groups of functional or behavioural traits and to quantify their importance for litter mortality and growth. The primiparous sows were reared in individual pens with straw on the floor. Their activity was measured automatically by computer vision and we used CLR data of daily time budget on postures and standing activity (eating, drinking, exploring). Sow reaction to humans and piglets was assessed by on-farm notations. We measured 100 maternal traits and grouped them into 11 blocks : X1 Farrowing performance and environment; X2 Body reserves at maternity entry; X3 Udder quality; X4 Reactivity at maternity entry; X5 Reactivity at farrowing; X6 Standing activity before D0; X7 Postural activity at D0; X8 Postural activity after D0; X9 Standing activity before D0; X10 Standing activity at D0; X11 Standing activity after D0. Predictors of the proportion of dead piglets and litter weight gain calculated for three periods after birth (D0-D1, D1-D3 and D3-D7), were adjusted for the effects of breed and litter size. Multi-block partial least squares analyses were applied. The hundred traits explained 85% of the variation in the mortality block and 79.6% of that in the growth block. X3 and X7 had a significant effect ($P < 0.05$) and together explained ~25% of mortality and ~30% of growth. X10 explained 15.8% of mortality ($P < 0.05$) and X2, X5, X9 had a contribution $\geq 9.8\%$ each. Time spent eating and exploring at D0 influenced mortality and growth significantly. Time spent lying on the side at D0 and newborn aggression also influenced litter mortality. Teat functionality and diameter influenced growth. Sow behaviour plays an important role in early piglet production.

Session 74

Theatre 6

Efficiently recording of sow's activity patterns through embedded computer vision

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The assessment of a sow's maternal behavior relies on manual annotations which are time consuming and are susceptible to an observer's bias. This maternal behavior and particularly the level of activity of a sow is linked to piglet growth and to preweaning mortality. The aim of this study was to provide an overview of the data collected with the MoSBReal device, a user-friendly tool that automatically records changes in sow postures during their stay in the farrowing unit. Thirty devices (60 cameras) were installed in 10 selection farms to collect images from Landrace and Large-White sows. In total, 168,589 images coming from 275 sows were collected over 8 postures: kneeling, standing, sitting, lying on their left or right side with or without showing their udder and ventrally. A classification model, based on YOLO v11, was trained on 154,780 images. The algorithm was tested on 13,809 new images and had an overall precision of 91.2%, from 83% to 98% depending on the posture. Using this model, the activity of 13 sows was recorded between the third day before farrowing till the tenth day following it. The activity profile of those sows was studied. The number of daily posture changes was peaking for nine sows on the day of farrowing and the day before for the other four. When looking at the time spent in each posture, we found that on the day of farrowing sows spent 53.8% of the time with the udder showing. After farrowing, sows spent significantly more time in those postures (81.45% on the next day). Through this sample some diversity of activity profile has been observed. By studying the activity profile of our 275 sows we could find a new way to improve maternal abilities and weaning performances with it.

Effectiveness of a custom-trained model for pose estimation analysis to detect snout-tail interactions under field conditions

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Tail-biting in pigs during the growing period poses challenges for animal welfare and economic sustainability in livestock farms. This study aimed at detecting snout-tail interactions in a large confined environment under field conditions. The dataset included video recordings of 35 post-farrowing pigs housed in a single pen over a 3-week period. A total of 100 frames were randomly selected and annotated to track 7 key points per animal (one on the snout, one on the head, one on each ear, two along the dorsal axis, and one on the tail). Frequent occlusions and clusters of animals in close proximity posed challenges for individual pig identification. Advanced pre-processing and cropping techniques were applied to address these challenges. Three commonly used PyTorch-based neural networks — DlnetStride32Ms5, ResNet101, ResNet50 — implemented in the software DeepLabCut (DLC), were tested for computational efficiency, and accuracy and robustness of key point tracking. To enhance reliability in pose estimation, a confidence score cutoff (p_cutoff) of 0.6 was applied. Key points with confidence scores below this threshold were excluded from the analysis to minimize tracking errors. The excluded key points were primarily associated with occlusions and rapid animal movements, often leading to misdetections or low-confidence predictions. Among the tested models, DlnetStride32Ms5 demonstrated the highest accuracy, training speed and reliability, with more than 90% of key points having a confidence score above 0.6. Trained on 95% of the dataset with 100 epochs, this model achieved excellent results, with a precision (mAP) of $92.87 \pm 7.37\%$, recall (mAR) of 94.08 ± 7.03 , and an average RMSE of 6.67 ± 0.55 pixels on the test set, after applying the p_cutoff . Despite some high RMSE values, the accuracy of head-to-tail interconnection reached 0.89 ± 0.03 , indicating consistent detection performance. These results highlight the potential of pose estimation analysis for advancing the detection of tail-biting events in pigs.

Session 74

Theatre 8

Study of automated identification of dairy cows using computer vision and RFID technologies

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The aim of this study is to develop an automatic identification and tracking system for milking cows in smart barn by integrating computer vision and RFID technology. The primary goal of the research is to train a deep learning model that detects and tracks each animal in the barn through visual data that can be integrated and augmented with the ID provided by the RFID. The trial is conducted at the University of Bologna experimental barn, where Holstein cows wear RFID collars, and three antennas are placed at the front feeding trough to recognize their IDs. The model is trained and tested on a dataset through a collection of one month of continuously recorded video. The proposed methodology involves generating bounding boxes around detected cows using a deep learning object detection model. We perform automatic labelling by matching the coordinates of the bounding box to the positional data from the RFID antennas, using .csv logs. This involves extracting coordinates from the output of the deep learning model and matching them with the spatial coordinates transmitted from the RFID system. Each detection is assigned to a unique animal ID. Using an automated labelling process with large amounts of unlabelled data, it decreases the need for manual annotations and accelerates model training. We assess performance in detecting individual cows and correctly identifying them using precision, recall, F1-score and intersection over union (IoU) as the statistical analyses. The evaluation between visual detections and RFID data is analysed for tracking accuracy measure. Overall, this study provides a new approach to real-time animal identification and behaviour monitoring for future smart barns. The synergy between computer vision and RFID technology provides a window into the behaviour of individual animal, and achieves a higher level of precision livestock farming, welfare management and data-driven decision making in dairy farms.

Automated Monitoring of Dairy Cow Behaviour and Feed Competition Using Computer Vision

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Lying and standing times are key indicators of dairy cow welfare. The stall standing and comfort indices are commonly used as indicators of lying behaviour and overall welfare. Standing and lying times are readily obtained using leg tags, however, deploying tags on every animal can be expensive, and it does not provide location information. Continuous observation by trained observers over extended periods can provide a more robust and representative measure but is labour intensive and not practical in a non-research setting. To address this, we utilise low-cost RGB CCTV cameras, typical of systems already available on farm, to train a YOLOv8 object detection model to identify lying and standing cows, achieving AP-50-95 scores of 0.83 and 0.781 for lying and standing respectively. The observed average daily lying time of 12 hours and 33 minutes $\pm$ 1 hours 28 minutes aligns with existing literature. Competition at the feed face alters eating behaviour and is a risk factor for welfare issues and contributes to rumen dysfunction. Competition for food exacerbates the effects of food sorting resulting in an imbalanced diet for both dominant and subordinate cows. Additionally, restricted access to the feed face may prolong standing times increasing risk of lameness. Using homography, we transform side view images of cattle into a top-down perspective, to measure proximity to the feed face and infer feeding behaviour. Hence the proposed approach enables measurement and analysis of standing, lying, and feeding behaviours. The models were trained and deployed in a high-turnover maternity barn, allowing assessment of impact of population size on feed face utilisation. Using distance-based measures, we quantified crowding at the feed face, which is a proxy indicator of feed competition. This method provides a real-time, non-intrusive, and cost-effective approach to monitoring welfare by detecting changes in eating, lying, and standing behaviour.

Session 74

Theatre 10

Tracking and Behavior Classification of Individual Dairy Cows in a Multi-Camera Setting

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The monitoring of individual dairy cows in loose housing systems is a key goal of precision dairy farming. In recent years, video cameras have been increasingly used to detect and track the location and behavior of cows, with commercial systems now being developed. However, these systems are often very restrictive in terms of camera positions and angle, and multiple cameras represent a further challenge due to animals transitioning between areas. Another challenge is the re-identification of individual cows, which often relies on extensive model training, or identification via RFID at fixed locations. This study aims at establishing a setup for the long-term tracking and behavior classification of individual dairy cows with minimal human input by combining image processing with an indoor real-time location system (RTLS). Eight cameras were installed in a dairy research barn compartment with approx. 60 animals. The deep learning pipeline for image processing consisted of an object detection model (YOLOv8) trained on over 20,000 bounding-box annotated images, a pretrained tracking algorithm (BoT-SORT) to connect detections across video frames, and a classification model trained on 17,000 cropped images of individual cows to distinguish between lying, standing and eating. The detection model performed well with a mAP50 of 0.98. The classification model's overall accuracy was 0.96, but it showed problems when distinguishing between feeding and standing in the alley. By combining these results with the RTLS, which records a cow's x and y coordinates relative to the barn floor once per second, cows could then be assigned the nearest cow-ID based on their boxes' overlap with the cubicles and troughs visible in the image. In a next step, this monitoring pipeline will be used to record the lying and feeding behavior of cows over longer periods of time, and identify connections to diseases like lameness. This work was funded as part of the "DaNuMa" project with NextGenerationEU funds from the European Union by the German Federal Ministry of Education and Research (16DKWN038B).

Stress indicators in cow-bonded calf rearing after a short-term separation of cow and calf on day 8

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In traditional husbandry, calves are separated from their mothers within 24 hours after birth and bottle-fed. Cow-calf bonded rearing allows calves to suckle from their mother or a foster cow for up to 30 weeks, promoting natural bonding. Stronger bonding leads to challenges at later separation. This study examined cow and calf's activity and feed intake after seven days of full-day contact. Data were collected at the dairy farm Veitshof of the Technical University of Munich (TUM) from cows (n = 12) and calves (n = 12) in the first 10 days after birth. Collars were used to track cow's rumination, feeding, and lying times, as well as pedometers for movement patterns in the barn. Body weight, milk intake and salivary cortisol levels were measured in calves before and after separation. The preliminary statistical analysis included descriptive statistics and linear models. Results indicate a decrease of rumination and lying time and increased activity in cows after separation. Calves struggle transitioning from frequent small udder feedings to two larger (≥ 5 L per feeding) nipple-bucket feedings. Salivary cortisol levels increase in calves 48 hours after separation ($p \leq 0.05$). Based on the preliminary results, it can be concluded that the separation is challenging for both, the cow and the calf. However, dam's separation stress seemed to last longer and more intensively, evidenced by more days of noticeable change in their behavior afterward.

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Theatre 12

AI That Sees Pain: A Transparent Approach to Equine Welfare

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Recognizing pain in horses is challenging due to subtle expressions. This study presents SHIC-XE, a 3D deep learning framework for objective equine pain detection. Unlike conventional AI, SHIC-XE enhances transparency by mapping attention to anatomical regions where pain signs are recognized by the AI model, helping veterinarians understand the AI's decision-making and fostering trust in its assessments. SHIC-XE utilizes deep learning to process equine video data, with attention mechanisms highlighting anatomical regions associated with visible pain expressions. It aggregates attention patterns on a 3D horse prototype, visually indicating where the model detects pain signs. The system was tested on two datasets with different pain induction methods to assess performance and generalization. Results showed 95% F1 for the first dataset and 89% F1 for the second. Cross-dataset validation achieved a perfect F1 score, demonstrating robustness. 3D-SSIM analysis confirmed high consistency in attention maps, reinforcing the system's ability to identify biologically relevant pain indicators. By integrating explainable AI into precision livestock farming, SHIC-XE enhances diagnostic accuracy, reduces subjectivity, and improves equine welfare. Its adaptable pipeline enables broader applications in automated pain assessment for other species.

The Road to Enhancing Poultry Behavior Analysis Through Mechanistic Modeling

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Mechanistic modeling utilizes our conceptions of natural processes to better understand the systems modeled. These models describe the elements within a dynamic system to determine influential factors. Such models are scarcely used for animal behaviour but could be implemented to explain spatial distribution. A novel model was built to reproduce data on the distribution of brown- and white-feathered pullets on perches, platforms and ramps within 24 experimental pens. Videos were collected biweekly from 4-18WOA pullets housed in groups of 10. Videos were analyzed using scan samples at 15-minute intervals during the light period and hourly intervals during the dark to determine the mean number of pullets at each timepoint on each location. This model was written as a set of ordinary differential equations describing movement of pullets on and off each of 6 locations within the pens: the floor(F), ramp(R), high perch(HP), low perch(LP), high platform(HL), and low platform(LL). First-order kinetic equations were used to describe the flow in and out of each location. A fitting algorithm was used to adjust k values and minimize the residual sums of squares between the observed and predicted bird numbers at each location. Concordance correlation coefficients(CCC) were calculated to determine the model fit for each location. The CCC values showed the model generated the best fit for nighttime data, specifically for HP(0.91), HL(0.82) and R(0.79) which could be due to the high use of these locations and minimal movement in the dark period. The fitted model parameters were analyzed to compare strains and ages. It was hypothesized that parameters would describe differences in the pullets' inclination to use various roosting structures within the pens during the dark period. We found that, presumably due to the high number of parameters, the model may have been overfitted, and the individual parameters did not carry any meaningful significance. Future iterations of this model should aim to identify key structures and parameters before fitting. Application of mechanistic modeling to describe a physical dynamic system such as the distribution of animals in space could expand our knowledge and offer new perspectives on the underlying motivations behind animal behaviours.

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Poster 14

Data Acquisition And Training Of An Automatic AI System For Scoring Tail Lesions of Pigs at the Abattoir

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Tail biting poses significant challenges to animal health, welfare, and the economic viability of pig farming, particularly in view of the on routine tail docking restriction. Monitoring the prevalence and severity of tail lesions at the slaughterhouse (SH) is a valuable tool for welfare assessment across the pork production chain. The aim of this study was to acquire an adequate amount of data acquisition and preparation as the first step toward training an AI-based system that will be deployed in abattoir for the automated digital assessment of tail lesions. A comprehensive evaluation of 2,666 heavy pigs' carcasses was conducted by 5 independent evaluators at an Italian SH in the slaughter line. Tail lesions were assessed using a standardized scoring system: 0=no lesions, 1=healed scars or superficial lesions, 2=deep injuries, fractures, dislocations, partial or complete loss of tissue. 1,469 tails were photographed from 3 different angles, and then used to score tails lesions using the newly developed Tail Scoring Application by six evaluators, five of whom had previously completed scoring in SH. Cohen's kappa coefficient was used to analyse the inter and intra evaluators agreement at the SH and from the pictures. Overall, Cohen's k coefficients were 0.74 for the assessment at the SH, 0.68 for the picture's assessment and 0.79 for the picture's assessment considering only those evaluators who were also present at the SH. The intra Cohen's k coefficient for assessing the same tail at the SH and from pictures was 0.761. This study suggests that even if tail scoring is a challenging task, the training of the evaluators can improve the consistency agreement. These results can be used as a valuable basis for the further development of the AI model for the detection of tail lesions in pigs at the SH. Research supported by Tailscan Project, EU Pilot Projects & Preparation Actions (PPPA) – ANIMALWELFARE-2022-TAILEDOKING

Smart sips: sensor-based monitoring of daily water consumption to identify heat stress in weaned piglets
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This study investigated heat stress (HS) in weaned piglets (n=72, 24 pigs/pen) by analysing their average daily water consumption (WC) recorded with water flowmeters (Picomag/ Endress+Hauser) in comparison to the mean daily temperature-humidity index (THI) and mean daily animal-specific indicators (ASI). Latter ones comprised the following parameters: lying in the manure area (MA) and without body contact (BC) in the whole pen evaluated by hourly video analysis during a batch (39 days) in summer as well as the respiratory rate of 18 animals per day (RR). Using data from combined temperature and humidity sensors on pen level (DOL 114/dol-sensors A/S), THI was calculated and categorised into group 1 (<75- no HS) and group 2 (>75- warning and danger for HS) (De Prekel et al. 2024; Lucas et al. 2000, NWSCR 1976). In group 1 (n=30 days), WC was 66.5 litres/day with ASI: RR 50 breaths/minute, MA 25% and BC 16.67%. In group 2 (n=9 days), WC was 73.7 litres/day, with ASI: RR 53 breaths/minute, MA 37.50% and BC 20.83%. THI values with warning and danger for HS were reflected well by WC and ASI. In the next steps, a larger data set will be analysed to take a closer look at the potential of the automatic evaluation of weaned piglets' water consumption as an indicator of HS. The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany. The Federal Office for Agriculture and Food (BLE) provides coordinating support for digitalisation in agriculture as funding organisation, grant number 28DE109E18.

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Poster 16

Listen for welfare: detection of welfare issues using a pig-scream detector in weaner pigs

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Early detection of stressful situations in pigs is crucial to maintain animal health and welfare. In a pilot study, a detector software was created to identify scream-linked tail biting events in audio- and video recordings (doi:10.1016/j.atech.2024.100582). In this study, the detector was used to detect total screams per day (SD) in a batch (39 days) of weaner pigs (n=144). Sound was recorded with one microphone (AXIS T8351 MkII) in the middle of a compartment with six pens. Biweekly examination of skin (SL) and tail lesions (TL) was carried out to calculate the sum of lesion-scores per day (SLS/TLS). The proportion of hanging tails (HT) was determined daily and eight tail biters were removed by the farm staff during the daily inspections. SD were compared between day of biter removal and the following day by calculating the mean, minimum and maximum difference. The maximum SLS, TLS and HT were assigned to a specific day in the batch. In total, 1,161 screams were detected, with a maximum of 99 SD on day 34. SLS, TLS and HT were lowest at day of weaning. Maximum values were observed on day 3 for SLS, day 25 for TLS and day 37 for HT. Removing the biters reduced SD (mean -8.7 SD, max -40 SD and min -2 SD) on the next day. The detection of pig screams has the potential to identify welfare compromises at compartment level. Due to overlapping events (e.g. rank battles, tail biting, resource struggle), the relationship between SD and event is not always clearly defined, but the method is suitable for indicating ongoing problems within groups of weaner pigs. The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, grant number 28DE109E18 and 2822ZR014.

Application of drones in health monitoring of beef cattle

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Introduction: Effective health monitoring of beef cattle in extensive pasture systems is essential for early detection of health issues, such as infections, fever, and heat stress, enabling timely intervention and reducing economic losses. Current monitoring relies on subcutaneous RFID chips that record body temperature and transmit data to a central unit when within range. However, the limited range of stationary systems, particularly in large pastures with varied terrain, restricts real-time data collection. This study explores integrating drones with existing diagnostic infrastructure to enhance monitoring efficiency and response time. **Materials and methods:** Initial tests assessed a system comprising: Subcutaneous RFID chips in the head and neck, recording temperature changes, Collar transponders activating chips and transmitting data to a central unit, Intermediary stations collecting high-frequency data. The next phase will integrate drones equipped with receivers capable of collecting transponder data mid-flight, reducing limitations related to stationary system range. Drones will also be equipped with thermal imaging cameras to enhance health monitoring by detecting early-stage health disorders, inflammation, and heat stress. Additional studies will analyze the impact of drone presence on cattle behavior. **Results and discussion:** Preliminary tests indicate that the current stationary system enables body temperature monitoring 1-2 times daily, with frequency increases triggered by elevated temperatures or manual intervention. However, large pastures and varied terrain reduce data collection efficiency due to GSM connectivity limitations. Severe heat stress conditions, as indicated by THI above 80, were associated with increased body temperatures in affected individuals. Drones offer a solution to these limitations, allowing for near real-time data collection across large areas. Thermal imaging cameras will further refine health assessments, enabling early detection of disease symptoms. The integration of drones into cattle monitoring systems will enable faster detection of health anomalies and reduce labor costs. The ability to monitor cattle remotely and continuously provides a crucial advantage in mitigating the effects of heat stress and disease outbreaks.

Multistage Ear Tag Detection – An AI-Based Approach for the Automatic Identification of Individual Animals Based on Ear Tags in Pigs

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Accurate collection and assignment of animal-specific data are crucial for livestock health monitoring and the detection of potential issues. This is particularly essential in pig farming, as a decreasing staff-to-animal ratio and an increasing shortage of labor have further intensified the demand for automated animal identification. In this context, utilizing computer vision and ear tag information for identifying individual animals holds significant potential. With this study, we address current limitations of existing ear tag identification methods by employing a sequential model framework in which four independently trained object detection models extract robust animal and ear tag features and generate an individual animal ID proposal. By incorporating commercially available low-cost ear tags, we first assess the performance of each model independently, with all detection models exceeding an mAP:0.95 score of 0.97. In the second evaluation stage, the overall effectiveness for individual animal identification was assessed using new data samples from both a familiar camera perspective and a new camera perspective. As a result, the proposed method achieves a precision value of 0.99 in the known camera environment, demonstrating that most generated ID proposals are accurate. In the unknown environment, our approach achieves a precision of 0.91 and a recall of 0.90, which highlights the generalization capability of the presented method. The findings of this study show the potential of vision-based ear tag identification techniques, which can be extended to other species and further be applied to enhance existing multiple-animal tracking methods. This work is supported by funds of the German Federal Office for Agriculture and Food (BLE) (grant numbers: 28DE109G18 and 2823ZR016).

Use of animal body temperature measurements in monitoring heat stress

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Heat stress significantly affects animal health, welfare, and productivity, leading to reduced feed intake, impaired immunity, and mortality. The Temperature-Humidity Index (THI) is commonly used to assess thermal load. While thermoregulation involves physiological mechanisms like vasodilation and sweating, prolonged exposure to high THI can disrupt these processes, causing hyperthermia. This study analyzed the relationship between THI and body temperature measured via subcutaneous microchips in beef cattle. The study was conducted on 35 Limousine cattle in the Świętokrzyskie region, Poland, in June 2024. Body and skin temperature was measured via subcutaneous microchips. Temperature and humidity was collected via a weather station. Cattle had pasture access with shade and water during the day and were housed in semi-open barns at night. Body temperature was recorded at 4-6 hour intervals, with intensified monitoring every 30 minutes if it exceeded 39°C. THI was calculated based on environmental data. Between June 18 and July 3, THI exceeded 80 on several days, particularly June 19 and June 26-30, with ambient temperatures of 30.1–37.5°C and humidity between 31.1–60.9%. During these periods, 15% of the herd exhibited body temperatures above 39°C (39.1–39.4°C) and elevated skin temperatures (32.5–37.3°C), indicating heat dissipation challenges. On other days, moderate heat stress (THI 72.0–81.9) was observed. Only 6% of the herd exhibited hyperthermia (39.1–39.7°C), with surface temperatures exceeding ambient conditions, suggesting ongoing heat accumulation. These findings highlight the relationship between elevated THI and hyperthermia incidence. Integrating body temperature monitoring with THI enhances heat stress detection and management. Real-time monitoring enables timely interventions such as increased water access and shade provision, minimizing heat stress effects. Continuous THI tracking allows farmers to optimize environmental conditions, improving animal welfare and productivity. Future studies should investigate individual heat tolerance for thermal resilience.

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Theatre 1

When is a livestock system sustainable? Insights from transdisciplinary research

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Livestock systems have conventionally been classified, and analysed, according to types of feed needed and livestock commodities produced. While such classifications have proven very useful from a production point of view, their shortcomings for sustainability research are evident when contrasting the differences between mostly circular and mostly linear livestock systems. When considering them, a broad typology of five systems can be applied: i) extensive grazing, ii) intensive farming reliant on the territory, and iii) backyard systems, for traditional circular systems, and iv) improved pastures, and v) Confined Animal Feed Operations, for modern linear systems. Criteria for evaluating and comparing their sustainability require the use of indicators that, although of general use and acceptance, offer challenges in how they are applied and also interpreted, as with agroecological principles that struggle to capture the sustainability of mobile systems. Adequate scales must also be applied to convey realistic messages, both to describe the situation on the ground and to deliver effective policy orientations – to avoid known challenges such as e.g., interpreting carbon footprints without an ecosystem contextualization. Such scales, often socio-ecological in nature, need specific integrative research approaches that can consider evidence from a variety of disciplines, and that also implicate farmers to capture field realities. The example of pastoralist systems shows how data from the past help understanding current dynamics, but also the importance of not approaching critical thresholds in order to avoid collapses.

The Environmental Assessment Landscape: Toward Comprehensiveness or Fragmentation?

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The supply of environmental assessment information in the global agri-food system has increased rapidly over the past two decades. At the same time, demand from citizens, consumers, policymakers, and certain industry stakeholders has grown at a similar pace. This increase in both supply and demand—spanning different scales (e.g., product, firm, or country), covering both negative and positive aspects (e.g., greenhouse gas emissions, deforestation, or ecosystem services), addressing various governance types (e.g., norms, policies, or markets), and linked to different agri-environmental schemes (e.g., practice-based or result-based)—has resulted in a fragmented and sometimes confusing landscape. Some authors have even described this increase as “fast and furious.” Grass-based ruminant farming systems illustrate this complexity, as they generate both negative environmental impacts (e.g., CO₂-equivalent emissions, high land use occupation and energy consumption) and positive contributions, such as the preservation of semi-natural habitats and the provision of regulating and cultural ecosystem services (e.g., erosion prevention and aesthetic landscapes). Reducing this fragmentation presents significant challenges in terms of concepts, methods, and practical implementation. The first challenge is conceptual: developing frameworks that integrate the diverse disciplinary perspectives needed to assess livestock farming’s varied contributions (e.g., ecology, sociology, economics). The second challenge is methodological: identifying ways to simultaneously account for negative impacts and positive contributions (e.g., CO₂-equivalent emissions and ecosystem services). The third challenge is operational: ensuring that assessment procedures are not hindered by data limitations, high costs, low credibility, or stakeholder resistance (e.g., Earth observation tools may be perceived as intrusive). To advance concepts and methods, we argue that assessment procedures should ultimately support decision-making. Finding a right balance is crucial, between the comprehensiveness needed to capture the full scope of assessment issues, and the conciseness required to ensure clarity and usability for decision-makers.

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Theatre 3

From the classical “three-pillars” vision of sustainability to new frameworks for assessing the effects of farming systems enhancing poultry welfare

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Livestock farming systems (LFS) face numerous environmental, social, and economic challenges, all encompassed by the concept of sustainability. Over the past two decades, the scientific community has developed many methods and tools to assess LFS sustainability (e.g., the OVALI method for poultry). These multicriteria approaches are built on several theoretical and methodological considerations, such as designing the conceptual framework, weighting the criteria, or aggregating the results. In the past decade, societal concerns regarding animal welfare (AW) in the poultry sector have risen sharply. Consequently, evaluating the effects of practices designed to improve AW, on various sustainability criteria (e.g., production costs, labour, environmental footprint) has been a significant challenge for researchers and stakeholders. For instance, in conventional chicken production, the COCORICO method introduced a new conceptual framework that departs from the traditional three-pillars view of sustainability. In this method, animal-friendly practices implemented in chicken farms (e.g., the European Chicken Commitment) are indeed evaluated across four pillars: AW, economy, environment, and farmer. Similarly, scientists involved in the European Partnership Animal Health & Welfare are developing since 2024 a generic framework to assess, in all livestock species, the impacts of practices enhancing AW. This goal, however, raises several challenges. Firstly, experts from various disciplines and different livestock sectors must agree on a shared vision of sustainability. Secondly, methodological choices must be addressed, in particular those regarding the weighting and/or the aggregation of results. These experiences underscore that each multicriteria assessment framework is unique and results from a lengthy, complex process involving many methodological considerations and choices. This work was co-funded by the European Union’s Horizon Europe Project 101136346 EUPAHW.

DairyMix: multicriteria assessment for circular mixed dairy farming systems

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Dairy production is a key backbone of European agriculture but heavily relies on external inputs, lacking resilience and circularity. When dairy production is recoupled with grasslands and diversified crops, it relies more on on-farm produced feed and provides valuable ecosystem services. The aim of DairyMix (www.dairymix.eu) is to define region-specific concepts for sustainable and circular crop-forestry-livestock systems for dairy production. To assess the environmental performance of a wide range of dairy farms in Argentina, Brazil, France, Germany, Ireland, Italy, Norway and Poland, Life Cycle Assessment was carried out, to be integrated in a multicriteria assessment with economic and social sustainability indicators. Crop and whole-farm models were linked to assess circularity indicators. Agroforestry practices were evaluated, and in-depth interviews were carried out to assess the socioeconomic factors determining farmers' environmental choices. The project results are incorporated into the DairyMix online platform. In contrast to "one-fit-all" solutions, DairyMix presents a range of options for the sustainability of farming systems for dairy production, favouring the adoption of informed decisions on environmental, economic and social sustainability for circular and integrated crop-forestry-livestock in different European and Latin-American regions.

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Theatre 5

Exploring the drivers of productivity in organic multi-species livestock farms across Europe

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Farm diversification has been proposed as a way to increase agricultural productivity while reducing environmental impacts. However, the case of livestock diversification has remained under-explored, in particular the levels and drivers of agricultural productivity in organic multi-species livestock farms. Here we addressed this lack of information by assessing the livestock, land and labour productivity of 96 organic multi-species livestock farms across Europe. We aggregated each farm's production of commercialised crop and livestock products into an agricultural production unit (kg protein) and an economic unit (income in €). We then calculated the agricultural productivity of each farm per unit of livestock (LU), land (ha) and labour (annual working unit; AWU). We found that organic multi-species livestock farms across Europe had an average agricultural productivity of 128.7 kg protein/LU, 139.2 kg protein/ha and 4255.1 kg protein/AWU, and economic productivity of 2756€/LU, 2411€/ha, and 62907€/AWU. We also identified the drivers of each productivity indicator. We used Elastic Net, a penalised regression, to relate farm productivity indicators to farm-related explanatory variables. The model had an $R^2 = 0.61$ with an average mean squared error of 0.02. The main explanatory variables varied to some extent for each productivity indicator and functional unit, indicating the existence of synergies (variables uniformly related -either positively or negatively- to several indicators) and trade-offs (variables positively related to some indicators and negatively related to others). Feed self-sufficiency was an example of synergy, being negatively related to all indicators and functional units. Conversely, stocking density led to a trade-off, being positively related to agricultural and economic productivity per unit area, but negatively related to agricultural productivity per LU. Euros per kg of protein, reflecting the marketing strategy of the farm, was negatively related to agricultural productivity but positively related to economic productivity.

Assessing the sustainability and ecosystem services of farming practices of dairy cattle farms

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As part of the Italian Living Lab activities within the HE Project RE-LIVESTOCK (GA N° 101059609), this study examined the sustainability of agricultural practices adopted by selected dairy farms. The multicriteria analysis focused on key environmental, economic, and social aspects to identify potential trade-offs between sustainability dimensions. To achieve this, the RE-LIVESTOCK Tool, an online software based on the existing PG-Tool, was employed. Through a multicriteria survey, this flexible tool provided a comprehensive assessment of farm and land management practices, offering valuable insights for farmers, technicians, researchers, and land managers. The tool generated a sustainability score ranging from 1 to 5, providing a structured evaluation of different farming dimensions using 13 indicators covering areas such as soil and water management, animal welfare, landscape features, and social capital. The study assessed ten semi-intensive dairy farms located in the Tuscany region, Italy. Farm sizes ranged from 40 to 1600 hectares, with livestock units per farm varying from 24 to 500. The most common breed was the Italian Holstein. The results showed an average sustainability score of 2.84, ranging from 2.46 to 3.41 across all farms. Strengths were observed in soil management and animal welfare (with average scores of 3.3 and 3.85, respectively), while weaknesses emerged in nutrient balance (NPK budget, average score 1.90) and cropping system diversity (average score 2.35). Multivariate statistical analyses indicated clear distinctions in farm practices, particularly in soil management and economic performance, while also emphasizing the need for improved collaboration and social capital among farmers. In conclusion, while notable sustainability practices were observed, particularly in animal health and farming business resilience, significant improvements are needed in nutrient management and social collaboration to further enhance the overall sustainability. The RE-LIVESTOCK Tool has proven to be a valuable resource for identifying key areas and offering a pathway for farms to adopt more sustainable practices.

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Theatre 7

Semi-extensive dairy sheep farming and environmental impact: the key role of fresh forage and purchased feed in affecting environmental performance

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The present study investigated the environmental impact of dairy sheep farming, using LCA and multivariate statistical analysis to identify the key management practices affecting environmental performance in Mediterranean mixed crop-livestock systems. The average global warming potential (GWP) was 4.2 kg CO₂-eq kg FPCM⁻¹, with enteric fermentation accounting for 53.5% of total emissions, followed by fuels and electricity consumption (21.5%) and purchased feed (10.1%). The correlation analysis revealed a significant positive association between the use of purchased concentrates and key environmental impacts, including GWP ($r = 0.38$, $p < 0.05$), acidification ($r = 0.58$, $p < 0.05$), and eutrophication ($r = 0.50$, $p < 0.05$). Conversely, grazing time during lactation was negatively correlated with the use of purchased feed ($r = -0.66$, $p < 0.05$), indicating that increasing the share of fresh forage can reduce environmental impacts. The PCA identified two main components explaining 52.5% of the total variance. The first component (32% of variance) was strongly influenced by electricity and fuel consumption per hectare, which were positively associated with greenhouse gas emissions and resource depletion. The second component (20.5% of variance) highlighted the trade-off between high milk yields per ewe and environmental performances. Cluster analysis grouped farms into three distinct categories based on their environmental profiles. The first cluster was characterized by high environmental impacts and heavy reliance on external inputs. The second cluster exhibited moderate environmental efficiency with a balanced use of self-produced and purchased feed. The third cluster was associated with the lowest impacts, achieving high forage self-sufficiency and optimal grazing management. These findings highlight the role of feeding and farm management in mitigating environmental impacts. In Mediterranean semi-extensive systems increasing on-farm feed production, optimizing grazing systems, and improving forage quality can significantly reduce GHGs emissions per kg of FPCM.

Towards developing a shared methodology for the joint assessment of animal welfare and sustainability in livestock

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Livestock farming systems (LFS) play an important role in future food security, but they must tackle many sustainability challenges, including the growing societal concern for animal welfare (AW). Historically, sustainability assessment methods (SAM) focused on the three “pillars” of sustainability, i.e., economy, environment, and social, with AW encompassed in the social pillar. Consequently, such approaches rarely incorporated a comprehensive assessment of AW. However, there is a need to better understand the trade-offs between improving AW and other sustainability issues. This requires consideration of AW as an independent dimension on par with the other three sustainability pillars. In the framework of the European Partnership for Animal Health and Welfare (EUPAHW), we aimed to gather information about SAM applied to LFS. We developed two surveys and disseminated them to experts in the fields of sustainability, AW, multi-criteria assessment, and any combination thereof. The first survey aimed to identify and gather basic information about existing SAM. The second explored these in more detail, considering how metrics are summarised and aggregated in each method. To date, we have data on 23 different methods from eight countries and three multi-country consortia that consider at least one of the four previously mentioned pillars, including AW. However, only four (17%) of these consider all four pillars with equal focus. Out of the 12 livestock sectors for which we have data, the most-reported were broiler chickens (14 methods). Across all sectors, 16, 13, and 14 of the assessments could be applied to conventional, free-range, and organic production systems, respectively. Our presentation will focus on the conclusions drawn from these surveys, including the degree to which existing sustainability assessments encompass AW. This work is co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

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Sustainability of grassland-based dairy farms: a case study

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Grassland-based livestock farming is linked to several positive externalities, such as landscape maintenance, carbon sequestration, local biodiversity, and cultural heritage. However, local economic and climatic constraints can lead to land and low-input farms abandonment. This contribution aims at assessing the sustainability of low-input grassland-based farming, thereby supporting its environmental and economic viability. Farm structure and management data were collected from 41 grassland-based dairy farms in the province of Salzburg (Austria). A Principal Component Analysis identified 10 indicators: altitude, organic, farmland area, field size, cuts on permanent grasslands (PG), husbandry system, herd size, milk yield (MY), outdoor access, share of PG. K-means clustering was then performed, obtaining 3 clusters. Cluster 1 represented conventional (85%), medium sized (16 ha) farms at mid altitude (507 m asl), with small PG fields (1.35 ha), medium sized herds (18 cows), tie stalls, zero concentrate self-sufficiency (CSS), and both the lowest outdoor access (61 days/year – d/y) and milk price (0.42€/l). Cluster 2 described higher altitude (741 m asl), organic, small farms (11 ha) and fields (1.4 ha), with low arable land and temporary grasslands (TG) (15%), small herds (10 cows), medium loose housing (43%) and outdoor access (155 d/y), low CSS (15%), and higher milk price (0.48€/l). Cluster 3 referred to mid altitude (544 m asl), organic (90%), largest farms (35 ha) and fields (1.84 ha), with low arable land and TG (10%), largest herds (31 cows), loose housing (100%), the highest outdoor access (301 d/y), CSS (22%), and milk price (0.5€/l). Clusters were similar for the other factors considered. In conclusion, Cluster 3 showed the highest future viability, with loose housing, the highest milk price and feed self-sufficiency, maintaining a strong link with the territory. Cluster 2 achieved good milk price and feed self-sufficiency, with higher tie stalls, and the smallest herd size, due to the higher altitude. In a long-term scenery, Cluster 1 has the lowest viability, potentially improvable through investments to enhance its durability.

Carbon footprint is weakly linked with ecosystem services of dairy farms

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Livestock farming, especially ruminants, are frequently perceived as a major cause of global warming. Aiming at mitigation, legislative requirements and marketing activities refer to product-based sustainability assessments such as carbon footprints (CF). Apart from that, it is widely recognised that multiple other functions and services of agricultural land use are equally important. Thus, it is essential to uncover and address potential trade-offs and synergies between these different areas of sustainability. Therefore, we assessed CF as well as ecosystem services (ESS) provided by agricultural land use of 89 predominantly grassland based dairy farms in the regions of Upper Austria and Salzburg. The ESS selected for assessment included net food production, drinking water potential, erosion prevention, plant biodiversity and recreation potential. Data were collected by an online survey, taken from the Integrated Administration and Control System, freely available GIS data, remote sensing, and literature. Results show a great variability between farms for the assessed ESS. Overall, associations between CF per kg energy corrected milk (ECM) and ESS indicators are rather low ($R^2 < 0.14$). Positive correlations of product-related CF with plant species richness and recreation potential indicate potential trade-offs. Contrarily, net food production, drinking water potential and erosion prevention are not related to the CF per kg ECM. The high variability suggests necessary further evaluation to identify driving factors. A preliminary analysis reveals livestock density, external nitrogen input, proportion of low intensity grasslands and higher elevations being farm characteristics important for ESS. These findings suggest that CF alone is not an appropriate metric to assess multifunctional dairy systems and highlights the necessity of diverse indicators for sustainability assessment. Acknowledgements: The authors would like to thank SalzburgMilch GmbH for the cooperation and partial funding of the research. Keywords: dairy, carbon footprint, ecosystem services, multifunctional agriculture, sustainability trade-offs

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Assessing the vulnerability of French livestock production to climate and socio-economic change

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Climate change poses challenges to French livestock sector, but existing knowledge about its impacts and adaptation strategies vary according to livestock farming systems and regions. The aim of this study is to analyse how climatic and socio-economic changes affect French livestock farming and how these effects vary according to geographical contexts, animal species (ruminant and monogastric) and systems (e.g., pasture-based or landless). To answer this, the study relies on grey literature, including project reports, and expert opinions, which provide insights from those most familiar with regional conditions. A dynamic tracking method was used to cover diverse farming systems and all metropolitan French regions. In addition, 15 semi-structured interviews were conducted with experts, such as veterinarians, agricultural advisors, project managers and researchers, selected for their contributions to relevant projects and expertise in livestock farming and climate change adaptation. The data were organized in a grid of analysis that categorises impacts based on their typology, the climate-related stressors causing them and the sectors affected (e.g., animal feed, livestock performance, and water and soil resources), to compare results across regions and livestock systems. Preliminary results show that heat stress is a common problem in all regions: 77% of the surveyed sources indicated negative impacts on animal welfare and productivity. However, other issues, such as emerging diseases, appear more region- and sector-specific (5% of sources). A literature gap was identified regarding the impact of climate change on monogastric livestock production in the French regions, as most studies focus on ruminant systems. This study offers a comprehensive overview of the challenges climate change poses to the French livestock sector and shows how these challenges vary by region. It provides a territorial perspective focusing on animal species, farming systems and stages of the production chain. It provides a base for future discussions between value chain actors on the impact of climate change on livestock farming.

Integrating Animal Welfare into Sustainability Assessments: Insights from the European Partnership on Animal Health and Welfare (EUPAHW) Network

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Animal welfare (AW) is essential for a sustainable farming system, and it should align with the economic, environmental, and social pillars of sustainability. However, many current sustainable systems fail to incorporate AW, with AW-friendly practices seldom integrated into livestock sustainability strategies. To address this gap, a 3-year project as part of EUPAHW (2024-30) and launched in 2024 aims to integrate AW into existing sustainability assessment tools, identifying synergies and trade-offs between AW and other sustainability components. This initiative brings together experts in AW, economics, sociology, and ecology to develop a science-based approach, evaluating best practices, and selecting priority case studies. The project includes both terrestrial and aquatic species and encompasses, when possible, all aspects of livestock systems, from farm management to transport and end-of-life conditions. In addition to scientific inquiry, the project places a strong emphasis on dissemination, ensuring that research findings translate into practical, evidence-based insights for a wide range of stakeholders, including policymakers, farmers, NGOs, veterinarians, universities, and training centres. This strategy will be closely integrated with the dissemination activities of EUPAHW (Work Package 5 -Communication, Exploitation, Education and Dissemination), fostering a dynamic and effective exchange of knowledge. This work is co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

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Poster 13

Split-feeding as a sustainable feeding strategy for improving egg production and quality, nutrient digestibility, and environmental impact in laying hens

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Nutritional management is a critical aspect of commercial egg production, as laying hens require precise nutrient intake to maintain high productivity and egg quality. Consequently, split feeding, which involves adjusting nutrient composition and limestone particle size at different times of the day, offers a more holistic solution to better match the bird's nutrient requirements during the egg formation process. Recent studies suggest that split feeding strategies not only improve productivity and egg quality but also hold promises for addressing the growing environmental concerns associated with poultry farming. Therefore, we aimed to explore the effects of split feeding on production performance, egg quality, nutrient digestibility, odor emissions, and economic benefits in laying hens. A total of 468 Hy-Line Brown hens, aged 63 weeks, were divided into three dietary treatments over 13 weeks. The control group received a single basal diet, while two split-feeding groups (TRT1 and TRT2) received diets differing in nutrient composition between the morning and afternoon. TRT1 maintained the same levels of metabolizable energy, standardized ileal digestibility amino acids, calcium, and phosphorus as CON, while TRT2 had 5% lower metabolizable energy, standardized ileal digestibility amino acids, and calcium, and 15% lower phosphate. Data were analyzed using the GLM procedure of SAS, and the significance of differences between means was tested by Duncan's multiple range test. Results showed that TRT1 reduced the incidence of downgraded eggs by 19.3% in the later phase of the experiment (9–12 weeks) compared to CON. Both TRT1 and TRT2 showed reduced diet costs by 6% and 15%, respectively. Additionally, split feeding improved nutrient digestibility, reduced harmful gas emissions, and resulted in a 4.5% feed cost savings per kilogram of salable egg production compared to CON. These findings suggest that split feeding could enhance egg quality, reduce environmental impact, and contribute to the sustainability of poultry farming.

Life Cycle Assessment of equine production in marginal areas

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Italy's inner areas, culturally and ecologically valuable regions, are threatened by land abandonment and environmental degradation that negatively affect agricultural viability and animal and vegetal diversity. As a part of the SUNEQUMAR project on the innovations contributing to the resilience of extensive livestock farming in Centre South Italy (Molise Apennine), besides the geolocalization of grazing equines for a better evaluation of pasture quality, a cradle-to-gate Life Cycle Assessment (LCA) is carried out following ISO 14040/14044 standards. The LCA approach quantifies the environmental impact, climate change, resources use, eutrophication, land and water use, by the means of a robust Life Cycle Inventory database from primary and secondary data. Primary data on inputs and outputs of crop and livestock systems were collected on site (in the field) and secondary data were obtained from literature and databases (mainly ECOINVENT®). The assessment was carried out with 1 kg of live weight as the functional unit. The project multidisciplinary frame investigated in inner areas of Centre South Italy is intended to be used in the development of predictive models addressed to fragile ecosystems for improved sustainable land use and livestock management. Best practices and future-proof management will be generated with respect to environmental issues for a sustainable resilience of agro-alimentary sector in inner marginal areas. The research is supported by PRIN project 20224L4WSR Sustainable Innovations in Equine Production of Marginal Areas (SUNEQUMAR), funded by Next Generation EU. (CUP: H53D23005120).

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Poster 15

Carbon Footprint of dairy small ruminant and cattle production systems in Greece

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This study evaluates the carbon footprint of dairy small ruminant and cattle farms in Greece, within the framework of the Climate Farm Demo (CFD, Grant ID: 101060212). CFD is a pan-European initiative that brings together pilot demo farms across 28 countries, representing diverse pedo-climatic zones. Its primary goal is to accelerate the adoption of Climate-Smart Farming practices and solutions by EU farmers, with the aim of adapting agricultural production systems to climate change. For this purpose, the study involved 9 intensive dairy farms, 2 semi-extensive and intensive dairy goat farms, and 8 dairy sheep farms ranging from semi-extensive to fully intensive systems, which were recruited as demo farms. In-person interviews were conducted on each farm, collecting data on herd size, production, management, breeding, waste management, nutrition, crop cultivation, and energy and water management. Each interview lasted approximately 2 hours, and diet samples were also collected to ensure accurate diet formulation. After data curation, the carbon footprint per farm, per animal, and per kg of milk was calculated using the Agrecalc Cloud tool (Edinburgh, UK), a state-of-the-art cloud-based farm carbon calculator that employs higher IPCC Tier methods to model carbon footprints. The results indicated that both cattle and small ruminant demo farms were within the EU carbon footprint range in terms of CO₂ equivalents per kg of milk. However, robust data on the Greek average is limited for comparison. Interestingly, the carbon footprint per kg of milk was lower for high-yielding cows, as was the carbon footprint per cow, suggesting that intensification of production systems can improve both performance and sustainability. Simulations of different scenarios across the demo farms revealed that key factors influencing the carbon footprint included precision feeding, manure management, and fertilizer use, particularly in the case of home-produced feedstuffs.

Development of consequential life cycle assessment methods for evaluation of European livestock systems
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Life cycle assessment (LCA) is a well-known methodology to estimate the potential environmental impacts of products or services throughout their life cycle. Attributional LCA (ALCA) is a popular approach for this purpose, which aims to determine the environmental loads attributed to the processes directly used or physically connected with the product used; however, ALCA does not fully account for the broader, system-wide consequences of implementing a specific strategy or change in demand beyond the immediate boundaries of the production system. Consequential LCA (CLCA) is used to overcome this disadvantage. The UNEP-SETAC Life Cycle Initiative, supported by the United Nations, establishes that “the consequential approach attempts to provide information on the environmental burdens that occur, directly or indirectly, as a consequence of a decision”. To date, little information is available regarding the advances of CLCA methods, within evaluations of current livestock systems, and a lack of consistency regarding the application of CLCA hampers evidence-based debates on the future of livestock in Europe. To better inform future policy making, improved evidence-based knowledge is needed for evaluating the consequences of livestock systems. The objective of this review was therefore to develop environmental CLCA methods for farm-to-fork evaluations of European livestock systems. The literature search and extraction of information was carried out in a systematic and thorough manner following the PRISMA framework. The included CLCA methods were evaluated by indicator weightings, impact allocation, substitution effects, and functional units. Results were assessed from twenty-one articles, organised by their methodological approach, i.e. (1) System expansion and substitution, (2) Exploratory Scenario modelling and (3) Economic model-based. The applied methods were discussed, and suggestions were given towards appropriate approaches for the assessment of European livestock systems.

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A conceptual framework of integrating animal welfare into life cycle assessment of livestock systems
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Life Cycle Assessment (LCA) approaches are widely used to assess the impacts of production, identify hot spots, and analyse the consequences of change for a product or system. The use of LCA has broadened over time, from an initial focus on quantifying environmental impacts to the exploration of social impacts via Social LCA. Social LCA includes the analysis of economic costs through Life Cycle Cost Assessment, and the development of integrated Life Cycle Sustainability Assessment. To quantify the sustainability for livestock systems, LCA provides an integrated approach, which encompasses environmental, economic, and social dimensions, and recognise animal welfare as a key social component. However, the integration of animal welfare within LCA remains underdeveloped, with no consensus on suitable indicators for assessment. Advancements in animal behaviour and welfare science, particularly the shift towards positive animal welfare, offer opportunities for refining LCA methodologies. Incorporating indicators that capture welfare at both individual and group level could enhance the comprehensiveness of sustainability assessments for livestock systems. In an on-going project, we aim to suggest a conceptual framework for incorporating animal welfare into LCA of livestock systems. The study combines a systematic literature search and a delphi-styled workshop. Expected results include: (1) Identifying the current approach of assessing animal welfare within LCA for livestock systems, (2) Elucidating the knowledge gaps in the current approach of assessing animal welfare in LCA, (3) Suggesting a conceptual framework for better integration of animal welfare into LCA based on (1) and (2) and workshop outputs, (4) Presenting examples of animal welfare indicators that are suitable for on-farm application and evaluating the criteria for their inclusion into LCA.

Environmental impact of fattening beef production on different flooring systems in Veneto region

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Italy is one of the largest beef producers in the European Union, with production concentrated in the northern regions, particularly around the Po Valley. The Veneto region, in the eastern part of Po Valley, has the highest beef stock density in Italy and is a hotspot for air pollution due to emissions from industry and agriculture, as well as the region's unique topography. Beef production in Veneto is intensive, primarily focused on fattening specialized breeds imported from France. Weanlings are raised in cow-calf systems on pasture before being transported to Veneto, where they are typically fed ad libitum on maize silage-based diets. This study evaluates the environmental impact of two housing systems for the most common breeds in this system: Limousin bulls and heifers fattened on slatted floors and Charolais bulls and heifers housed on deep bedding. Emissions were analysed at both farm and regional levels, considering the annual import of animals. Since the system boundary focuses on Italian production, the functional unit was 1 kg of body weight gain. Preliminary results indicate that Global Warming Potential (GWP100) and Eutrophication Potential (EP) were higher in deep bedding systems, while Acidification Potential (AP) was higher in slatted floor systems. Differences in EP and AP were related to variations in fertilization in crop production between farms. As this study only considered the Italian production phase, emissions from manure management were the dominant contributor to GWP100 in deep bedding systems, with this effect more noticeable at the regional level. Management practices significantly influenced GWP100 results. While our findings highlight the environmental impact of housing systems, on the other side our previous research suggests that deep bedding offers significant animal welfare benefits. Future environmental policies should take a comprehensive approach, balancing emission reduction strategies with livestock welfare considerations, mainly where trade-offs exist between sustainability and animal welfare.

Challenges and best practices towards higher welfare standards in beef cattle: results from the European Partnership for Animal Health and Welfare

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A renovation of the EU legislative framework on beef cattle welfare is needed. Major knowledge gaps concern the implementation of best practices (BPs) based on management, resource-related, and animal welfare variables. Through literature search and expert opinion, welfare threats (WTs) for suckler cows and heifers, fattening cattle, breeding bulls, suckler calves, and dairy-beef calves were collected. End-of-career cows were also included, but no data was found on BP for the on-farm management. WTs were classified into the 5 welfare domains and according to the production systems. For each WT, BPs were recognised as possible solutions/preventive measures. Domains that grouped a higher number of WTs were "Environment" for dairy-beef calves (20/29) and fattening cattle (25/33) and "Behaviour" for dairy-beef calves (21/29). BPs primarily addressed domains of "Behaviour" (57/163), "Environment" (54/163) and "Health" (40/163) and some of them softened more than one or two WTs. Few BPs, such as "controlled temperature and humidity" addressed at least three WTs: "cold stress" in dairy-beef calves and "heat stress", "locomotory disorders (including lameness)", "prolonged hunger", and "prolonged thirst" for both dairy-beef calves and fattening cattle. Our results show that managing welfare risks in beef cattle farms is complex but allow to identify the BPs where focusing efforts would be most effective. This work was co-funded by the EU Horizon Europe Project 101136346 EUPAHW.

Territorial indicators of circularity and ecosystem carrying capacity for redefining livestock's role in the food system

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Livestock systems play an essential role in the food system but also face growing scrutiny regarding their environmental footprint. Traditional environmental impact assessments emphasize eco-efficiency—minimizing resource use and emissions per unit of animal-sourced food. This approach overlooks the systemic role of livestock in the food system, including their contributions to nutrient cycling, soil fertility, and the upcycling of biomass inedible to humans. To ensure livestock production operates within planetary boundaries, it is essential to shift from a narrow focus on efficiency to a broader perspective that integrates spatial circularity and ecosystem carrying capacity. Geo-SOL, a territorial mass-flow food system model is presented. It integrates detailed, context-specific livestock husbandry systems modelling and uses a spatially explicit module to derive novel indicators of spatial circularity and ecosystem carrying capacity. By modelling livestock's impact spatially, Geo-SOL links livestock production to ecological processes that define local carrying capacities and ecosystem services. This approach allows for a more comprehensive assessment of how livestock interacts with regional resource availability, nutrient flows, and land use constraints. Preliminary results from modelling of selected storylines of the EU-funded project PATHWAYS will be presented. They explore how different adjustments in animal husbandry systems, aligned with different PATHWAYS livestock sustainability narratives, impact these territorial indicators. The analysis will focus on the implications of these indicators in comparison with other sustainability metrics, highlighting their potential to provide a more systemic perspective on livestock's role within food systems.

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Theatre 2

Behind the Scores: Evaluating Sustainability Constructs and Farm Structural Factors in European Livestock Systems

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Sustainability assessments rely on composite scores to evaluate farm performance across environmental, economic, and social dimensions, but their reliability is often overlooked. This study examines the Public Goods Tool (PGT) results across 106 European livestock farms, assessing its reliability and the role of key structural farm variables in driving sustainability. Given Europe's diverse livestock systems, ensuring indicator robustness is essential for effective policy evaluations. Using Cronbach's alpha (α), we analyse the internal consistency of the PGT's sustainability constructs. Results reveal high reliability for constructs like Profitability ($\alpha = 0.94$) and NPK Budget ($\alpha = 0.87$). In contrast, others, such as Animal Welfare ($\alpha = 0.33$), Energy & Carbon ($\alpha = 0.44$), and Landscape & Heritage ($\alpha = 0.42$), lack consistency, indicating a need for refinement. Logistic regression shows that structural farm variables significantly impact sustainability performance. For instance, growing more diverse crops enhances system security, while higher livestock densities negatively affect environmental dimensions. Our findings highlight the importance of reliability testing in sustainability assessments and the impact of structural farm characteristics on evaluation frameworks. Refining low-reliability constructs and integrating key farm variables could enhance the PGT's applicability for sustainable livestock farming in Europe.

Multiscale scoring method for socioeconomic sustainability assessment of value chains

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Several frameworks and tools have been developed to measure societal and business progress toward sustainability. This work builds on the concept of planetary boundaries, recognizing the need for changes in the livestock sector to remain within the "safe operating space for humanity." The livestock sector could transition following different pathways such as agroecology and organic farming, however it is necessary to extend the performance evaluation to socio-economic aspects and to the whole livestock value chains (VC), including the processing and distribution phases. The final goal of this study is to co-design a multiscale method to evaluate the socio-economic sustainability of post-farm gate livestock value chains. The three dimensions that we will observe are techno-economic, socio-cultural and governance sustainability. The themes characterizing these dimensions in livestock VC can be numerous. Therefore, European sectorial experts were asked to choose the most relevant to be assessed. The identification and prioritization of sustainability themes were achieved using an online two-round Delphi Study. The final assessment method considers 22 themes, and associated to each theme, one or two indicators were selected after a review of available frameworks, primarily Sustainability Assessment of Food and Agriculture systems (SAFA). In the second round, an ordinal ranking of the themes was obtained with a best-worst scaling experiment and then converted into a system of numerical weights to be used in the assessment. The refinement and validation of the assessment method will be carried out through an empirical application to animal product VC, collecting primary data by interviewing VC actors. Researchers and policymakers could benefit by comparing phase by phase livestock VCs bringing similar products to market and identifying areas for improvement in socioeconomic sustainability. This research has been developed within the PATHWAYS project, funded by the European Union's Horizon 2020 Research and Innovation Programme.

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Theatre 4

Relationship between livestock intensification and feed-food competition in diverse European broiler and cattle farms

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Livestock production accounts for a significant share of global feed and land, raising concerns about resource competition with humans. Monogastrics are considered more feed efficient and land productive than ruminants. But this may vary with intensification and if we consider what can be used for human nutrition (arable land and human edible protein). This study aimed to assess feed efficiency and land productivity using different metrics in 213 farms—82 broiler, 78 dairy, and 53 beef farms—from very diverse systems in 9 EU countries. Considering key farm inputs, we grouped farms according to their intensification level (6 broiler, 7 dairy, and 5 beef farm groups). We calculated protein efficiency as kg of protein produced per kg of total protein (PE1) or per kg of human-edible protein (PE2) in feed, and land productivity as kg of protein produced per ha of total land (LP1) or per ha of arable land (LP2). As expected, PE1 and LP1 were higher in broiler than in dairy and beef farms (Median: 0.41 vs. 0.22 vs. 0.09 and 410 vs. 248 vs. 86 kg/ha, respectively) and increased with intensification. However, PE2 was higher in dairy than in broiler and beef farms (0.94 vs. 0.43 and 0.39) and LP2 was higher in broiler and dairy than in beef farms (410 and 404 vs. 173 kg/ha). PE2 and LP2 increased with intensification in broiler farms but decreased in dairy farms; the most extensive dairy farms requiring no human edible protein. In conclusion, intensification enhances protein efficiency and land productivity and broilers are more efficient than cattle. But, when considering the use of human-edible feed inputs, dairy systems are more efficient than broilers and their efficiency is higher in low input systems. These results will be used in an overall assessment covering product quality, animal welfare, and environment.

Economic resilience of broiler farms in two different countries

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Modern consumers demand safety and quality in foods of animal origin, often associated with extensive farming systems. This perception exists for all species, including broilers, which in most cases are housed in high-density barns. Under the mEAT quality project (Grant Agreement No. 101000344 – mEAT quality), innovative strategies have been investigated to meet the above-mentioned societal demands, environmental requirements, and economic needs of entrepreneurs. Within this framework, the factors of extensiveness of production are a pivotal issue and the project proposes a step-by-step analysis of them. As a first step, the impact of these factors on the intrinsic quality of meat has been assessed. Data have been collected from conventional, free-range and organic farms and they have been processed through standardized sustainability assessment protocols. Data collected from thirty Polish and thirty German breeders have drawn a general description of the farm, highlighted some key environmental issues – mostly related to animal welfare –, and offered economic and productivity information. The economic protocol provided key information on economic performance and its variability according to the degree of extensiveness of broiler farms. Moreover, a qualitative survey has been combined to the traditional technical questionnaire, in order to detect economic resilience through appropriate parameters. This approach aims to describe the bargaining power of the farmer within the value chain and explores the ability of the farm to withstand and overcome negative events. Responders have been asked to score (from 1 “poor”, to 5 “very high”) a list of more than twenty factors and abilities affecting their entrepreneurial skills, resilience of resources, robustness of training and propensity to innovate. The incidence of different responses has been compared both between countries and between production systems. The overall goal of this analysis is to bring to light a new perspective, now shared by more research on the approach of economic sustainability, which is not only related to costs, but looks at the entire value chain and the role of the farmer.

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Theatre 6

Life Cycle Assessment of European broiler chicken systems

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Broiler chicken production systems in Europe are characterized by a wide range of configurations in terms of input intensity, animal rearing conditions (ARC, indoor vs outdoor), animal traits (AT like growth rate and final liveweight (LW)). This study aimed to assess the environmental impact (Life Cycle Assessment) associated with broiler chicken reared in various European systems. A cradle-to-farm gate model was applied, with 1 kg LW and 1 m² of land as functional units and global warming potential (GWP), freshwater eutrophication potential (FEP), non-renewable energy use (NREU), land use (LU) as impact categories. Data originated from a total of 91 farms located in Belgium (20 farms), France (28), Italy (25), Poland (10) and Switzerland (8). Farms were grouped according to a gradient of intensification in 6 systems, based on ARCs and ATs. The systems ranged from outdoor rearing farms using slow growing strains (OutSlow, 24 farms), to indoor rearing farms using fast-growing strains and producing light (IndFast, 15) or heavy broilers (IndFastH, 35). On average, a production cycle lasted from 35±1 d (IndFast) to 82±7 d (OutSlow), with a final LW from 1.9±0.2 kg (IndFast) to 3.0±0.2 kg (IndFastH). On average, 1 kg LW was associated with 2.57±0.72 kg CO₂-eq (GWP), 34±7 g P-eq (FEP), 17.1±4.9 MJ (NREU), 3.49 ±2.61 m²/y (LU), whereas per 1 m² of land 0.97±0.39 kg CO₂-eq (GWP), 13.1±6.7 g P-eq (FEP), 6.5±2.8 MJ (NREU). Indoor systems evidenced lower impacts per 1 kg LW, especially LU (-63%) but higher per 1 m², especially FEP (+106%) than outdoor ones. Within both indoor and outdoor systems, ATs showed a complex influence on environmental impacts. In conclusion, environmental impact profiles varied in complexity across systems. In perspective, impacts should be assessed together with the intrinsic quality of chicken meat from these systems and other extrinsic quality traits such as animal welfare. Acknowledgements: INTAQT project, Horizon 2020, agreement n°101000250.

A Living Lab to address heat stress in dairy goats: impact of a nutritional intervention on milk yield and quality

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The frequency and intensity of extreme weather events are increasing, with a severe impact on livestock husbandry. Thermal stress impacts on animal welfare and is responsible for significant productive and economic losses. In the H2020 PATHWAYS project we established a Living Lab in Spain to co-develop with farmers associations potential solutions to deal with heat stress in dairy goats. The aim of this study was to evaluate the impact of a specific dietary supplement on primiparous dairy goats under heat stress conditions. Thirty Murciano-Granadina dairy goats from a commercial farm were used. They were randomly assigned to two treatments (n=15): control concentrate + alfalfa (CTL), and control concentrate with a methionine hydroxy analogue (HMBi) and a high-solubility magnesium oxide buffer (HS). The trial started on July 1st and ended on October 30th, 2023. Individual daily milk yield was monitored, and temperature and relative humidity at the barn and milking parlor were recorded hourly using sensors. A total of eight samplings for milk composition were performed every fortnight. Moreover, 50 activity collars validated for dairy goats were used during the trial to monitor behavioral pattern (resting, eating, walking). The results were statistically analyzed using repeated measures GLM. Milk yield, protein, and the fat + protein contents were significantly higher ($P < 0.001$) in the HS diet compared to the CTL diet, with values of 2.71 vs. 2.20 kg/day for milk yield; 94.5 vs. 74.9 grams/day for protein production, and 215.4 vs. 176.6 grams/day for fat + protein, respectively. Fat production was higher ($P = 0.006$) in the supplemented animals (119.1 vs. 101.1 grams/day). THI values were higher ($P < 0.001$) during July and August compared to September and October, 75.4 and 68.6, respectively. A significant correlation was identified between activity behaviors and the THI; treatment effect results are pending. Supplementing the diet of primiparous lactating goats with a HMBi and a magnesium oxide buffer positively impacts milk production and quality under stress conditions Acknowledgements: H2020-PATHWAYS project (101000395)

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Effects of quantity and quality of space on hair cortisol, hair dehydroepiandrosterone (sulphate) and their ratio in growing-finishing pigs

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Pig husbandry systems across the world can differ greatly in characteristics such as the pig breed used, type of feed and the quantity and quality of space (i.e., enrichment) provided to the pig. These differences may have impact on (chronic) stress indicators like cortisol. Within the H2020 project mEATquality (No.101000344), we studied whether more space and better quality of space leads to changes in hair cortisol (HC), hair dehydroepiandrosterone (sulphate) (HDHEA(S)) and their ratio in husbandry systems with varying extensification levels in Poland, Denmark and Italy. In Poland, quantity of space (1 (control) vs 2 m²/pig) was evaluated on two intensive commercial farms (n=132). In Denmark, quantity of space (0.7 (control) vs 1.4 vs 2.1 m²/pig) and quality of space (straw (control) vs straw, wood logs, feed bags and ropes, and vs straw with roughage) was evaluated (n=410) on an intensive experimental farm. In Italy, quantity of space (1 vs 3 m²/pig or 2.4 vs 7.2 m²/pig) and quality of space (access to shade and branches or not (control)) was evaluated (n=312) on three extensive commercial farms with outdoor access. Hair samples were collected for all pigs prior to slaughter and HC and HDHEA(S) were measured by an in-house ELISA. In most experiments both HC and HDHEA(S) was lower in pigs housed with more space than pigs housed with less space regardless of the extensification level, indicating that more space per pig resulted in a lower allostatic load during the growing-finishing period than when less space per pig was provided. Quality of space did not result in differences between HC, HDHEA(S) and HC/HDHEA(S) ratio, likely due to relative small differences in quality of space between the treatment and control groups. Overall, sufficient quantity of space was important in reducing the allostatic load in pigs in husbandry systems with different extensification levels, but quality of space did not seem to affect their allostatic load in any of the tested systems.

Monitoring grassland biodiversity using farmer-derived data: opportunities and challenges

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Biodiversity in European grasslands has declined due to multiple drivers, including management intensification and over- or under-grazing. Policies in the EU and UK, such as the EU Biodiversity Strategy, aim to restore biodiversity. However, assessing the effectiveness of these policies requires effective biodiversity monitoring, which comes with practical challenges. For example, who should do the monitoring, and what methods should be used? This study evaluates whether farmer-recorded data can be useful in monitoring grassland biodiversity. We investigated this through literature review, farmer surveys, and field data collection. The review evaluated previous studies on farmer-derived biodiversity data, such as results-based payment schemes. An online survey targeted UK Pasture for Life (PFL) farmers to explore constraints and opportunities. Additionally, we tested simple field protocols in the UK, Sweden, and Romania, to compare farmer-recorded data with expert botanical assessments. Of our 25 survey respondents, most farmers reported that they monitored biodiversity, but typically on an infrequent or ad-hoc basis, and few used recording schemes or apps. Time constraints were the main barrier, followed by a lack of technical expertise and suitable protocols. When asked about incentives, most farmers favoured an easy monitoring protocol. Our fieldwork tested such protocols and found that farmer-reported counts of non-grass plant species in 5×5m quadrats correlated closely with expert botanical assessments. However, the use of farmer-recorded indicator species was less reliable. Our findings highlight the need for simple, farmer-friendly biodiversity monitoring protocols. A straightforward metric, such as counting flower species during the appropriate season, could be an effective tool while encouraging greater farmer engagement in biodiversity conservation.

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Theatre 10

Joint assessment of milk quality, its environmental impacts and ecosystem services: emerging concepts and methods

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More and more attention is being paid to farming practices by social demand. So, when assessing the quality of animal products, it is necessary to consider both intrinsic (i.e. relative to the product) and extrinsic (i.e. relative to farming practices) quality. Therefore, we intend to identify new concepts and methods to jointly assess the intrinsic quality (IQ) of animal products, their environmental impact (EI) and the ecosystem services (ES) they provide in order to help the farmers choosing the most appropriate practices. We performed a systematic literature review from a research equation that selected references published after 2000 that consider at least two of the three dimensions of quality (i.e. IQ, EI or ES). We identified 255 eligible primary articles and 17 relevant reviews focusing on various food, diets and agricultural systems. None assessed jointly IQ, EI and ES. Then we identified 7 reviews dealing with concepts and methods. The 5 reviews focusing on IQ and EI only considered the nutritional quality in order to orient consumers' choices. They do not consider others IQ dimensions, as sensory, technological or sanitary quality. EI assessment relies mostly on life cycle assessment (LCA) using nutritional scores as functional unit to take IQ into account. Nevertheless, reviews argue that a single score cannot account for the diverse functions of food, while a functional unit is supposed to measure the ability of a product to accomplish its expected function. An alternative method proposed consists in converting the nutritional quality of the product or the diet into its endpoint impact on human health. In the 2 reviews considering EI and ES, no method to jointly consider ecosystem services assessment (ESA) with LCA makes consensus. A major difficulty to tackle is that LCA historically used product as the functional unit (e.g. 1 kg), whereas ESA focused on ecosystems (e.g. 1 ha). This first step in our literature review illustrates the difficulty in assessing jointly IQ, EI and ES even if the concepts and methods we seek are essential to help organize information and contribute to more sustainable dairy farming decisions and practices.

Exploring the nutrient density of animal sourced foods on environmental footprint: a systematic review of functional units

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Nutritional life cycle assessment (nLCA) is a sub-field of life cycle assessment (LCA) which integrates both environmental impacts and the nutritional and health effects of a product. Traditional LCA uses mass based functional units to express carbon footprint (e.g., kg CO₂e/kg of liveweight/deadweight), whereas nLCA uses nutritional functional units to express carbon footprint. The primary aim of this study was to uniquely identify and compare nutritional functional units of animal sourced foods in relation to nLCA and environmental footprint using systematic review methodology. Medline, Scopus and Web of Science were searched using a strategy with multiple inclusion and exclusion criteria. Articles were screened and independently assessed by a first and second reviewer. Any discrepancies were resolved using a third reviewer. A total of 59 studies were identified to be included in the systematic review with 40 of the papers being published in the last five years (2020-2024). Key data extracted included environmental impact, public health indicators and nutritional functional units of animal sourced foods. From the included studies, several functional units were identified including protein, protein quality (e.g., Digestible Indispensable Amino Acid Scores), omega-3 fatty acids and Nutrient Density Scores. While many studies examine nutritional function units, few studies further consider the impact of non-nutrient factors (e.g., bioavailability, amino acid balance, digestibility). This study has identified useful nLCA indicators (functional units) that may be able to inform the position of animal sourced foods in society with regard to nutrition, health and environmental footprint.

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From Coop to Table: How Genetics and Environment Influence Broiler Meat Quality

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The quality of poultry meat is shaped by genetics and rearing conditions. This study examines how genotype (JA757, JA787) and housing system (free-range GR1 vs. indoor GR2) influence broiler breast meat quality. While slaughter, carcass, and breast muscle weights showed no significant differences, key meat quality traits were affected by environmental factors. Housing conditions influenced pH at 48 hours post-slaughter ($p = 0.04$) and color parameters b and C ($p = 0.0001$), suggesting environmental effects on pigmentation and biochemical processes. Water retention properties varied, with JA757 exhibiting higher thaw loss, while cooking-related water loss was affected by both genotype and environment. Texture analysis showed that genotype influenced Young's Modulus, while housing conditions affected the 20–80% range. Notably, Spaghetti Meat was significantly linked to genotype ($p = 0.0001$) and housing ($p = 0.01$), with JA787 and indoor birds showing a higher prevalence. However, Wooden Breast and White Striping were not influenced by these factors. Fat content was significantly affected by housing and genotype-environment interactions, whereas protein and moisture content remained unchanged. These findings indicate that genotype had a limited effect on carcass traits, while rearing conditions played a crucial role in pH, color, water retention, and fat deposition. Understanding these interactions can help optimize rearing systems to enhance meat quality while ensuring ethical and sustainable poultry production. This study was conducted as part of the mEATquality project, funded by the EU's Horizon 2020 Research and Innovation Program (Grant No. 101000344).

Meat quality traits of pigs housed with increasing space availability

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Space allowance for fattening pigs was tested in two experimental trials, as part of the mEATquality research project, funded by the European Union's Horizon 2020 research and innovation programme under grant agreement N° 101000344. The experiments were carried out on heavy pigs intended for Italian PDO ham, and replicated twice. In the first trial, 72 pigs of the Italian Duroc x Large White crossbred were kept indoor, undergoing 1, 2 and 3 m²/pig space allowances (i.e. 24 pigs per treatment), where 1 m²/pig is the minimum space allowance for pigs over 110 kg of live weight, according to Directive 2008/120/EC. The second trial was conducted in an organic farm with 91 commercial hybrids (Topigs TN70 x Fomeva K-line) with space allowances of 1,36 m²/pig indoor and 1,05 m²/pig outdoor, 2,6 m²/pig indoor and 2 m²/pig outdoor and 3,9 m²/pig indoor and 3 m²/pig outdoor, where 1,3 m²/pig indoor and 1 m²/pig outdoor is the minimum for organic pigs up to 110 kg of liveweight, according to Regulation (EC) 889/2008. No differences were detected between the average values of growth and slaughter performances for the investigated space allowances. In each trial, 60 pigs (20 per treatment in two replicas), were sampled at slaughter and their carcasses analysed for % lean meat estimate, muscle and backfat thickness, slaughter weight and dressing %. The left loins were analysed for their intrinsic quality traits including pH 24h, pH 48h, drip loss, CIE colorimetric indices L*, a*, b*, and derived parameters chroma C*, and hue angle h°, thawing and cooking losses, slice shear force (SSF) and fracturability (SSf) and composition. In the first trial significant differences were found in the quality traits of the loins for thawing loss, SSF and SSf, showing the positive effect of the increase in space allowance in decreasing thawing loss and improving tenderness. The second trial, did not generate significant differences in the intrinsic quality of the tested pork loins; the SSF and SSf values were aligned with those measured with the highest space allowance of the first trial.

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Poster 14

Lower stocking density in pigs: welfare, growth rate, and meat quality

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Space allowance is a crucial factor in the welfare of fattening pigs. European Union regulations define minimum space requirements for pigs at different growth stages to ensure animal well-being. This study aimed to investigate the effects of different space allowances on growth performance, carcass traits, and meat quality in commercial hybrid pigs. Two experiments were conducted using 102 and 80 pigs, respectively, with three different space allowances: the current minimum (1.0 m²/pig), a planned increase (1.5 m²/pig), and a doubled space allowance (2.0 m²/pig). Pigs were housed in intensive systems. Key parameters collected before and after slaughter included average daily gain (ADG), final body weight, carcass characteristics and intrinsic meat qualities such as pH, texture, water-holding capacity, and fatty acid composition. Results demonstrated that pigs with 1.5 m²/pig exhibited the highest ADG in both experiments, suggesting that moderate increases in space benefit growth performance without significantly impacting slaughter weight. Differences in carcass traits were noted primarily between sexes rather than space groups. Meat quality parameters, including pH, water-holding capacity, and texture, showed minimal variation across space allowances, aligning with previous studies that found limited impact of indoor space modifications on pork quality. The fatty acid profile also remained largely unchanged across groups, indicating that increased space alone does not significantly alter meat composition. Overall, the findings suggest that while increased space allowance improves growth performance, particularly at 1.5 m²/pig, it does not substantially enhance meat quality. Future research should explore economic feasibility and consumer willingness to pay for welfare-improved pork products to support policy changes. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 101000344.

Evaluating manure impact methodologies within Life Cycle Assessments (LCA) of livestock systems and products

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The environmental impact of livestock production is of critical concern, with manure management being a significant contributor to greenhouse gas (GHG) emissions, accounting for approximately 10% of total agricultural GHG emissions globally. Manure supports soil fertility, however its management causes GHG, in particular methane (CH₄) and nitrous oxide (N₂O). LCA is a key tool for quantifying these impacts in livestock systems. However, the lack of methodological harmonization in manure-related emissions assessments poses challenges for comparability and accuracy across LCA studies. This study undertook a harmonisation approach to assess LCA methods on manure emissions in livestock systems and production chains focused on: i) Assessing current LCA methodologies; ii) identifying key emission drivers, iii) supporting sustainable livestock systems, iv) providing recommendations for LCA practitioners. An initial pool of 29,151 papers was collected. After screening, 263 papers remained, with 48 focused on manure emissions in LCA. The LCA method harmonization used criteria selected through expert panel and workshop: transparency, completeness, fairness and acceptance, robustness, and applicability, leakage inclusion for anaerobic digestion, accuracy in GHG emissions from manure storage, treatment, and animal housing. While IPCC Tier 1 approaches dominate due to their simplicity, Tier 2 and Tier 3 methodologies provide greater accuracy by incorporating detailed system-specific parameters. However, the higher data requirements of these advanced methods limit their broad applicability. Further, inconsistencies in system boundary definitions, emission factor selection, and nitrogen dynamics modelling further complicate direct comparisons between LCA studies. As innovations are developed to reduce emissions, future LCA improvements could balance accuracy and applicability by refining emission factors and process models while integrating advancements in observation and crop-livestock assessments.

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Poster 16

Improved welfare vs. meat quality: impact of enrichment and stocking density on broiler chicken

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The typical traits of modern fast-growing broiler chickens are high growth rates and a high proportion of breast muscles in the carcass. This study aimed to implement access to enrichment (perches vs. no perches) and two stocking densities (41 vs. 35 kg/m²) and its interaction with sex of the birds. Most of the examined slaughter traits were affected by sex, as well as the interactions between sex, use of perch, and stocking density. None of the fresh meat quality traits exhibited differences solely due to the use of enrichment. The yellowness index was greater in males compared to females ($b^* = 10.7$ vs 10.1) and in the 41 kg/m² group compared to 35 kg/m² ($b^* = 10.7$ vs 10.2). The pH_{thawed} was higher in the 35 kg/m² group compared to 41 kg/m² (5.93 vs. 5.89). Males and females differed in two meat texture parameters. Sex had no effect on the chemical composition of chicken meat. The meat from enriched conditions contained more moisture and less protein compared to the control group. Males exhibited a higher incidence and greater severity of myopathies compared to females. In conclusion, environmental enrichment and stocking density do not impact slaughter traits or myopathies, but do affect certain aspects of chicken meat quality. Research financed by European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 101000344.

More Meat, Fewer Cows: How Detailed and Context-Specific Herd Modeling Unlocks Unexplored Synergies in the European Food System

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Global and regional food system models often oversimplify cattle production and fail to distinguish between dairy and suckler systems. This makes it challenging to assess how improved herd management strategies influence total food output. One such strategy is extending the productive lifespan of dairy cows. This reduces the need for replacement animals while increasing the number of calves available for fattening. Another strategy is the dual-use dairy production system—dual-purpose breeds or a combination of sexed semen for replacement animals and meat-genetic crossbreeding for non-replacement animals—leading to fattening calves with the genetics for meat production. Rather than being limited to veal, they can be raised to maturity, contributing to overall bovine meat production. Most models overlook that calves are traded, failing to capture the cross-border effects of herd management changes. A context-specific herd model covering all NUTS2 regions in the EU, the UK and Switzerland, is a part of the GeoSOL baseline within the PATHWAYS project. It reconstructs regional herd dynamics using statistical data, explicitly differentiating between dairy and suckler herds and the different fattening schemes while accounting for live trade. It evaluates the impact of extending dairy cows' productive lifespan on beef production under different fattening and slaughter strategies, including the adoption of dual-use production systems. Results indicate that extending the productive lifespan of dairy cows within a dual-use system can enhance beef production compared to current systems. When this additional meat from the dairy sector replaces beef from suckler herds, total meat output can be maintained with fewer cattle overall. Accounting for herd structure and trade dynamics allows to move beyond simplistic narratives that assume reducing livestock to mitigate environmental impacts comes with a proportional reduction in bovine meat consumption. More detailed and context-specific models are essential to unlock synergies within the food system, minimizing trade-offs and supporting the development of balanced and pragmatic PATHWAYS for a sustainable livestock management.

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Poster 18

Participatory approaches for sustainable livestock production

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Livestock systems play a crucial role in meeting global demand for animal products but are increasingly criticised for their negative environmental and societal impacts. Future livestock production systems must maximize positive contributions while mitigating adverse effects. Participatory approaches, which actively engage stakeholders in decision-making and give the affected parties a voice, offer a means to align livestock systems with local needs and to enhance sustainability. This study explores the characteristics and benefits of participatory approaches used to enhance the sustainability of livestock production. The study utilises examples from the EU-funded project PATHWAYS (www.pathways-project.com; GA 101000395). The research employs both quantitative (surveys) and qualitative methods (focus groups, farm networks and Living Labs). Surveys were conducted to assess the opinions of relevant stakeholders on ecosystem services. They were preceded by stakeholder analyses to identify those that are involved in or are affected by decisions. Focus groups were used to gather in-depth insights from international actors from the Multi-Actor Platform of PATHWAYS, including discussions on visions for sustainable livestock production in 2050. Farm networks were established to facilitate the exchange of knowledge around a certain theme of innovation. The project PATHWAYS works with farm networks in 15 Practice Hubs focusing on innovative practices related to sustainable livestock production (dairy, beef, pork, poultry, sheep and goat). Complementing these Practice Hubs, 15 Living Labs were established to test practical improvements in on- or off-farm farmer-initiated trials. Findings indicate that participatory approaches enhance collaboration, stakeholder ownership and informed decision-making in the livestock production sector. By integrating diverse perspectives, these methods contribute to more sustainable livestock systems that better align with stakeholder needs and capabilities.

Responsible Development and Deployment of AI and IoT in the Dairy Industry, bridging gaps between research and implementation: The WELL-E Initiative

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Responsible AI that properly addresses real-world stakeholder needs is at the heart of the R+I Chair in Animal Welfare and Artificial Intelligence. WELL-E approach relies on the use of IoT, computer vision, and machine learning to improve our ability to detect and monitor changes in animal welfare earlier than possible with visual methods, and to generate predictions to aid on-farm decision-making, ensuring that resources and efforts are focused on the animals most likely to succeed in the long term. Grounded in industry partnerships, members of the dairy community sit on both the scientific and management committees, where they contribute to research orientations, leading to the co-creation of research projects and initiatives. This collaborative approach not only ensures the applicability and relevance of all research carried out, but allows for continuous feedback between the research and industry environments, creating a true digital living lab that can grow with the Chair. Launched in 2023, our team has been conducting pilot research on two research farms and working to build functional and resilient data collection infrastructure for implementation on commercial dairy farms throughout 2025. Through a federated learning approach, this deployment ensures robust continuous monitoring across a network of farms, while respecting data confidentiality and cybersecurity. We have developed a framework for the study of animal behaviours and emotions, presenting a paradigm shift for both annotation and data analysis based on continuous and heterogeneous data sources. As we grow our network of partner farms, we will continue focussed studies on research farms, allowing for continuous exchange between the two contexts. This collaborative approach promotes the responsible integration of new technologies to the industry and empowers endusers to be at the forefront of these new developments, ensuring their sustainability and reinforcing the importance of stakeholder participation in innovative scientific research.

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Theatre 2

ICAR/IDF initiative: Guidelines and reference standards for using rumination sensor data in animal health and welfare recording

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On-farm sensor data offers new opportunities for animal health and welfare assessment when used along the dairy value chain, thus improving transparency and sustainability in the dairy sector. The aim of the joint ICAR IDF initiative is to develop guidelines to facilitate use of sensor data for improving animal health and welfare. The joint initiative consists of different stakeholder groups, scientists, breeding organizations and manufacturers and has the goal to develop: Definitions and terminology for health conditions and behaviors measured with sensor systems Standards to facilitate data exchange along the dairy value chain following acknowledged ICAR and IDF principles Guidelines for best practices on data collection, handling and analysis for use in genetics, health and welfare assessment Recommendations and protocols for testing sensor performance First results from the use case sensor-based rumination data will be presented, recommendations on use of rumination data, key performance indicators and references standards for animal health and welfare assessment given. The close collaboration between the relevant stakeholders in the joint ICAR/IDF initiative enables development of guidelines considering industry needs while promoting use of sensor data along the dairy value chain.

Optimizing Machine Learning (ML) models for automated detection of calf behaviors

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The use of ML models to automate the identification of behavior promises tremendous benefits in the management of farm animal health, welfare and productivity. Little is known about how to best optimize the development and deployment of these models. We examine training ML models to recognize behavior from data generated by an Inertial Measurement Unit (IMU) sensor device attached to calves using human-labelled video recording as our ground truth. Our focus was on how to optimize the IMU data stream provided to the model as well as how different types of ML models affect model reliability. Digital filtering (eg. decimation, rotational transformations) of the IMU data were systematically varied as well as different ML model structures to test the impact of these manipulations on model outcomes. Data was collected from paired housed calves (4 calves) with neck mounted IMU, and video recorded for 76 consecutive hours. Posture (stand and lie), was coded from the video using focal sampling and continuous recording. AgNotate, a C++-based annotation platform, was used to merge camera and IMU data, aligning annotations across the time series to improve behavioral labeling for model development. Half the coded data from all calves was aggregated and used to train models under different IMU data conditions. Then, the other half of the labeled data from each individual calf was used to test the different models for specificity, sensitivity, and accuracy on a per-animal basis, allowing for 8 replicate assessments of each of the different manipulations. Model performance improved with both increased sampling frequency and increased IMU data signal-to-noise ratio. IMU data offers several orders of magnitude better temporal resolution (1 to 10Hz) compared to conventional video recording (<30 Hz) when used to define ethograms and, in particular, captures transitions between behaviors readily, which may have more predictive value. Findings highlight the importance of integrating high-frequency IMU data and effective filtering methods for more robust and scalable ML applications in PLF and welfare measurement, ensuring accurate, automated monitoring of animal behavior.

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Theatre 4

Development and validation of a triaxial accelerometer for behavior monitoring in Murciano-Granadina goats

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The use of accelerometers has been consolidated as an effective tool for monitoring animal activity, allowing the recording and analysis of behaviours, which provides key information on the health and welfare of livestock. However, its application to goats presents specific challenges due to their specific behaviour and speed of movements. This study aimed to validate a collar-integrated tri-axial accelerometer for behavioural monitoring of Murciano-Granadina dairy goats under intensive husbandry conditions. The research was carried out in an experimental farm (UPV) with eight adult dry goats, housed and equipped with collars containing a tri-axial accelerometer (Digitanimal, S.L.). The goats were observed daily by video cameras in daylight hours, obtaining a total of 115 hours of observation, manually labelled with Boris software. The behaviours tagged were eating, walking and inactive. A multilayer perceptron-based predictive algorithm with initial performance accuracy of 0.9 was obtained, which was subsequently incorporated into the devices and validated in a new phase of observation and tagging process, reaching promising scores related to behaviour prediction in goats. These results validate the potential of accelerometers to collect data that contribute to the analysis of animal welfare and health.

Heavy breathing by accelerometer-based recordings is associated with body temperature, rumen and blood metabolites of Fleckvieh dairy cows during heat stress: A cluster-analytic approach

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This study investigated the use of accelerometer-based sensors to monitor heat stress (HS) in dairy cows by detecting heavy breathing (HB), which is a key thermoregulatory response. A total of 32 mid-lactating Fleckvieh cows were analyzed using k-means clustering based on HB data from SenseHub® sensors over 35 d (July-August). Two clusters were identified: high HB (HIGH, n=10) and low HB (LOW, n=22). Daily records included the temperature-humidity index (THI), milk yield (MY), HB, rumination, and eating time. Body Condition Score (BCS), blood, and rumen fluid samples were collected, along with rectal temperature (RT). Data were analyzed by GLIMMIX procedure of SAS. HIGH cows had longer ($P<0.01$) HB (174 min/d) than LOW cows (54 min/d). THI breakpoints for HB were 73.65 for LOW and 70.90 for HIGH cows, suggesting that HIGH cows experience HS at lower environmental stress conditions than LOW cows. In fact, HIGH cows showed higher RT ($P<0.05$) than LOW on d 17 (39.07 vs. 38.71 ± 0.09 °C; THI=70) and 28 (38.94 vs. 38.66 ± 0.09 °C; THI=75). There were no differences in MY and its composition, except for greater milk urea in HIGH cows ($P<0.05$). HIGH cows had higher plasma GGT, albumin-to-globulin ratio, but lower zinc, suggesting reduced liver function and innate immune response, intensified inflammation, and dehydration. Plasma β -hydroxybutyrate was higher ($P=0.05$) in HIGH cows, indicating increased lipid mobilization. Indeed, HIGH cows had lower BCS than LOW cows ($P<0.05$). HIGH cows had greater rumen D-lactate and total volatile fatty acids content, suggesting poorer rumen fermentation. Overall, this research highlights the utility of sensors for early detection of HS in dairy cows through HB, which relate with key physiological and metabolic parameters. This insight can assist farmers in optimizing cooling systems to mitigate MY losses during HS while potentially guiding genetic selection programs for improved heat tolerance.

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Theatre 6

Improving individual energy requirement prediction in dairy cows by the integration of physical activity data

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Physical activity significantly impacts energy expenditure and varies among dairy cows. New technologies, such as portable sensors, enable individual and continuous activity data collection, allowing for its integration into feeding strategies. This study aimed to determine cows' activity profiles to model the relationship between physical activity and energy expenditure. Hourly movement counts were recorded from 48 free-stall-housed Prim'Holstein cows over a 0 to 120-day postpartum period. Missing data (~0.5%) were filled using linear interpolation (zoo R package) between same hours of consecutive days. Activity durations were derived from time budgets found in the literature. Cows were categorized as lying when hourly activity values were below the median, as walking when above the 60th percentile, and as standing otherwise. Daily activity durations (walking, standing, Lying) were smoothed (FDA R package) to reduce noise. PAM Clustering (Partitioning Around Medoids – cluster R package) was performed on the mean and coefficient of variation of walking and lying durations for two periods (0-15 days and 15-120 days) to assess whether activity profiles can be predicted early in lactation. PAM clustering with dynamic time warping distance (Dtwclust R package) was applied to individually centered walking duration time series to identify variations in activity curve shapes. Three activity profiles (1 = "high", 2 = "medium", 3 = "low") were identified, mainly based on daily walking duration. They were predicted using the data of the first 15 days with 77% accuracy. Two distinct curve shapes of walking duration (A = "high-fall-increase" and B = "progressive increase") were associated with previous profiles. Cows from profile 1 were mostly associated with the A shape (77%) while cows from profiles 2 and 3 were more often associated with the B shape (respectively 69% and 92%). These activity findings will be used to enhance energy requirement modeling and test various individualized management strategies of dairy cows.

Identifying shade-seeking behavioural triggers in Holstein heifers for improved heat stress management and welfare

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New technologies, such as precision livestock farming (PLF) and machine learning (ML), are transforming the livestock sector by enhancing efficiency, improving animal welfare, and enabling data-driven decision-making for better farm management. In the current context of climate change, identifying behavioural triggers in response to heat stress events is an essential prerequisite for improving animal welfare and optimising farm management strategies. Therefore, by including behavioural triggers in predictive models, the development of context-specific heat mitigation strategies, cooling interventions, and farm management decisions could be facilitated. To this end, this study investigates the environmental conditions that trigger shade-seeking behaviour in Holstein heifers, using PLF technologies and ML algorithms. In contrast to the THI value of 72, our results reveal that for this specific context, a THI of 76 acts as a behavioural trigger for shade-seeking behaviour. In addition, it seems that the heat load during the last 4 days, also defines animal behaviour at a given moment. Our findings also reveal an adaptation window, where behavioural responses stabilise after extended heat exposure, potentially indicating acclimatisation of the animals to heat. Moreover, our results underscore the importance of cumulative THI values rather than relying solely on momentary observations. In contrast to one-fits-all solutions, understanding the nuanced and context-specific responses of animals is key for their welfare, resilience, and productivity in environments where heat stress compromises livestock production.

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Theatre 8

Detection and characterization of disturbances in the trajectory of production and behavioral data for dairy cows

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Technologies available in precision breeding facilitate the continuous collection of zootechnical and behavioral data at an individual scale. Analysis of these data could help monitoring animal welfare and production. In order to determine if methodologies of data analysis can detect and classify disturbances linked to events referenced by animal keepers, a supervised approach using the smoothing functions B-splines (FDA R package) was compared to a non-supervised approach using a Gaussian kernel smoothing (UpDown package). The analyzed data were milk yield (MY), dry matter intake (DMI) and standing time (ST) automatically collected during the first four months of 40 dairy cows' lactations. Moreover, with FDA, the disturbances characteristics were recorded: duration, maximum and minimum differences between smoothed curves (i.e., reference curve being more smoothed than disturbed curve), and intensity (i.e., difference between maximum and minimum). Event type (health (H), feeding (F), reproductive (R)) effect on these variables was tested using a linear mixed model. Detection of disturbances was more sensitive with FDA than UpDown (respectively, MY: 6 vs 3%; DMI: 8 vs 1%; ST: 7 vs 0%). The specificity was similar between these two methods for all variables ($\geq 99\%$). The maximum difference of ST disturbances was higher for feeding events than health events ($P = 0.01$), while for DMI disturbances it was higher for feeding events than reproductive events ($P = 0.02$). The duration of MY or MSI disturbances was longer than that of ST, regardless event type (MY: 10 ± 5 days, MSI: 11 ± 6 days, ST: 7 ± 4 days, $P < 0.001$). The intensity of disturbance was more important for ST than MY or MSI, regardless event type ($P < 0.001$). Based on these results, the FDA method seems more appropriate to detect all kinds of disturbances than UpDown. However, it is necessary to refine the determination of parameters detection (threshold for selecting disturbances, degree of smoothing), as well as the choice of variables to be able to better classify the disturbances.

Validation of a new textile sensor band for heart rate variability assessment in calves

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Heart rate variability (HRV) has been studied as an indicator of stress in various species, including dairy calves, where significant changes in HRV were linked to cow-calf separation, isolation, and disbudding. Given HRV potentialities, feasible technologies need to be implemented for on-farm use. A promising tool are wearable belts with given their comfort and the possibility to be worn or tape with reduced animal handling and stress. The aim of this study was to examine if a smart textiles biotechnology (Smartex) could be compared to a Standard base-apex ECG for measuring HRV in dairy calves. Twenty healthy Italian Holstein females calves (20-60 days old) from one dairy farm were recruited. Standard base-apex ECG and Smartex ECG were simultaneously acquired for 16 minutes in the standing, unsedated, unclipped calves. The ECG tracings were taken while the animals stood still. After parameter extraction, many tests in time, frequency, and nonlinear approaches were used to compare Smartex to traditional base-apex ECG systems. The Bland-Altman test was used to assess the level of agreement between the two instruments, and a linear regression analysis was used to assess the association between them. A general agreement between the two systems was confirmed by both the statistical approaches. In particular, regression analysis showed an r^2 coefficient higher than 0.9 for the following HRV parameters: mean RR, SDNN and LF/HF, indicating the most stable relationship between the two systems. Smart textiles biotechnology can be used for HRV measurement in calves as a possible animal-based measure for animal welfare assessment.

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Theatre 10

The Potential of Infrared Thermography in Precision Dairy Farming

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Precision livestock farming is becoming increasingly popular in animal production, favouring non-invasive techniques for monitoring animal health and welfare. Infrared thermography presents a promising and effective solution for detecting hoof diseases and lameness. Hoof problems and lameness remain major problems on dairy farms significantly impacting animal welfare, reducing milk production, and increasing breeding costs, ultimately leading to substantial economic losses. Lameness can generally be identified in three ways: firstly, through locomotion scoring system; secondly, via routine hoof trimming; and thirdly, during other activities such as herding. Early detection of infectious conditions and prompt treatments are crucial for preventing lameness. Effective prevention strategies help reduce the pain and discomfort associated with lameness, improving overall animal welfare. An increase in temperature is a clear indicator of inflammatory changes within the body, as inflammation leads to increased blood flow to the affected areas. Infrared thermography, as a non-invasive diagnostic tool, enables the visualisation and assessment of variations in surface temperature, making it a valuable asset in dairy farm management. The potential of infrared thermography in detecting specific hoof conditions and preventing lameness has been well documented in previous studies. Findings suggest that combining infrared thermography with artificial intelligence-based predictive models holds great promise for developing automated real-time monitoring tools for hoof health. However, several factors influence temperature readings on a cow's feet, including ambient temperature, contamination, measurement angle, disease presence, and varying levels of lameness, all of which significantly affect the accuracy of readings. While there is a considerable potential, challenges remain, such as lack of well-defined temperature thresholds, insufficient real-time measurements, and difficulties with animal identification, etc. Further research is needed to develop optimal integration of different methods to improve the accuracy and efficiency of infrared thermography for detecting hoof disease and lameness.

Enhancing Livestock Welfare to Heat Stress characterizing physiological responses in Marchigiana trough PLF and omics combination

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Livestock welfare is crucial for sustainable agricultural production. This study investigates resilience and welfare under heat stress also in local adapted breeds to identify biomarkers for breeding resilient animals through Precision Livestock Farming (PLF). We monitored 30 Marchigiana (MAR), 20 Holstein (HOL), and 30 Limousine (LIM) cattle, measuring key physiological parameters using IoT sensors. Blood samples for hematological and transcriptomic analyses were collected during heat stress peak (summer) and thermoneutral conditions (autumn), determining sampling days based on Temperature-Humidity Index (THI). Accelerometric data showed increased activity under heat stress, though animals rested longer compared to thermoneutrality conditions (-0.97 vs. -0.73, Spearman correlation). Blood tests revealed higher white blood cells (WBCs) in MAR and HOL, likely due to seasonal immune responses, while WBCs slightly decreased in LIM. HOL exhibited increased neutrophils, suggesting an inflammatory response, whereas MAR and LIM had eosinophilia, possibly due to summer parasites. Red blood cells and hemoglobin rise in MAR and HOL, likely aiding oxygen transport under heat stress. Transcriptomic analysis identified differentially expressed genes (DEGs) and miRNAs between heat stress and thermoneutrality. Gene ontology analysis linked DEGs to stress response, inflammation, and immune function, while DE miRNA targets were associated with transcriptional modulation and RNA packaging. Overall, our findings provide insights into cattle physiological responses to heat stress, supporting improved breeding strategies for resilience and welfare.

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Theatre 12

Impact of birth timing and heat stress on milk production in dairy cattle: a comprehensive analysis

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Heat stress (HS) in dairy calves poses a significant challenge, negatively impacting their health, growth, and impairs subsequent productivity. This study synthesizes recent research findings to assess the effects of environmental conditions on calf welfare and to propose effective management strategies for alleviating HS. Data from the ResKuh project, which covers the Baden region with Baden-Württemberg (LKV BW) region as well as Alsace and the 3CE region: Alsace, Moselle and Haute-Marne departments from French region Grand Est, was analysed to highlight calf performance under varying temperature-humidity index (THI) conditions. The analysis reveals that calves are particularly susceptible to HS during critical growth phases. The study examined ~50,000 animals in LKV BW and ~90,000 animals in the 3CE, focusing on the effects of birth timing categorized by calving age into three groups: less than 28 months, between 28 and 33 months, and more than 33 months. Animals were further classified by birth period into summer (Jun 1 to Aug 31) and winter (Dec 1 to Feb 28). Results indicate that summer-born animals produce significantly less milk during their first lactation, with an average reduction approx. -171 kg Energy Corrected Milk (ECM) in 3CE and -558 kg ECM in LKV BW, particularly pronounced in those calving under 28 months (-340 kg ECM in 3CE and -443 kg ECM in LKV BW). By implementing evidence-based strategies, dairy producers can enhance animal welfare, improve growth rates, and ultimately increase the economic viability of dairy operations. These findings emphasize the substantial influence of birth period and calving age on early lactation performance, highlighting the necessity for improved management practices to mitigate HS and enhance living conditions. Future research should focus on the long-term effects of HS and explore how different breeds adapt to varying climatic conditions to ensure sustainable dairy farming practices.

Comparison of novel object test in cattle performed individually or as a group in the home pen

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There are a variety of behavioural tests to assess the individual personality of animals, which are mainly performed in specific testing environments. Because of this, behavioural tests are laborious and limited in sample sizes. In this study, novel object tests (NOT) were performed individually on dairy cattle with a trained human observer (NOT1) repeatedly with intervals of 4-8 month. Personality scores (PS) were derived from each NOT using principal component analysis: explorative (number and duration of contacts), bold (latency until first approach and contact) and sociable (number of vocalisations and latency until first vocalisation). Additionally, a NOT was performed on a group of cows in the home pen using a different object (NOT2). While cows were in the milking parlour the object was placed in the middle of the pen hanging freely at 1.5m height, accessible from all directions. The movements of the cows and the object's position were recorded using an indoor positioning system (metraTec GmbH), for up to two hours after the cows returned to the pen. Here, a contact was defined as a distance <1.2m between the position of the cow and the object. A linear model tested whether the measurements of NOT2 were associated with the PS of NOT1 (either the first or the latest), with the PS as dependent variable, lactation number (1: 1, 2: 2, 3: ≥ 3) and lactation day (1: <105, 2: 105-210, 3: >210) as fixed effects and a measurement of NOT2 as covariate. The results show no significant effects if using the latest PS of NOT1. However, using PS of the first NOT1, there was a positive effect of the mean contact length on the explorative trait ($p=0.009$, $n=37$), a positive effect of the latency to approach the object from 9m to 3m on the bold trait ($p=0.010$, $n=32$) and a positive trend of the mean number of cows being in contact with the object at the same time on the sociable trait ($p=0.099$, $n=29$). This study shows the possibility to assess animal personality in larger groups in production environment. However, it also shows the importance of ensuring the novelty in NOT even with larger intervals between then tests.

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Theatre 1

Work satisfaction as a component of social sustainability – results from Austrian dairy farmers

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Farmers are often affected by mental health issues. Work satisfaction is of high importance also in the broader context of social sustainability and, as recognised in the concept of One Welfare, interconnected with animal welfare. We hypothesized that farmers in innovative farming systems would show higher work satisfaction than those in reference systems. 116 dairy farmers from 50 family-run farms, 25 with cow-calf contact rearing (CCC, Innovative) and 25 with early separation (Reference) filled in a questionnaire with 5 themes on work/life satisfaction as well as physical and mental load (PML). Five-point or, for general life satisfaction, 11-point Likert scales were used wherein higher values correspond to higher satisfaction or, for PML, higher load. Principal Component Analysis revealed one factor for each theme. 85% of farmers were (very) satisfied (score ≥ 4) with their profession, 55% with their workplace and 83% with their life in general; no person was (very) unsatisfied (score ≤ 2) with those themes. PML was reported to be (very) high (score ≥ 4) by 20%. 33% were (very) satisfied with their free time, while 28% were (very) unsatisfied. To test for system differences, linear regression models were calculated including the independent variables farming system, gender, organic status, number of calves and of cows. Farmers on CCC farms assessed their PML lower (regression coefficient $B=-0.278$, $p=0.044$), but other themes were unaffected. Number of calves was negatively associated with free time ($B=-0.44$, $p=0.011$) and workplace satisfaction ($B=-0.02$, $p=0.013$). Our hypothesis was confirmed partly. The data revealed quite high satisfaction with life as farmer and life in general despite dissatisfaction with free time and high PML in some farmers in our sample. A bias due to convenience sampling can be expected. The data will be used for an integrated farm-to-fork evaluation considering animal welfare, social, ecological and economic sustainability in the scope of the project “COWLEARNING” (funded by FWF CM 400B).

Dairy farm systems analysis

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As part of the EU FreeWalk project, 41 farm and herd variables were assessed on 20 twin pair of cubicle – free-walk barn systems spread over six European countries during six farm visits in a two year period. It resulted in inclusion of 240 farm units (40 farms x 6 periods). About half of herds were grazing and a small number was farming organically, while the majority had a milking parlour. The goal was to discover useful relationships between variables illustrating farming systems. For this purpose, a clustering of variables was done using Principle Component Analysis (PCA, Varimax rotation). A component represents a set of variables (factors) that appear to be closely associated. It is common use to give that set of variables, the component, a meaningful name. Because variation between variables should not widely differ using the PCA method, the variables were standardized (mean divided by standard deviation). When looking at the results of PCA runs, the outcomes were quite easily to explain and reflect common scientific know-how about relationships at farm and herd level. This was encouraging. It was chosen to work with 7 components ($R^2 = 0.59$). The results indicate a strong clustering of variables associated to the component “grazing versus no grazing” and the same for “cow cleanliness/body scoring traits” and “fertility/calving interval traits”. Similarly, “organic versus conventional farming” characterizes the herd size and productivity level. Organic farms tend to have a bit more dirty cow teats and higher indications of mastitis. The freewalk housing system is associated with less leg injuries and lameness and tends to have a bit higher cell count compared to cubicle housing. Higher temperature and lower humidity in the barn are associated with lower milk fat and protein percentages. The use of 140 farm units based on 40 farms is not a most optimal method to use PCA, as well the inclusion of four binary variables, i.e. milking, grazing, housing and eco farming or not. However, two standard tests for checking the suitability of the dataset for PCA-use (Kayser-Mayer-Okin test for normality and Bartlett test of sphericity) did show satisfactory values.

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Consumer Perception of Climate Adaptation Strategies in PDO Cheese Production in France

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PDO (Protected Designation of Origin) cheeses rely on a strong territorial identity and the valorisation of local resources, as defined by strict specifications. Climate change challenges this balance, particularly in terms of feed availability, which directly impacts dairy farmers' ability to maintain PDO production standards. To adapt while preserving product quality and economic viability, farmers are exploring various adaptation strategies. As part of the ADAoPT research and development project, which helps PDO cheeses to adapt to climate change, we investigated consumer acceptability of these adaptation measures. Key questions included: Are these strategies aligned with consumer expectations? How might changes in production conditions affect the perceived quality and taste of the cheese? Two focus groups—one in a rural and one in an urban area—gathered insights from around 15 consumers on different adaptation strategies. AI-generated images helped assess both positive and negative reactions to potential changes. The workshops concluded with a willingness-to-pay exercise on Picodon PDO, evaluating the price consumers would be willing to pay for a cheese integrating one of these climate adaptation strategies. Findings reveal shared concerns across both groups. Some measures, like photovoltaic shade structures and water reservoirs, were viewed negatively mainly due to their landscape impact. Purchasing feed from outside the designated production area was strongly rejected as it contradicts PDO principles. The acceptability of grazing and outdoor access adjustments (e.g., keeping animals indoors in summer, allowing outdoor access in winter) raised concerns, despite already being practiced under certain climatic conditions. Interestingly, temporary production halts during dry periods, which would lead to cheese shortages in stores, were considered more acceptable. Meanwhile, introducing new forages (e.g., sorghum) sparked curiosity, particularly regarding potential changes in cheese taste. These insights highlight challenges and opportunities for PDO dairy sector adapting to climate change while maintaining consumer trust. Understanding consumer perceptions is key to designing strategies that align with market expectations and ensure economic sustainability and PDO values.

Operationalising "One Welfare" : challenge and opportunities

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The "One Welfare" concept emerged in the 2010s in recognition of the interdependence between human well-being, animal welfare and the environment. In the context of livestock farming, this approach could provide a way of mitigation of the sometimes contradictory constraints to which farmers are subject. The objective of this project was to lay the foundations for future operationalization of the concept. To this end, the first step consisted of building a multidisciplinary team and fostering mutual acculturation between the partners (ethologist, ergonomist, psychologist, sociologist, zootechnician, animal welfare specialist). To achieve this goal, a model for understanding the concept was developed based on the group's expertise and literature reviews. The second step consisted of a series of semi-structured interviews conducted with stakeholders (n=25; technicians, professional managers, researchers, economic stakeholders) in the cattle industry to gather their vision of the One Welfare concept, their opinions on the value of developing a methodology to support farmers based on this approach and their perspectives on how this methodology could be deployed. Finally, in a third phase, various interviews with farmers were conducted with the aim of conducting preliminary tests of a questionnaire designed to understand how farmers reason and manage the potential interdependencies between their own well-being, that of their animals, and the connection to their agroecosystem. Drawing on this preliminary experience, we propose to outline avenues of reflection for progressing towards the operationalization of a "One Welfare" approach in livestock farming.

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Dairy farm concepts targeting at societal appreciation: two cases in the Netherlands

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The dairy production chain is an important pillar of the European food system. It delivers a broad range of high-quality end products, and converts human inedible biomass into food. In the Netherlands, the dairy sector produces 14.7 billion kg milk/year, being 9.5% of EU production. The Dutch sector comprises over 14,000 farms and the dairy chain offers employment to 45,000 fulltime units. The export value is 10.3 billion Euro, with 77% being export within the EU. However, dairy farming does not only produce milk, but a diverse range of ecosystem services as well. These include other production services, like supplying biobased materials and energy; regulating and supporting services, such as water management and maintaining soil fertility; and social services, like care and recreation. In the Netherlands, dairy farming is the largest user of space. About 65% of the cultivated land is used for grass and forage crops, and 83% of the dairy farms applies grazing. Hence, dairy farming strongly determines the appearance of the cultural landscape. Prospects of farmers organisations and research institutes for the next decades indicate a considerable diversity in future dairy farming systems. This diversity will be a result of regional differences in for example soil type and agro hydrological conditions, and of entrepreneurial characteristics, economic feasibility and societal demands. The current challenges in the sector include low greenhouse gas and nitrogen emissions, adaptability to climate change, securing animal wellbeing, and fostering biodiversity. These societal challenges require a completely different cow house concept. This presentation highlights two dairy farms concepts which are currently being developed in the Netherlands. Innovation centre Dairy Campus is developing an "EVA barn" for 250 dairy cows. This concept aims to integrate emission-free housing, with high animal welfare standards, autonomy for energy and water, and a high level of automation to minimise labour input. Agro-innovation centre De Marke is developing an integrated farm approach with an 80 cow free-walk barn to foster animal wellbeing, low in emissions and able to produce different manure types that match the needs of the soil and crop rotation plan.

The movement towards socially responsible dairy farming in the USA

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Dairy farming has been under increased public scrutiny for up to two decades now in the USA. This scrutiny stems from (non)consumer interest in the origins of their food, and views on animal welfare and the environmental footprint of dairy production. The objective of this review is to give an overview of responses and proactive steps the US dairy sector has been taking to maintain a social license to produce. The USA is home to over 9 million dairy cows. While this number has not changed much for the last 2 decades, the number of dairy farms has decreased from over 70 000 to 26 000 today. Consequently, the average herd size has increased greatly. However, there is little evidence that the US public sees larger dairy farms as undesirable. Nevertheless, the low density of dairy farms means that few people have first-hand experience with dairy farms. Open house programs such as Breakfast on the Farm provide the public access to dairy farms and show how dairy production takes place. Two decades ago, the dairy sector was more contentious about public scrutiny of production practices and believed that public education was the main solution. Since then, the sector has embraced programs such as Farmers Assuring Responsible Management (FARM), that use science-based standards, verifications, metrics, and other tools to improve best management practices in areas of animal care, antibiotic stewardship, biosecurity, environmental stewardship, and workforce development. The goal is to continue to show customers and consumers that the dairy sector holds itself to the highest standards. The dairy sector is also responding to calls to reduce its environmental footprint by conducting lifecycle analyses and investing in impact reducing feed additives, digesters, genetics, and water saving technologies. The US dairy sector widely recognizes that socially responsible dairy farming must increasingly consist of practices that are good for planet, people, and profitability.

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Dairy sector in Ukraine

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In 2025 total nr of cows is 1177.7 thousand, which is 377.6 thousand less than just before beginning of the full-scale war. 68% of the cows is kept in households, which number has decreased significantly. The nr of cows in agricultural enterprises also decreased, but the share increased by 4.8% compared to 01.01.2022. In 2024, there were 1309 dairy farms. 84.7% of farms had less than 500 cows, but 55.2% of cows were kept on farms with more than 500 heads. Ukraine produced 7.2 million t of milk, which was 21.0% less compared with yr 2021. Milk production from households has decreased by 28.5% since 2021, milk production in agricultural enterprises has increased by 6.7%. In 2024, volume of milk delivered for processing reached pre-war levels totaling 3.223 million t. The quality of milk supplied for processing has generally improved. The amount of “Extra” milk in 2024 was 1.6-times higher than in 2021. This may be explained by the structural decline in smaller farms which result in higher overall quality milk. There are 120 registered dairy processing plants, with the biggest plant processing 600 t and smallest ones about 30 t of raw milk per day. All are privately owned and several are international dairy processors, of which 38 have a permission to export products to EU. Cooperative dairy processing is not developed due to consequences of the Soviet regime. Last 10 yr, small dairy farms have started developing small-scale dairy processing, so called craft production and added-value creation. Main challenges for dairy farming are: survival in the circumstances of the full-scale invasion; lack of personnel due to mobilization and forced migration; relocation of enterprises from war-affected regions; demining fields to ensure fodder production; electricity generators supply for farms and plants from blackouts affected regions and diversification of energy sources; adherence to anti-epidemic measures due to recent epizootic outbreaks; awareness raising and financial support of modernization of farms and plants to meet EU integration requirements on animal welfare and health, food safety, environment and climate, and a level playing field for agricultural trade with EU.

What Breeding Goal Should Organic Dairy Farmers Pursue? Results from a Willingness-To-Pay Study Among Consumers in Denmark, Germany, and Sweden

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There is a growing public focus on negative consequences of dairy production, including carbon emissions, biodiversity loss, and limited welfare for production animals. Many of these concerns can be significantly mitigated through innovation, such as enhancing traits in breeding goals. However, enhancing traits to address one issue may compromise the ability to effectively tackle other problems. For example, there may be a negative correlation between traits that help to limit carbon emission per kg milk and those that improve animal welfare. The organic dairy sector, in particular, is at a strategic crossroads, needing to identify the most important potential breeding goals to implement. This study aims to determine what organic consumers in three European countries—Denmark, Germany, and Sweden—are willing to pay (WTP) for four specific breeding goals. Questionnaire data were collected by the survey bureau Norstat (appr. n=1500 per country). WTP was studied for two products: organic cheese and organic milk. The four traits in the breeding goals were: Lower carbon emission (CARBON), Better animal welfare (WELFARE), Higher milk yield (YIELD), Healthier dairy products (HEALTH). WTP for the different attributes were estimated in random parameters logit models using the software NLOGIT 6. In all countries, and for both products, WELFARE, turned out to be the goal consumers were willing to pay the most for. HEALTH and CARBON had the second highest WTP while the WTP for YIELD was very limited. In Denmark and Germany, we found a positive relation between organic consumption and WTP for WELFARE and CARBON. In Sweden, a similar relation was not identified. Our results leave no doubt that, when innovating through breeding, organic dairy breeders and farmers should prioritize traits that enhance the ability of dairy animals to experience good welfare in organic production systems.

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Farmers' perspectives on grazing: a comparative study across eight European countries

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Grazing has been demonstrated to generate multiple benefits, including increased farm profitability, biodiversity conservation, cultural landscape maintenance, and improved animal welfare. However, the extent to which these benefits are realised is contingent upon the grazing intensity and how grazing practices are implemented, which are influenced by regional contexts and farmers' attitudes. This study investigated livestock farmers' perceptions on grazing as part of the EU-funded project Grazing4AgroEcology (GA 101059626) across eight European countries: France, Germany, Ireland, Italy, the Netherlands, Portugal, Romania, and Sweden. In Italy, two regions – South Tyrol and Sardinia – were selected to represent Alpine and Mediterranean environments. To achieve insight into farmers' perspectives, a comprehensive online survey was administered in 2023 in the respective national languages. The survey was disseminated through multiple channels, including newsletters, agricultural journals, and social media, yielding approximately 1,500 valid responses. It assessed farmers' perspectives on key aspects of animal husbandry, the perceived importance of external influences, and the primary constraints and incentives associated with grazing adoption. The results reveal significant regional differences in how farmers approach grazing and provide critical insights for policymakers, educators, and agricultural stakeholders. By identifying the factors shaping farmers' decision-making, these findings can support the development of targeted interventions to mitigate barriers and enhance the adoption of sustainable grazing practices.

Scientific research must contribute to a more objective vision of livestock farming

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Meat and dairy products, and more generally livestock, are the subject of incessant criticisms and attacks, often accompanied by significant amounts of misinformation. In this context, the Dublin Declaration on the Societal Role of Meat, published in 2022 and signed by more than 1200 experts, encouraged scientists in this field to alert policymakers, the media and the general public to the importance of building perceptions of livestock and dietary recommendations on solid scientific evidence. Scientists in animal science and related fields do so conscientiously, often at the cost of becoming the target of activist campaigns, aimed at discrediting troublesome scientific voices. Despite divergent views among signatories to the Dublin Declaration regarding how to implement scientific findings or on the nature or size of future livestock systems, and despite different contexts all over the World, there is a strong consensus on the critical importance of maintaining rigorous scientific approaches to ensure informed debate on livestock and its products. This shared commitment led to the publication of the Denver Call for Action, announced at the second International Summit on the Societal Role of Meat and Livestock in Denver in October 2024, where the latest scientific findings were presented. To further fuel the scientific debate and ensure that scientific evidence reaches key audiences in clear and accessible formats, the International Meat Research Foundation has created an open-access scientific library compiling key studies on these subjects. By leveraging multiple communication channels, this initiative aims to bridge the gap between research and decision-making, fostering a balanced public discourse and enabling well-informed choices about livestock production and its role in global food systems. Meeting the colossal challenge of feeding the world's growing population while minimising environmental impact will only be possible through the transparent application of scientific rigor, without dogmatism.

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How future breeders and advisers understand their position in relation to society's expectations

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This project Entr'ACTES seeks to gain a better understanding, from a sociological point of view, of what it means to be involved in livestock farming (LF) today, and the dynamics of change underway in a context where this activity is increasingly being questioned by society. The project aims to 1-Improve understanding of the challenges facing the LF, by producing knowledge on the perception of the LF and 2-Contribute to the sustainability of the LF by supporting changes in practices by taking greater account of societal issues. As part of this, we set up 5 focus groups to analyse how future breeders (FB) and future advisers (FA) perceive the profession: 3 focus groups with 32 FB (8, 11 and 13 students) and 2 focus groups with 20 FA (10 and 10 students). Each focus group was divided into 5 steps: 1-What is your vision of LF in the future? 2-What do you find easy and difficult to do in your future job? 3-What do you think will have the greatest impact on LF in the next 20 years? 4- What will your job be like in 2050? 5-Do you think you are sufficiently prepared for the future? The results show that, while the societal challenges are perceived by all (environment, animal welfare and food quality), there is a clear split in their perception of these challenges. The FB express annoyance and relativisation when faced with the constraints of taking these issues into account while the FA were concerned, worried and felt very much involved by these issues. As motivations, the FB emphasised the usefulness of the job, the FA their good technical knowledge and both underlined the appeal of new technologies. About fears, the FB emphasised the difficulties of setting up (access to land, cost), the profusion of regulations, competition between systems and countries and strained relations with society, while the FA mentioned lack of training in human and social relations, the increasing complexity of systems and the reluctance of breeders to change. In conclusion, the students enjoyed taking a step back through this exercise, but it is clear that FBs are more reluctant than FAs to commit to meeting society's expectations.

Integrating animal welfare as part of sustainability assessments

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In the pursuit of more sustainable animal production, more intensive production systems are often favoured because systems with high production efficiency, all other things equal, can be less damaging to the environment. This creates a risk of trade-offs, e.g. between reducing greenhouse gas emissions and promoting good animal welfare (AW). However, there are also production systems and best practises with opportunities for developing synergies between animal welfare and sustainability. To identify and further develop these potential trade-offs and synergies, proper tools for assessing multiple aspects of sustainability are needed. Several standard procedures already exist for assessing environmental impacts, e.g., life cycle assessments (LCA), but similar tools for AW assessment are not yet developed. We have reviewed the literature to evaluate methods used so far to integrate AW into sustainability assessments. To allow for comparison of AW impact across species and production systems, it is necessary to relate the AW score to a comparable functional unit, e.g. impact per kg produced meat. However, papers doing so report that the AW score is much more sensitive to the number of animals required to produce a functional unit, than to AW-promoting initiatives. Most papers mainly include indicators of negative AW, with the results that even a small reduction in AW will be magnified in species where many more animals are needed to produce a functional unit, compared to species where fewer animals are needed, e.g. poultry compared to beef cattle. The strong negative effect on the overall welfare of number of animals used per functional unit will be counterbalanced if the assessment also includes an equal number of indicators of positive welfare. The objective of our presentation is to discuss the further development of AW assessment tools in connection to sustainability assessments. This work is funded by “Danish Knowledge Center for Animal Welfare” and co-funded by the European Union’s Horizon Europe Project 101136346 EUPAHW.

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Poster 13

Consumer knowledge and market challenges for sustainable and artisanal cheese in Spain and France

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INTRODUCTION: The cheese industry in Spain and France plays a key role in cultural heritage and local economies, particularly in the POCTEFA cross-border region, where traditional cheese production is deeply linked to rural development. However, changes in consumer habits and challenges in the artisanal production sector require an in-depth analysis of market trends. **OBJECTIVE:** This study aims to diagnose the knowledge, requirements and opportunities related to food sustainability on the part of consumers and producers in the POCTEFA cross-border region of Spain and France. **MATERIAL AND METHODS:** The study followed a three-phase methodology: 1) Semi-structured interviews were conducted with both consumers in Spain and France; 2) Focus Groups with consumers in both countries; 3) Survey was launched to quantify consumer perspectives in both countries. **RESULTS AND DISCUSSION:** The research indicate that sustainability is not a decisive factor in the purchase of local and artisanal cheeses. 40% and 15% of consumers primarily prioritize taste and price, respectively. While economic and social sustainability do appear as secondary considerations, they are not explicitly recognized as sustainability-related factors. Instead, consumers express these concerns through motivations such as supporting small producers and contributing to the local economy. Environmental sustainability has little impact on cheese purchasing decisions. **CONCLUSION:** Despite being neighbours, Spain and France have distinct cheese consumption habits. However, sustainability plays a minor role in both, perceived more as support for local producers than an environmental concern. But it is true that France shows greater ethical awareness. The results show a need for producers to communicate these values in a way that consumer can prioritize.

New recommendations for housing and rearing calves in dairy cattle herds: Opinions of breeders and experts in Slovenia

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In the dairy sector, calves and other youngstock are often overlooked since their rearing does not bring direct profit. The society is critical of the immediate separation of calves from their mothers and the use of individual pens for unweaned calves. To align with the latest findings and best practices, the European Food Safety Authority (EFSA) has issued recommendations regarding calf housing and movement on dairy farms. New welfare trends include extended contact between mother and calf for the first 24 hours after birth, group housing in pairs or small groups of up to seven calves within seven days, and delaying the transfer of male calves to another herd until at least 28 or even 35 days after birth. These changes, which will impact cattle farmers, were discussed with agricultural consultants and dairy cattle farmers in focus groups. We organized three online focus groups and conducted an online survey. Our quantitative and qualitative findings indicate that consultants generally support the new EFSA recommendations, while Slovenian dairy cattle farmers express certain concerns and reservations. Cattle holdings in Slovenia are traditionally family-owned and small sized with an average number of 16.6 cattle. The biggest challenge for Slovenian dairy cattle farmers is the new EFSA recommendation regarding male calves, which should remain on the farm for a longer period (28 to 35 days after birth). Consultants mostly agree with this recommendation. However, they acknowledge that it entails higher feeding costs for male calves and additional space requirements, as current practices involve selling male calves at the age of 10 to 21 days. Farmers believe that three weeks is the maximum feasible age for Slovenian farms, as most male calves are transferred to neighbouring farms with suckler cows or nurse cows, and for these farmers it is preferable to add an additional calf to the nurse cow as soon as possible, as this minimizes potential issues related adaptation of calf to other cow.

Minor hoof trimming errors were associated with hoof lesions, but softer flooring in walking alleys mitigated their impact in dairy cows

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Lameness control is crucial for extending the productive longevity of dairy cows, with regular, preventive hoof trimming playing a key role. However, improper hoof trimming may negatively impact hoof health, and the consequences of specific trimming errors in different environments remain unclear. This controlled study in a Swedish research barn examined pressure distribution under the hind hooves of 113 Holstein cows (primarily in their 1st or 2nd lactation) after functional hoof trimming. The post-trimming pressure distribution was estimated in standing animals using an I-Scan system (Tekscan Inc., Boston, MA), and the pressure sensor 5250 CMP with 3.202 sensing elements per cm². The pressure sensor was placed on a specially made stainless steel plate (2 mm thick) and was covered with a 0.12-mm-thick polytetrafluoroethylene dipped glass-fibre fabric and a 1-mm-thick rubber cloth. Hoof lesions were recorded after the trimming at an average of 170 days of housing on different flooring types in walking areas: rubber (n=46), concrete (n=18), and mastic asphalt (n=49), under otherwise identical management conditions. Binary logistic regression analysis revealed a significant association between higher post-trimming hoof pressure and the development of moderate to severe sole haemorrhages. This association was evident in cows housed on hard floors (concrete and asphalt) for both peak pressure (P=0.023) and average pressure (P=0.033) but was not observed in cows on rubber flooring. However, overall sole haemorrhage prevalence did not significantly differ between flooring types. These findings suggest that trimming-induced pressure irregularities may contribute to sole haemorrhages, but softer flooring can help mitigate this negative effect.

Preliminary results from a survey on environmental perceptions of inner areas

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A web-based questionnaire assessed environmental impact perceptions and key farming challenges in marginal areas of Italy. The surveyed stakeholders (n=130) are mainly farmers, breeders, agronomists and researchers. The educational level accounts for 26.6% high school, 53.1% university degree, 18.5% higher education. Most of them are Italian (99.2%) and women (57.7%), with 62.3% aged 18-25, 23.8% aged 26-40, 10.8% aged 41-60, and 3.1% aged over 61. Geographically, 47.1% live in the North, 23.5% in the Centre, and 29.4% in the South of Italy. Out of 130 interviewees, 94.6% have an omnivorous diet, 2.3% are vegetarians and 1.5% vegans. Additionally, 5.2% are animal rights activists and 26% are environmentalists. While 60% of respondents felt to be fairly informed about protected areas, 46.9% had limited knowledge of productive activities' role and effects within them. The primary information sources are the Internet and social media (61.5%). Climate change (36.9%), hydrogeological disruption (29.2%) and an increase in wildlife (26.2%) are having a significant impact on respondents' daily lives. Major threats to protected areas are considered industrial and urban expansion (26.9%), pollution and littering (43.8% each), but also poor pasture management (20.8%). About 30% of respondents deemed national park policies effective. Among surveyed farmers (n=13), dairy cattle (Holstein, Jersey, Romagnola breeds) are raised in semi-intensive or semi-extensive systems mainly across rural areas of Italy. The average herd consists of 85 heads. The main challenges of breeding practices are extreme weather events, wildlife-related to crop damage, and fluctuating of public funding. The survey was approved by the Bioethics Committee of the University of Molise. The research is supported by project PRIN 20224L4WSR, funded by Next Generation EU (CUP: H53D23005120).

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Poster 17

Dairy Cattle Breeding Simulation Program (DCBSP) V.6.03: A Dynamic Environment for Teaching Animal Breeding and Genetic Improvement

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The Dairy Cattle Breeding Simulation Program (DCBSP) is a virtual environment for simulating dairy cattle herds, focused on teaching animal breeding and genetic improvement. Its origins date back to the late 1950s at Michigan State Univ. (East Lansing, MI, USA), where L.D. McGilliard developed the first versions of the program in FORTRAN language on punched cards. At that time, selection was focused solely on milk yield and it was not until the 1970s that M. L. McGilliard and D. Edlund (Virginia Tech Univ., Blacksburg, VI, USA) extended it to a multi-trait context. The 1979 version allowed simulation of up to 100 herds evaluated by selection indices, and was used internationally in 49 universities. Later, J.F. Medrano (Univ. California-Davis, CA, USA) coordinated and promoted the program updates from the 1990s onwards, including BLUP evaluation without herd limits (v.3.4., 2007) and genomic evaluation (v.5.07., April 2016). The inclusion of fertility rate as a reproductive trait in the genetic/genomic evaluation framework of DCBSP makes a very relevant change in the simulation scheme, since for the first time it allows selection for a phenotype that directly affects the contribution of the cows in the herd. In this sense, both the intentional selection on fertility trait and the indirect selection (by genetic correlation with the other phenotypes) can directly affect the open days of each cow/calf and, consequently, the number of calves available for selection in each round. The possibility of inseminating with sexed semen (with or without additional economic cost) seeks to provide an additional and realistic option for the management of future replacement. In parallel, web-based supports were developed for the interaction between users and the DCBSP. The DCBSP v.6.03 and its web support allows a practical and realistic approach to teach animal genetic and genomic selection including productive and reproductive aspects of a dairy herd with a special emphasis on selection for production traits.

Future-proof housing of hyper-prolific lactating sows

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Objective There is a growing global human population and demand for animal protein. This leads to emphasis in future pig production on the production being sustainable and continuously improving the sustainability. Elements to include and improve are production efficiency, pig welfare, social acceptance, job satisfaction and environmental impact which needs to be addressed in the way we house our sows and piglets. **Production efficiency** Increasing piglet survivability reduces the number of sows needed to maintain production level. A reduction in herd size of one sow reduces the annual emission by 521 kg of CO₂e and if including feed production, it's an annual reduction in emissions of 1,550 kg CO₂e. Besides genetic improvements in piglet survivability and litter gain, piglet survivability can be improved by ensuring sufficient colostrum and milk supply for the piglets and supporting the sows nursing capability. This can be done by including research in nutrition of neonatal babies and by increasing our understanding of innate piglet behaviours and preferences and meeting the piglets' need especially in the first days of life when they are most vulnerable. **Pig welfare** Loose housing of lactating sows is a very important step in improving sow welfare. Reduction in use of confinement from day 4 post farrowing and until weaning will reduce the number of days in confinement by 47-50 days a year for each sow; so for every ten sows that is 470-500 days annually without confinement. This is a very important improvement as when confined sows must eat, drink, rest and dung in the same position which they would never do when not confined. In addition, loose housing improves the piglets' access to the udder and reduces fights for teats. **Environmental impact** Understanding the sow behaviour enables designing pens with either a partly solid flooring and reduced surface area of slurry which reduces the ammonia emissions and enables frequent emptying of the slurry, thus reducing methane emissions. Or if using fully slatted flooring, there are upcoming technologies which reduce the slurry surface and support frequent emptying of tanks. **Perspective** A sustainable concept of housing lactating sows can attract skilled labour and increase job satisfaction where 'proud to produce' can become another important element of license to produce.

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Theatre 2

Impacts of three types of farrowing pens (crate, temporary confinement with lift crate and temporary confinement) on the performance and behaviour of sows and piglets

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A trial was performed on 264 sows from 11 different batches of 24 sows. In each batch, 8 sows were housed in a farrowing crate (C) and the others in a pen with temporary confinement from the Monday of the farrowing week until 5 days after farrowing, with (TL, N=8) or without a lift function (T, N = 8). Video recordings were made of sows from each treatment in several of these batches for a total of 25 sows per treatment, to monitor farrowing duration, time taken for four piglets to access the udder and occupation of the pen by the piglets for 24h. They were also observed during 4 hours, the morning before the stalls were opened (D5), and at D6 and D8 in order to register sow postures, the frequency of suckling before and after the stall was opened, and the occupation of the pen by the piglets. Direct observations were performed on all sows and litters at about 10 days to assess the quality of the mother-piglets relationship through 10 scans during 4 hours. Changes in litter size, piglet weight and causes of mortality were recorded from birth to weaning. The data were analysed using R software with linear models (packages glmmTMB and lme4) after variable transformation if necessary. Causes of death and variables from direct observations were analysed by Chi-square. Litter size did not differ among treatments at birth. The lift function decreased losses during the first two days ($P < 0.01$), but the difference was no longer significant at D5 (resp. 13.0%, 13.5% and 15.3% for C, TL and L) and at weaning (resp. 17.0%, 19.2% and 20.5%). We recorded fewer losses due to crushing in TL (20.6% of causes) and more losses due to weak piglets (47.1%) before stall opening. After opening, crushing was a more frequent cause of loss for T (42.6%) than for C (12%), TL being intermediate. Piglet weights were similar among treatments throughout lactation. After opening the stalls, sows spent more time standing ($P < 0.05$), and some of them lay close to the nest, enhancing the bond with their piglets. There were, nevertheless, no effects on the duration or frequency of suckling. The high variability in farrowing duration observed with the temporary crate raises questions about when to restrain sows in these systems.

Animal welfare inspections in organic pig farms in Germany: Measures, Thresholds and Training

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Regulation (EU) 2018/848 on organic production and labelling of organic products mentions the contribution to high animal welfare standards as an important objective of organic livestock farming. In addition, consumers' expectations of animal welfare on organic farms are high. Nevertheless, and despite regular inspections, animal welfare issues in organic farms are not always reliably identified and solved. The project "Animal welfare monitoring in organic agriculture — improving animal welfare and making it transparent (<https://bit.ly/OeKoTier>)" aims to develop a standardised inspection concept comprising primarily animal-based measures for the annual animal welfare inspection in organic farms. This concept should enable the reliable identification of farms with severe animal welfare issues in order to initiate improvements and, if necessary, derive measures and sanctions. In a large project team including three research institutions, four organic associations and three inspection agencies proposals for pigs, sheep, goats, cattle and poultry are being developed. For this purpose, indicator sets for the on-farm assessment by inspectors, including animal-based measures, other findings and resources, were elaborated. For pigs, the animal-based measures mortality, lameness, bursitis, manure on the body, body condition score, runts, tail lesions, ear lesions, skin lesions on the head and carpal joints in suckling piglets, hospital pigs as well as lung and liver findings from meat inspection were selected. In addition, an evaluation framework with intervention thresholds for each indicator will be developed to classify the results of animal welfare inspections. These thresholds refer to the prevalence above which inspection agencies should take further action to clarify potential violations of Regulation (EU) 2018/848. As part of the project, the feasibility of the developed inspection concept will be tested in 40 organic pig farms. In addition, online and on-farm training materials on the concept will be designed for inspectors.

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Theatre 4

The effect of social experience on aggressive interactions and play behaviour when introduced to a large group of unfamiliar pigs

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In recent years, interest has grown for new innovative pig housing systems keeping finisher pigs in large (<450 pigs) groups. The housing system includes a centrally placed sorting scale, facilitating handling of individual pigs within the large group. However, knowledge about social interactions and pig welfare in the housing system is limited. This study aimed to investigate aggressive interactions and play behaviour during the first 72 hours post mixing when introduced to a group housing system in the finisher unit. The study was conducted on a Swedish commercial farm in 2024. Three treatments were applied with one replication (202-293 pigs/batch): (1) pigs remained in their own litter (8-20 pigs/pen) kept intact during the nursing and weaner phase, (2) pigs were grouped with one other litter from birth (17-31 pigs/pen) kept intact during the nursing and weaner phase, and (3) pigs were mixed during the weaner phase according to weight at 50 days of age (9-15 pigs/pen). Each batch was moved to the finisher group housing system at about 80 days of age. Aggressive interactions and play behaviour was observed after mixing, using scan sampling at 5-minute intervals, during 1 hour on 12 separate occasions from mixing until 72 hours post mixing. Preliminary results indicated presence of aggressive interactions throughout the observation period in all treatments, with a significant decline over time, particularly within the first 2 hours. Pigs in treatment 1 showed significantly more aggressive behaviour the first two hours post mixing compared to the other treatments ($p < 0.05$). Play behaviour occurred in all groups, with significant more play outs registered for treatment 3 in comparison to treatment 1 and 2 ($p < 0.05$). To conclude, increased social experience during the weaner phase seems to have a positive impact regarding aggressive behaviour and occurrence of play behaviour when later being introduced to a large group of pigs in the finisher unit compared to pigs that have only been raised in their own litter.

Impact of housing design and animal level ventilation on pig growth and air quality

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Housing design and ventilation management in pig fattening systems may affect production performance and air quality, mainly under heat stress conditions. This study evaluates the impact of floor type and auxiliary ventilation on pig growth and air quality. The experiment was conducted at the experimental farm CITA-IVIA (Segorbe, Spain) with 128 Duroc x (Landrace x Large White) pigs, divided into a control group (C) with slatted floor and an experimental group (EXP) with mixed floor (33% solid) and additional ventilation at the animal level in the last feeding phase. Animal growth, performance and emissions of NH₃ and CO₂ at the room level were measured. Dräger X-node sensors were installed to measure CO₂ and NH₃ concentration. To determine emissions, a mass balance approach was followed, following the VERA protocol. Ventilation rates were estimated using CO₂ mass balances. CO₂ production was calculated using the formulas of CIGR (2002), based on heat produced by the animals, their estimated weight and the ambient temperature. Initially, the EXP group showed lower weight and feed conversion rate, but these differences disappeared over time. Additional ventilation did not affect growth of the animals. Regarding emissions, the EXP room reduced CO₂ due to better ventilation but increased NH₃ by 40%, due to higher slurry exposure in the solid zone and higher volatilisation. In conclusion, although floor type and ventilation did not significantly influence animal performance, they have a clear effect on air quality. This highlights the need to develop management strategies that balance the reduction of emission and optimise animal production.

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Theatre 6

Greenhouse gas emissions and slurry pollutants across different pig genetic lines

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Swine production contributes to greenhouse gases (GHG) emissions and boosts ammonia release. Most of this gas pollution comes from slurry emissions (e.g. CH₄ and N₂O). This study aims to characterize the GHG and ammonia production and its relationship with production traits across pig genetic types. A total of 762 pigs from 9 different crossbreeds were raised from 80 to 165 days old at IRTA facilities. Also, the effect of 8% dietary protein reduction was tested in one of the experimental groups. Individual body weight and feed intake were recorded throughout rearing, and room air and slurry composition were measured at three time-points. Least-square means comparison and residual correlation analyses were performed. The concentration of GHG (g of CO₂ equivalents per m³ of indoor air) and ammonia (g/m³ of indoor air), as well as the total phosphorus and total ammonia nitrogen in pig slurry (g/kg), varied across genetic lines (coefficient of variation: 30-35%). Excluding the animals within the protein trial, the genetic type with the highest average daily feed intake and growth rate had the highest air concentration of methane and ammonia. Also, the genetic group with the highest feed intake yielded the highest total phosphorus concentration in slurry. Residual correlations indicated a positive association between the pigs' body weight at sampling time and the indoor concentration of CO₂ equivalent, driven by CH₄ and N₂O room concentration. In turn, CO₂ equivalent concentration tended to be negatively correlated with that of ammonia. Feed efficiency, measured as feed conversion ratio, was not significantly associated to slurry or air pollutant concentration. Dietary protein reduction lowered the concentration of slurry ammonia nitrogen by 20% and air ammonia by 50% without significant impairment in production performance. These results support the link between performance outcomes and pollutant concentrations (in both air and slurry) and dietary protein reduction as an effective mitigation approach to lower the release of nitrogen compounds in growing pig barns.

Exposure to ammonia and particles: how to prevent and protect workers in pig farms from respiratory risks?
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People working in pig farms are exposed to concentrations of ammonia (NH₃) and particles that can cause respiratory problems such as coughing, asthma or bronchitis. To limit this, two categories of measures can be implemented: the preventive one aimed at reducing the concentrations of pollutants in the air and the protective one to protect workers. The lack of information on these measures prevents their implantation on the ground. The objectives of this study are (1) to identify the preventive measures applicable in farms and the protective measures available on the market and (2) to evaluate them (effectiveness, ease of implementation, cost). The preventive measures were identified via a review of the scientific literature (102 references) and surveys of 13 breeders and employees. The protective measures available on the market were identified by carrying out research with the main suppliers of respiratory protection masks and through discussions with MSA preventers. The means of prevention and protection best suited to pig farms were selected using a multi-criteria grid and the opinion of breeders. At the end, 60 preventive measures have been identified (human: 9, organizational: 23, technical: 28). Eighteen protective measures have been selected and evaluated in breeding conditions: Technical sheets have been written per measure (preventive and protective ones) including its principle, an associated price range as well as all of the evaluation criteria identified. Opinions of breeders and the experts of the project have been integrated to these sheets. The availability of these technical sheets to pig farmers should help provide them the necessary information to encourage them to better integrate air quality and occupational exposure in their working conditions. The next step of the project is the development of a smartphone application allowing farmers to assess the air quality in their buildings and find appropriate prevention and protective solutions.

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Theatre 8

Pig growth, body composition and nutrient balance with local protein sources no mineral phosphate, but with amino acid and phytase supply

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Formulating diets for growing pigs that are low in protein (CP) and phosphorus (P), with minimal use of imported protein-rich feedstuffs and mineral phosphates (MP), while meeting digestible amino acid (dAA) and phosphorus (dP) requirements, is a challenge for sustainable pig production. This study formulated grower (40 kg) and finisher (60 kg) diets using local protein sources and no MP and evaluated effects on growth, body composition and nutrient balance. Forty-eight Swiss Large White pigs (12 blocks of four female or castrated male littermates) were housed in one pen and assigned to 4 iso-energetic ad libitum fed diets: Control (C), N-, P- and NP-. In N-, soybean meal was replaced with more rapeseed cake, protein beans and with five additional limiting synthetic AA to minimize CP, while maintaining dAA levels as in C. In P-, MP was removed and Ca:dP was maintained as in C by adjusting CaCO₃. Diet NP- combined both N- and P-. All diets included 500 FTU/kg of microbial phytase. Compared to C, N- diets contained 3% (grower) and 0% (finisher) soybean meal with 140 g/kg (-10%) and 116 g/kg (-15%) CP; P- diets had 3.4 g/kg (-20%) and 3.0 g/kg (-22%) P. Daily individual feed intake and BW at the start, diet change, and end were recorded. Nutrient excretion was calculated by subtracting nutrient intake from body accretion, determined via dual X-ray absorptiometry at the start, diet change, and end. Statistics included sex, N and P effects and their interactions. No interactions between N- and P- were observed ($P > 0.05$). Overall, growth performance remained unaffected. Body composition remained unchanged across diets, except for reduced ($P < 0.001$) bone mineral content and density (-8% at diet change, -6% at end) in P-. N and P excretion were reduced ($P < 0.01$) in grower and finisher periods by N- and P-, respectively, leading to overall reductions of 20% N and 25% P. These findings show that formulating diets without imported protein sources and MP successfully minimized N and P excretion while maintaining growth and lean tissue composition. Further research is needed to optimize microbial phytase supply in MP-free diets to mitigate bone demineralization.

Association between litter of birth traits and sow longevity

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This study aimed to investigate possible associations between sow longevity and litter of origin traits. Data used was collected between 2019 and 2023 in 11 European L03 PIC commercial multipliers. Lifetime performance records were available on 44909 females. Data included females' dam parity [i.e. primiparous (n=30144, PP) vs. multiparous (n=14765, MP)], litter of origin total born (TB) and born alive piglets (BA), and percentage of females selected as replacement gilts from each litter. For each female, parity at removal (i.e. P0 to P3+), and removal reasons were available. Data were analysed via univariable Cox regression survival models with contemporary group (i.e. farm, year and month when entering the herd) included as random effect. Females born from PP sows were more likely to be removed before P3+ (HR=1.17, CI=1.10-1.25, P<0.0001) and were more likely to die due to health (HR=1.54, CI=1.27-1.87, P<0.0001) and leg (HR=1.42, CI=1.13-1.78, P=0.003) issues when compared with females born from MP sows. Higher TB and BA were associated with improved longevity (P<0.0001) likely due to higher selection intensity. Indeed, a decreased relative risk of death was observed when selection intensity increased. Results suggest that special attention should be given to gilts born from gilts during their developmental period to improve their longevity. Results also highlight the importance of maximising gilt selection options. Further research is necessary to understand the biological mechanisms behind the associations observed in this study and their practical implications in terms of genetic lag and farm profitability.

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Theatre 10

Metabolomics identifies novel biomarkers for fat deposition, feed efficiency and growth in Italian

Large White pigs

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The Italian heavy pig sector relies on key traits that need optimization: feed efficiency, as pigs are slaughtered at a heavier weight; growth, as they have a minimum slaughter age; and the right fat proportion, since their meat is used for high-quality dry-cured hams. Advances in omics suggest that plasma untargeted metabolomics may provide biomarkers to improve evaluation accuracy. This study analyzed over 700 Italian Large White pigs, with random residuals extracted from genetic indexes routinely collected for selection. Pigs were grouped into five pools based on the trait of interest: feed conversion ratio (FCR), average daily gain (ADG), backfat thickness (BFT), lean cut (LC), and ham weight (HW). Machine learning, including the Boruta algorithm, identified discriminant metabolites associated with these traits. The SNP-based heritability of key metabolites was estimated using GEMMA. Boruta analyses detected 8 to 26 discriminant metabolites per trait, with only partial overlap. The most common were sphingomyelins, leucine-, isoleucine-, valine-, and tyrosine-derived metabolites. The highest metabolite overlap was found between HW and LC, sharing ~50%, especially amino acids. The average heritability was 0.25(±0.11), with three metabolites exceeding 0.5. These findings highlight the trait interplay and demonstrate metabolomics' potential in identifying biochemical markers for growth, feed efficiency, and fat deposition. Such metabolites could enhance selection precision for high-quality dry-cured ham production. The research was supported by the Italian PRIN2022 project FEEDTHEPIG, funded by the European Union – NextGenerationEU under the National Recovery and Resilience Plan (PNRR) – Mission 4 Education and research – Component 2 From research to business – Investment 1.1 Notice PRIN 2022 PNRR (DD N. 1409 del 14/09/2022), proposal code P2022FZMJ9 – CUP J53D23018310001.

Rethinking boar taint and quality detection towards a more sustainable pig production

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Raising entire males is the most profitable, sustainable, and animal welfare-friendly approach to growing pigs. However, the main obstacle to entire male pig production is the lack of an efficient, on-line solution for sorting tainted meat at the abattoir. Currently, the human nose is the most common method used, but it suffers from issues of subjective accuracy and challenges in maintaining trained employees. The objectives of this study were to examine the potential of a new system (advanced optics and AI) as an on-line method for detecting boar taint and other quality measures; assess human performance; and determine whether meat quality is affected by higher levels of boar taint metabolites (Androstenone and Skatole). Entire males at three commercial abattoirs (n=20,000) were sorted by human nose on-line, as well as 133 male pigs were evaluated by human nose laboratory at IRTA's research abattoir. Of which, 400 positive samples and 400 negative samples were sent for laboratory analysis of Skatole and Androstenone. Advanced optics and AI demonstrated promising potential for on-line sorting of meat for boar taint and other meat quality measures, with models showing accuracy ranging from 90%-100% compared to chemical analysis and common scaling methods. Human nose results, in comparison to laboratory analysis for Androstenone and Skatole, differed between locations and methods; sensitivity: 50.2%-90.3%; specificity: 48.1%-90.4%. No meaningful significant differences in meat quality were observed between tainted and untainted meat. These results highlight the complexity of using the human nose as a method and the importance of adopting an objective sorting method. This may be a direction for a future of raising entire males as an alternative to surgical castration. Part of this work received funding from PIGWEB, the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101004770.

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Theatre 12

Towards an automated analysis of climatic influences on outdoor area usage by fattening pigs

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In organic farming and in various national animal husbandry certification programs, access to outdoor areas is mandatory for fattening pigs in the EU. The benefits associated with the provision of outdoor areas include the expansion of functional areas and the interaction with diverse climate stimuli and can therefore contribute to animal welfare. The aim of this study is to automatically deduce activity or resting behavior of the animals, throughout a continuous animal detection and a posture classification process. The data used to investigate the pigs' usage of these areas is collected through the TiPP – "Transparency in Pig Production" project. A total of 336 pigs are analyzed over two fattening periods between December 2024 and July 2025 on a pig farm in Lower Saxony, Germany. The pigs are housed in two compartments, each with two pens and pen-specific outdoor access. To monitor the outdoor usage, four cameras have been installed both indoor and outdoor. The analysis is carried out by two successive convolutional neural networks, trained with YOLOv11. The first model detects pigs in the outdoor area, while the second model classifies the behavior of each pig into activity ("not lying") or resting ("lying") behavior. First preliminary results, compared to a manual time sampling have highlighted the positive performances of the model (precision: 0.95, recall: 0.96, F1-score: 0.95). The next stages of this study will include additional information in form of climate data to extend the current results of the behavioral analyses and provide information on how climate conditions can affect the behavioral preference of pigs. Furthermore, the results could also be used to control and/or improve the design of functional areas in the pen and the outdoor area. This project is supported by the funds of the Federal Ministry of Food and Agriculture (BMEL) (grant number 2823ZR016).

Effect of supplementary water provision to weaned piglets on performance and behavior

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Piglets that do not consume enough water are at a higher risk of dehydration, which negatively affects solid feed intake. The present study evaluated the effect of additional water supplementation for 7 days after weaning on growth, and water and feeding behavior of weaned piglets. A total of 954 TN70 x Rattlerow Seghers Maximus piglets were housed into two identical compartments according to sex. One compartment contained 16 pens: 7 control (C) and 7 test pens (T) with 35-36 piglets per pen, and 2 pens housing each 20 smaller or less viable piglets (S). In all pens, drinking water was provided by a nipple (7 per pen; flow rate 0.7 Lit/min). In test pens additional water was provided one week after weaning using a clickfeeder which was refilled twice a day up to 7 liters. The piglets were weighed at pen level on day 0 (weaning day), 7, and 42, with the S-piglets excluded from the weighing on day 42. The usage of the feeding trough, standard and additional water supply was monitored (min/day/piglet) in two pens per compartment (1 C and 1 T) using a Dahua and Copeeks camera system. Daily feed and water intake were indirectly measured based on the administered amounts from day 0 to 42. No significant differences in daily growth (g/d) were observed between C and T piglets, both from day 0 to 7 (C: 96, T: 88, $p > 0.05$) and from day 0 to 42 (C: 373, T: 382, $p > 0.05$). Piglets from group T were using the clickfeeder for an average of 25 min on day 0, gradually decreasing to 6 min on day 7, while using the standard water supply less frequently during this period compared to group C (C: 7.5 vs T: 5 min/day). The latter trend was also observed during the week after the clickfeeder was removed (C: 5 vs T: 4 min/day). However, the overall water intake (Lit/day) was similar between treatments from day 0 to 7 (C: 0.7, T: 0.7) and day 0 to 42 (C: 1.7, T: 1.6). Daily feed intake (g/d) also showed minimal numerical differences between treatments from day 0 to 7 (C: 243, T: 254) and day 0 to 42 (C: 606, T: 579). In conclusion, in the present farm, providing additional water in a clickfeeder one week after weaning had no significant effect on piglet growth and appears to have little impact on water and feeding behavior.

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Theatre 2

Evaluation of the attractiveness of an alfalfa ball and its effectiveness in interrupting post-weaning tail-biting episodes

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An experiment was conducted to assess the attractiveness of a high-density dehydrated alfalfa ball weighing 20 kg (Luzapeak©, referred to as BL) and its effectiveness in preventing or interrupting episodes of tail biting. The trial took place in two post-weaning rooms, each consisting of 16 pens with 10 pigs. The experimental design involved a two-factor cross: the type of enrichment, comparing BL (CL treatment) to a suboptimal object commonly used in farms (compressed organic material chew disc, CS treatment), and the tail length, either full tail (QE) or docked tail (QC). To test the effectiveness of BL in interrupting a tail-biting episode, an intervention protocol was defined for the CS condition in case of tail biting: additional enrichment in the form of either BL or a natural material rope was provided. The analysis of variance did not show any significant differences in zootechnical performance between the CL and CS conditions. BL was significantly more attractive than the provided discs and chains. Among the nine occurrences of tail-biting, height of them were in the CS treatment, and only one in the CL treatment. BL appears to be an interesting enrichment material for preventing and interrupting tail-biting episodes, and further trials should be conducted to validate this hypothesis.

Facial expressions of lactating sows reveal greater well-being and more appeased situations in pen than in crate

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Facial expressions can reflect an animal's emotional responses to disturbances in its physical environment. This study involved 12 Large White sows, each reared in two different conditions during 2 successive farrowings and lactations, in a crate (CB) at third parity and then in a pen (CL) at fourth parity; they had experienced each condition at the previous parities. In each condition, their reactions to highly predictable (feeding) and unpredictable but known events (iron injection, weaning) were measured by 2 methods for image production, facial images (N=240 CB and 210 images in CL taken) with a digital camera by a single operator before/after each event, and image extraction from video records to cover the +/-10 min period around 2 to 3 feeding events per sow (N=2090 in CB and 2325 in CL at ~18 d after entrance). Each image was annotated by a single observer. Uni-trait binomial logistic models correcting for the random effect of the sow were applied to explain head position, ear orientation, eye opening and mouth opening, the presence of tears and the presence of mouth drooling. Compared with crate, sows in the loose condition had their heads up (image: 72.9% vs. 65.2%), their ears flapped (left ear: image 44.0% vs. 14.9% / video 26.2% vs. 22.4%, $P=0.02$), and more marked symmetry of the ears (video: 70.2% vs. 54.5%; $P=0.0001$) and eyes (95.5% vs. 89.1%; $P=0.002$); they were actively anticipating meals and showed a decrease in attention after meals. In the blocked condition, the sows showed signs of increased vigilance as compared to blocked sows, such as ears pricked up in various events (image: 75.6% vs 46.1%) or backwards at mealtime (34% vs 22%) and eyes more open (image: 55.7% vs 31.9% $P<0.001$) and more mouth drooling before meals. A multivariate analysis to integrate the different facial features into the different responses and better characterise sow variation is currently underway. Facial expressions can be used to decipher the emotional state and mood of sows.

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Theatre 4

Impact of cleaning and disinfection protocol on health and performance in French pig farms

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Technical and economic performance are directly affected by biosecurity and hygiene practices in pig farming (HURNIK 2005, CORRÉGÉ, 2011). Good cleaning and disinfection practices reduce the sanitary load in the rooms, thereby reducing the risk of diseases and clinical signs impacting zootechnical performance (GBPH, 2023). To our knowledge, there is no recent study of the impact of the cleaning and disinfection protocol on the performance and health of pigs based on data collected in the field. For the year 2023, technico-economical performance, technical performance of the Pass Cheptel software, carcass data at slaughterhouse and Pig Connect audit results were collected from 598 farms in western France and cross-referenced with the results of an annual compliance audit. Farms carrying out a complete cleaning and disinfection protocol (soaking, washing, detergent, rinsing, disinfection, drying) have a higher (61.54% vs 61.35%, $p<0.05$) and more homogeneous ($p<0.05$) muscle ratio than those using an incomplete protocol (at least one of the steps is not followed). This difference can be linked in particular to active drying (complete protocol: 61.56% vs incomplete with drying: 61.42% vs incomplete without drying: 61.35%, $p<0.05$). The farms using a complete protocol also tend to have a reduced technical 8-115kg FCR ($p=0.098$). Farms with a complete protocol have fewer total seizures (0.18% vs 0.27%, $p<0.05$), fewer seizures for respiratory reasons (0.34% vs 0.43%, $p<0.05$) and fewer seizures for joint reasons (1.32% vs 1.83%, $p<0.05$) at slaughter. Farms that carry out active drying during their protocol also have fewer total seizures (complete protocol: 0.17% vs incomplete without drying: 0.28%, $p<0.05$) and fewer seizures for joint reasons (complete protocol: 1.15%, incomplete with drying: 1.35% vs incomplete without drying: 1.85%, $p<0.05$) at slaughter. This study validates the impact of a complete cleaning and disinfection protocol on the technical and economic performance of farms. It encourages farmers to be aware of the need to comply with all the steps of the protocol.

Impact of PRRSV and *Streptococcus suis* co-infection on the respiratory microbiome and mortality in piglets D. Schokker¹, S. Commandeur¹, S. Van Hemert¹, N. Stockhofe-Zurwieden¹

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Severe respiratory and systemic diseases still occur in pig husbandry, posing ongoing challenges for veterinarians and farmers. Porcine reproductive and respiratory syndrome virus (PRRSV) and *Streptococcus suis* (*S. suis*) are critical pathogens in swine, and their co-infection can result in severe respiratory and systemic disease. The objective of this study was to explore the changes of the respiratory tract microbiome of piglets with or without a co-infection by PRRSV and *S. suis*. The study involved infecting either conventional piglets or Cesarean-Derived Colostrum-Deprived (CDCD) piglets. Within each Three groups were made: Control, *S. suis* infection, and PRRSV with *S. suis* co-infection. The respiratory tract microbiota was characterized using a metagenomic approach, including four tissues: nasal, tracheal, tonsillar, and pulmonary. The comparison of conventional to CDCD background revealed differences in the 'baseline' microbiomes. In addition, the *S. suis* exposure group compared to control, showed a dysbiosis and decreased resilience, leading to overgrowth of *S. suis*. Moreover, the presence of PRRSV worsened these effects on the microbiome. Co-infected piglets with CDCD background showed increased mortality rates, compared to conventional background piglets in the co-infected group. These findings were corroborated by data on presence of pneumonia. This study underscores the intricate relationship between microbial dysbiosis, viral co-infections, and host immunity.

Exploring the effects of dietary amino acids levels on the behaviour and performance of pigs

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In this study, we determined the effects of varying dietary lysine (Lys) (first limiting amino acid (AA) in most pig diets) and tryptophan (Trp) (primary precursor to serotonin) levels on the social behaviour and performance of growing pigs, using commercially relevant levels of AAs on a commercial farm. 2,293 PIC Camborough® barrows (mean start weight 11.87 ± 1.35 Kg) were used in a randomised complete block design (block = wean date and weight) with a 2×3 factorial arrangement of feeding treatments: 1) Standardised ileal digestible Lys levels at 100% (HL) or 80% (LL) of PIC Lys requirement at the average point of the feed phase, and 2) Trp:Lys ratio of 0.210 (HT), 0.185 (MT) or 0.160 (LT). Pigs were randomly allocated across the 6 treatment combinations over 3 start dates (wean dates). Pig behaviour and skin lesions were recorded for 10 weeks. Feed intake and weight gain were recorded. Only ear lesions (EL) occurred frequently enough for analysis. LL pigs showed more ear biting behaviour than HL pigs ($p=0.032$, LS mean = 0.114 and 0.093 ear bite behaviours/observation days). Lys and score week interacted on the number of pens showing ear lesions ($p<0.001$), HL pigs showing lower incidences of EL at score weeks 1 & 7. Lys and Trp:Lys ($p=0.030$) and Lys and score week ($p=0.010$) interacted on the number of pens showing severe ear lesions (SEL). Pigs on HL/MT and HL/LT had lower incidences of SEL and incidences of SEL increased more rapidly over time for LL pigs, resulting in higher incidences of SEL for LL pigs at score week 7. Treatment interacted with feeding phase on ADFI ($p<0.001$), ADG ($p<0.01$) and FCR ($p<0.001$), with pigs on the LL/LT diet performing poorest compared to other treatments. Results suggest that low lysine levels increased ear biting behaviour and that diets low in both Lys and Try:Lys negatively impact performance. Tail biting was not common in this population, but the potential remains for Lys levels to affect this (and other) behaviour in other populations and so deserves further exploration.

Effects of protease supplementation on growth performance and intestinal function in weaned piglets fed low digestible protein sources

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The present study investigated the impact of dietary fermentation extract supplementation on the growth performance and intestinal function of weaned piglets fed a diet containing high levels of low digestible protein sources compared to a standard commercial piglet diet. A total of 180 weaned piglets (21 days old, Duroc × Large White × Landrace cross-breeds) with an initial body weight (BW) of 6.27 ± 0.45 kg were randomly assigned to three dietary treatments: (1) a standard commercial positive control (PC) diet containing 22.2% soy protein, 5% whey protein, and 3% fish meal; (2) a negative control (NC) diet with 30.1% soy protein, omitting whey protein and fish meal; and (3) the NC diet supplemented with 0.02% fermentation extract (NC + FE). The diets were formulated to be iso-nitrogenous and iso-caloric and were pelleted under conditions of 0.4 MPa and 75°C. The experiment followed a completely randomized design with six replicate pens per treatment, each pen containing 10 piglets. Statistical analysis was performed using one-way ANOVA, with differences between means assessed by Duncan's Multiple Range Test ($P < 0.05$). Feeding the NC diet, which contained high levels of low digestible protein sources, significantly reduced ($P < 0.05$) final BW, average daily gain (ADG), and average daily feed intake (ADFI) by 3.1%, 8.2%, and 5.2%, respectively, compared to piglets fed the PC diet. Additionally, the NC diet significantly increased ($P < 0.05$) the feed-to-gain ratio and incidence of diarrhea by 3.4% and 88.3%, respectively. However, fermentation extract supplementation in the NC + FE diet restored final BW, feed-to-gain ratio, and diarrhea incidence to levels similar to those observed in the PC group, while ADG and ADFI improved ($P < 0.05$) by 4.6% and 4.3%, respectively. Regarding intestinal function, piglets fed the NC diet exhibited significantly lower ($P < 0.05$) duodenal villus height, crude protein digestibility, and gene expressions of duodenal peptide transporter PepT1 and jejunal amino acid transporter b0,+AT compared to PC. Additionally, blood urea nitrogen and diamine oxidase concentrations were significantly higher ($P < 0.05$) in the NC group. However, fermentation extract supplementation in the NC + FE group reinstated these parameters to levels comparable to PC, indicating improvements in intestinal development, protein digestibility, and nutrient transport efficiency. In conclusion, fermentation extract supplementation in diets containing high levels of low digestible protein sources mitigated negative effects on growth performance and intestinal function in weaned piglets. These findings highlight the potential of dietary fermentation extract in improving nutrient utilization and piglet health, supporting its use in swine nutrition.

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Theatre 8

Effect of lauric acid on sow and piglet performance pre- and post-weaning

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This study evaluated the effects of dietary supplementation with lauric acid (GoNutri Protect from Golden Agri) in sows and monoglycerides of lauric acid (GML) (GoNutri Protect Xtra from Golden Agri) in weaned piglets. A total of 50 sows (parity 2–6) were randomly assigned to one of two dietary treatments from day 100 of gestation until weaning: (1) a negative control (NC) and (2) NC supplemented with 2 kg/T lauric acid (GoNutri Protect from Golden Agri). Regardless of dietary treatment, sows farrowed an average of 19.1 total-born piglets and 17.8 live-born piglets, with an average live-born birth weight of 1,421 g. While lauric acid supplementation did not affect litter size at birth, it resulted in heavier piglets (+64 g, $P = 0.08$) and increased total litter weight (+2.1 kg, $P = 0.05$). Additionally, lauric acid improved litter vitality at birth ($P = 0.07$), although mortality within the first 24 hours post-birth was higher in the lauric acid group (+3.5%, $P = 0.02$). Pre-weaning performance was assessed after standardizing litters to 13–15 piglets. Piglets had an average weaning weight of 9.2 kg at 30.7 days of age, with a pre-weaning mortality rate of 8.2%. No significant differences in weaning weight, litter weight, or overall pre-weaning growth were observed between treatments. In the post-weaning phase, 72 piglets were selected and remained within their maternal treatment groups. They were fed either an NC diet or NC supplemented with 3 kg/T GML (GoNutri Protect Xtra from Golden Agri). A tendency for higher average daily feed intake (ADFI) during the first two weeks post-weaning was observed in the GML group ($P = 0.07$), leading to numerically higher average daily gain (ADG). Feed conversion ratio (FCR) and faecal consistency scores did not differ between treatments. These findings suggest that dietary lauric acid may enhance birth weight and early vitality but does not impact overall pre-weaning growth and survival, while GML supplementation may support higher feed intake and growth post-weaning.

Impact of dietary electrolyte balance on lactating sow performance, inflammation, and gut integrity under heat stress

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Heat stress is a major challenge in lactating sows, as it negatively affects feed intake, milk production, immune function, and overall reproductive performance. One potential nutritional strategy to mitigate these adverse effects is the optimization of dietary electrolyte balance (dEB), which regulates acid-base homeostasis and thermoregulatory responses. This study aimed to evaluate the effects of different dEB levels on lactating sow performance, thermoregulation, inflammatory and oxidative stress markers, and gut integrity during peak summer heat stress conditions. A total of 40 lactating sows were assigned to four dietary treatments with varying dEB levels (230, 250, 270, and 290 mEq/kg). Increasing dietary dEB levels during heat stress linearly increased ($p < 0.05$) average daily feed intake during lactation and improved ($p < 0.05$) piglet weaning weight. Blood pH decreased ($p < 0.05$) linearly with rising dEB levels, while hair cortisol content showed a decreasing trend ($p=0.09$). Blood concentration of IL-1 β tended to decrease ($p = 0.071$) with increasing dEB levels, and IL-10 showed a tendency to peak (quadratic, $p = 0.066$) at 250 mEq/kg before declining. Behavioral analysis showed a quadratic response ($p < 0.05$) in standing behavior, peaking at 250 mEq/kg, while position changes decreased ($p < 0.05$) linearly with increasing dEB. Beta diversity analysis revealed significant differences in unweighted UniFrac PCoA between 270 and 290 mEq/kg dEB groups. The abundance of actinobacteriota phylum tended to decrease ($p=0.084$) linearly, however, the abundance of major phyla including firmicutes, bacteroidota, spirochaetota, planctomycetota, and proteobacteria were unaffected. The abundance of bacteria at family and genus level was not changed. In conclusion, a dEB level of 270–290 mEq/kg appears to be the most beneficial for maintaining sow performance and piglet growth during heat stress conditions.

Effect of a sensory functional feed additive on the behavior and feed consumption of piglets at weaning

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At weaning, piglets experience various challenges which can lead to reduced feed intake. The aim of this trial was to investigate the effects of a new functional feed additive on piglets' behavior and feed intakes at weaning. The trial was conducted in a French commercial farm. A total of 528 weaned piglets (27 days old) from 4 batches were divided in 2 experimental treatments: 1) Control, standard pre-starter feed, 2) Control feed + a novel feed additive (blend of functional aromas and a novel core) included at 350 g/tonne of feed from weaning (d.0) to d.14 post-weaning (pw). Piglets were blocked by sex and weight at weaning. Piglets were housed in pens of ~33 pigs and were monitored according to the farmer's practices. Piglet feed intakes were recorded by pen from d0 to d14 pw. Four pens per treatment and per batches (16 in total) were filmed from d0 to d1 pw. The cameras used were GoPro and the videos were analyzed using BORIS free software with a blind observer. Behavior was analyzed for 6 hours (4 hours after weaning and 2 h at 24 hours after weaning) every 30 seconds via a group scan sampling using a previously established ethogram. A total of 720 behaviors were scored per pig per pen. Data were analyzed using a Chi-square test and Anova in Jamovi free software. Time budgets were made on pigs' activity period. Time budgets were significantly different between the two groups ($P\text{-value} < 0.05$). The differences were as follows: during the 6 hours of observation, piglets receiving the additive spent a lower percentage of time lying down compared to control piglets (48 vs 56%, $P\text{-value} < 0.001$), piglets receiving the additive spent a higher percentage of time eating and rooting compared to control piglets (26 vs 17%, $P\text{-value} < 0.001$ and 2.1 vs 1.8%, $P\text{-value} < 0.05$, respectively). Time budgets were not significantly different for the following parameters: fighting, walking and interacting with an object/pig ($P > 0.05$). In addition, additive supplementation increased the average daily feed intake per pig from d0 to d14 pw by 4.2% compared to control piglets. In conclusion, supplementing weaned piglets with a feed additive based on functional aromas and a novel core can increase feed intake pw and feeding behaviors during this challenging time.

Towards a comparative approach on animal and plant parasitology: assessing convergences and perspectives and preparing an international symposium

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Despite their major impact on human, animal and plant health, research on animal and plant parasites or pests still largely follows separate paths. However, these parasites share many physiological, biological, genetic and molecular commonalities. Comparative parasitology, as an emerging discipline aimed at exploring the similarities and differences between animal and plant parasites or plant pests, opens up new perspectives by bringing these two worlds together. Comparing the mechanisms of host-parasite interactions in plants and animals allows the identification of new therapeutic targets and integrated control methods. Thus, comparative approaches and interdisciplinary collaborations are essential to advance our overall understanding of parasitism. First result based on bibliography underline the potential of this One Health approach. So, we started an international Brazil–France project which one aim is to organize a symposium in Lyon in 2026 to foster dialogue between animal and plant parasitologists or plant pests' specialists around conceptual convergences, methodological opportunities and common perspectives in both disciplinary fields. This symposium could focus on: mechanisms of host-parasite interactions, ecology and evolution of specificity, community dynamics, parasitism and life history traits, new diagnostic and control tools. We, therefore, invite specialists interested to join forces to: 1-Identify common research questions and barriers to overcome; 2-Set up collaborative projects and funding applications; 3-Conduct work crossing approaches and models; 4-Supervise interdisciplinary internships; and 5-Contact us to participate in the symposium preparation. In the short term, these collaborations will allow a fruitful sharing of concepts and methods. In the longer term, they will lay the foundations for integrative research opening up major perspectives in fundamental and applied parasitology.

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Theatre 12

Reframing salivary oxytocin as a biomarker: a bibliometric analysis of research trends, theoretical frameworks and future directions

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Salivary oxytocin is proposed as a non-invasive biomarker for positive emotions and welfare. The assumption is that peripheral oxytocin reflects central release and can objectively quantify sociality and well-being. This aligns with early, optimistic theories of oxytocin's prosocial and stress-reducing effects, yet decades of research have challenged the universality of these claims. The salivary oxytocin field largely adopted this perspective, despite growing evidence that oxytocin's effects are highly context-dependent and shaped by individual and environmental factors. This study presents a bibliometric analysis of 322 publications in humans and farm animals to examine the evolution of salivary oxytocin research, including publication trends, citation patterns, and keyword evolution. Our findings indicate that: (1) Early theoretical assumptions about oxytocin's prosocial and stress-reducing effects continue to shape the field, especially in livestock, despite evidence for broader physiological roles; (2) Limited integration between human and animal research persists, despite oxytocin's evolutionary conservation; and (3) Methodological challenges remain a key concern, yet inconsistencies are often attributed to measurement limitations rather than a re-evaluation of theoretical frameworks. These patterns have implications for animal welfare research, where salivary oxytocin is increasingly used as a welfare indicator. The current approach may oversimplify the oxytocin-welfare relationship, reinforcing a unidimensional interpretation of oxytocin as a marker of positive affect. Instead, we propose a broader framework viewing oxytocin as an indicator of adaptive capacity, reflecting physiological and behavioral responses to social and environmental challenges. Our findings highlight the need for greater theoretical integration, methodological refinement, and interdisciplinary collaboration to advance salivary oxytocin research. Future studies should move beyond traditional prosocial narratives and consider oxytocin's complexity and context-dependent nature when evaluating its potential as a biomarker.

Domains of greatest threat to welfare and potential best practices for mitigation in rabbit farming: results from the European Partnership for Animal Health and Welfare

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According to EFSA, rabbits can face twenty welfare consequences under farming conditions, varying in frequency and duration based on animal category and housing system. To improve rabbit welfare, some best practices (BP) can be adopted, but structured information is still lacking. One aim of the EU Project EUPAHW is to fill this gap. Starting from literature search and expert opinion, welfare threats (WT) to rabbit welfare for growing rabbits, breeding does, and kits were collected. The WT were classified into welfare domains (health, behaviour, nutrition, mental and environment) and grouped into subcategories according to the main hazards they are linked to (e.g., management, housing conditions or genotype). Then, BP were identified for each WT and assigned to relevant production systems in which they are or can be implemented. The domains that grouped a higher number of WT were "behaviour" for growing rabbits (13/37) and "health" for kits (7/15) and breeding does (16/42). Most WT (45/94) for all animal categories fell into the "housing conditions" sub-category. The BP primarily addressed the domains of Health and Behaviour but rarely mitigated more than one or two WT. As a matter of fact, few BP (1/29 for growing rabbits; 2/29 for breeding does) addressed at least three WT, namely: "reduced stocking density" for growing rabbits, "good climate control", and "good cleaning and biosecurity measures" for breeding does. For kits, only one BP out of 14 addressed two WT; none addressed three. Overall, our results show that managing welfare risks in rabbit farms is complex, but that some best practices can have an effect on several welfare domains or subcategories, on which it would be effective to focus efforts. This work was co-funded by the EU Horizon Europe Project 101136346 EUPAHW.

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Theatre 14

The Effect of Environmental Enrichment on Selected Physiological and Immunological Stress-Related Markers in Dairy Goats

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Farm animals are predominantly exposed to different stressors. Lately, we developed a minimally invasive method to measure stress via blood sampling to provide a reliable marker. Here, we aimed to evaluate the effect of brushes and stages, as an environmental enrichment to alleviate stress in goats. 24 mixed breed goats were divided into 2 groups according to farm's common physiological conditions (12 goats/group): milking and dry (milk-producing and non-milk-producing, respectively). Following 28 days of adaptation period each group was exposed to 10 days of environmental treatment (brushes and stages) or not (control) at different time points; thus, each group was served as its own respective control. At day 10 of each treatment, blood samples were collected from each goat and group. Gene study was done via qPCR using mRNA produced from isolated leukocytes and serological studies were done via ELISA using serum. Following the enrichment, we observed a reduction in reactive oxidative stress metabolites, advanced glycation end products (AGEs), and their binding protein (transferrin) in the dry goats, as determined by an ELISA. In contrast, no change in AGEs, along with an increase in transferrin levels, was observed in the milking goats. Moreover, oxytocin levels decreased in the dry and increased in the milking goats, while serotonin levels increased in the dry and remained unchanged in the milking goats. Additionally, gene expression of the cytokines, IL-6 and IL-1 β , and anti-oxidative proteins, lysozyme and transferrin (in peripheral blood leukocytes), as determined by qPCR, presented the same pattern: down-regulation in the dry or up-regulation in the milking goats. In conclusion, a reliable methodology was developed for measuring stress due to intensive husbandry farming in goats and to improve dairy goats' husbandry practice. Current environmental enrichment produced different responsiveness in goats correlated to their physiological status: beneficial effect in dry goats, detrimental effect in milking goats.

Microplastics in Agriculture: Linking Environmental Pollution to Animal Health Challenges

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Microplastic pollution is a growing environmental concern, with increasing impacts on agricultural ecosystems and animal health. The ECO(RE)ACT project addresses this issue by developing a strategic framework for microplastic reduction, monitoring contamination in irrigation water, soil, and animal feed, and establishing a Centre for Microplastics to advance research and public awareness. While the project focuses on contamination pathways and mitigation, emerging evidence suggests direct risks to animal health, requiring further study of physiological effects. Microplastics likely enter animal systems through contaminated feed, water, and inhalation. Once ingested, they may accumulate in the gastrointestinal tract, potentially disrupting the gut microbiome, nutrient absorption, and immune function, increasing the risk of inflammation and digestive disorders. Additionally, microplastics can carry toxic additives and pollutants, such as bisphenols and phthalates, which may act as endocrine disruptors, interfering with metabolism, growth, and reproduction. Prolonged exposure could impair liver and kidney function, disrupt lipid metabolism, and induce oxidative stress, contributing to metabolic disorders. Microplastics have also been detected in reproductive tissues, with potential links to reduced fertility and developmental issues in livestock. These disruptions not only affect animal health and productivity but also pose food safety concerns, as microplastics and associated toxins could enter the human food chain. Investigating the effects of microplastics on animal physiology is crucial for understanding their long-term consequences. Through the ECO(RE)ACT project's cross-border collaboration, we aim to bridge the knowledge gap between environmental contamination and animal health, providing evidence-based solutions for sustainable agriculture and food safety.

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Poster 16

Influence of different light colours on the resting behaviour of nursery piglets

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Pigs have two types of cones in their retina (dichromats). There is evidence that they perceive colours differently from humans. This study examined the influence of different light colours on the resting behaviour of nursery pigs at night. In total, the colour preferences of 36 nursery pigs for resting at night were tested in an experimental housing system in three batches (12 pigs per batch, 32 days per batch). Four resting boxes, facing each other in a 2 x 2 experimental design, existed. Each box was illuminated with one of four tested colours at a time (blue, green, red and yellow). The colours rotated among the four boxes every 24 hours to avoid a position bias. Video recordings were analyzed using scan-sampling technique with 10-minute-intervals (between 06 PM and 06 AM). Total number of pigs in a box and the individual preferences for resting behaviour were recorded. On average, 8.77 pigs rested in the boxes at each sampling point. The pigs rested in single boxes as follows (mean number of pigs per sampling point): Box 1: 1.96; box 2: 2.04; box 3: 2.41; box 4: 2.36. The analysis of the resting behaviour with regard to the offered colours led to the following results (mean number of pigs per sampling point): red: 2.70; green 2.57; yellow: 2.79; blue: 0.71. Individual analysis revealed that blue-lit resting boxes were avoided by all 36 pigs. It can be concluded that pigs perceive the colour blue differently than yellow, green or red. Blue could be perceived as bright and therefore avoided during resting behaviour. Resting under the colours yellow, green and red seems to be more comfortable for pigs. This could be because pigs have a weaker perception of these colours. Illumination of pens with different colours could be used in future as a tool for structuring pens in pig husbandry.

Effect of butyric acid salts on the intestinal permeability of weaned piglets*W. Kozera¹, K. Karpiesiuk¹, A. Woźniakowska¹, U. Czarnik¹, K. Dobiesz¹, G. Żak², M. Szyndler-Nędza², A. Mucha²**¹ University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 5, 10-719 Olsztyn, Poland, ² National Research Institute of Animal Production, ul. Sarego 2, 31-047 Kraków, Poland*

An important function of the intestinal barrier is to ensure the selective permeability and transport of molecules so that sufficient quantities of harmless components that are important for the functioning of the body enter the bloodstream. A key role in the control of paracellular permeability is played by the apical structures of the intercellular junction complex or tight junctions (Latin: zonulae occludentes, English: tight junctions – TJs), which form a selective diffusion barrier that regulates the paracellular flow of substances according to their charge and size. The test material consisted of a total of 24 piglets. The animals for the feeding groups were selected according to the analogue method and divided into 4 groups. During the experiment, the piglets in the control group received a basic mixture, while in the other groups they received the basic mixture with additives in the form of: 4000 mg/kg sodium butyrate, 2000 mg/kg encapsulated sodium butyrate, 2000 mg/kg mixture of calcium butyrate and sodium butyrate, protected in a lipid envelope. The results of immunoanalytical detection of the concentration level of the intestinal permeability marker zonulin (ng/ml), showed no statistically significant changes. However, there was a decrease in the level of the tested marker in groups of piglets fed compound feeds with added butyric acid derivatives, and an increase in the control group, in which the concentration of zonulin was 6,752 ng/ml. The lowest concentration of zonulin was recorded in group IV, in which piglets were fed feed with 2,000 mg/kg of a mixture of sodium butyrate and calcium butyrate in encapsulated form. In groups II and III, the concentration of zonulin was similar and was: 4,488 ng/ml and 4,022 ng/ml. Research Funded by the Minister of Science under “the Regional Initiative of Excellence Program”.

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Effect of butyric acid salts on biochemical and haematological indices in the blood serum of piglets*K. Karpiesiuk¹, A. Woźniakowska¹, W. Kozera¹, U. Czarnik¹, K. Dobiesz¹, G. Żak², M. Szyndler-Nędza², A. Mucha²**¹ University of Warmia and Mazury, ul. Oczapowskiego 5, 10-719 Olsztyn, Poland, ² National Research Institute of Animal Production, ul. Sarego 2, 31-047 Kraków, Poland*

The weaning phase is a stressful moment in the life of piglets. In the past, antibiotics were used to minimise the negative consequences of the weaning phase in animal husbandry, which was associated with the emergence of drug-resistant strains of bacteria that are insensitive to a specific antibiotic. Organic acids and their salts are among the preparations that protect the health of animals and maintain their performance. The aim of this study was to investigate the effect of butyric acid salts on biochemical and haematological indicators in piglet serum. The test material consisted of a total of 40 piglets that were weaned at 28 days of age. The animals for the feeding groups were selected using the analogue method and divided into 4 groups. During the experiment, the piglets in the control group received a basic mixture, while in the other groups they received the basic mixture with additives in the form of: 4000 mg/kg sodium butyrate, 2000 mg/kg encapsulated sodium butyrate, 2000 mg/kg mixture of calcium butyrate and sodium butyrate, protected in a lipid envelope. Blood samples were taken on the last day of the experiment. The inclusion of 2000 mg/kg coated sodium butyrate in the feed effectively ($P \leq 0.05$) reduced the concentration of total cholesterol and its HDL fraction as well as triglycerides in the blood compared to the control group. The addition of 2000 mg/kg of a mixture of calcium butyrate and sodium butyrate also caused a decrease in the concentration of triglycerides and the high-density cholesterol fraction. Under the conditions of the experiment carried out, the use of butyric acid derivatives in the feed mixtures led to an increase in the glucose concentration above the upper threshold value of the reference range. The addition of butyric acid derivatives did not lead to a significant differentiation of the mean values of the parameters of the morphological blood profile, but in individual cases to deviations of some blood parameters from the reference norms. Research Funded by the Minister of Science under The Regional Initiative of Excellence Program.

Body condition and rearing performance of sows in organic production

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Body Condition and Rearing Performance of Sows in Organic Production In organic farming the requirements for sows and farmers are very high. High rates of piglet mortality may occur and can lead to ethical conflicts (SCHILD et al., 2020). The study was conducted in the winter of 2024 on an organic farm. A total of 29 German Landrace sows were included. The following parameters were recorded, side fat thickness of the sows and the live weight of the piglets in the 1st, 3rd, and 6th weeks of suckling period. Additionally, the udder was evaluated. Statistical analyses were conducted using SPSS, employing univariate analysis and correlation analysis. The sows achieved a good level of total born (14.1) and live born (12.5) piglets. After six weeks of lactation, an average of 9.2 piglets were weaned. Piglet losses reached 24% in average (0 – 82%). The extent of these losses depended on litter size. In contrast to that, the number of litter and side fat thickness of the sow in the first week did not impact the level of losses. In the first week, the side fat thickness of the sows was 16.8mm±4.3, in the third week 14.8mm±3.9, and in the sixth week 12.8mm±4.0. Gilts and primiparous sows showed significantly greater side fat thickness at the first and second measurements compared to older sows. The mobilization of 4mm is consistent with conventionally kept sows. Again, there is a significant influence of age. Gilts and primiparous sows mobilize 5.1mm of substance, compared to 2.7mm of older sows. The weight of piglets increased from 1.7kg±1.1 on the fourth day of life to 6.4kg±4.6 in the third week, and 12.6kg±8.9 in the sixth week. Litters from sows with high mobilization of side fat exhibited significantly higher litter weights. No injuries to the udder were observed in any of the sows. The body condition and mobilization capacity of the sows were not problematic. However, it remains a significant challenge to limit losses, especially at farrowing and at the start of lactation (CHU et al., 2022). An essential aspect will be identifying the start of farrowing to reduce the number of sows farrowing outdoors.

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Botanical bioactives against *Ascaris suum*, the potential of tannin-rich plantsS. Kerros², A. R. Williams¹, S. Guillaume²¹ University of Copenhagen, Grønnegårdsvej 15, 1870 Frederiksberg, Denmark, ² Phytosynthese, 57 Avenue Jean Jaurès, 63200 Mozac, France

Gastro-intestinal nematodes are an important concern in animal production, negatively affecting animal performance and welfare. Conventional management prophylaxis leads to use regularly anthelmintics causing two main issues: resistance against a limited number of authorized molecules, and the use of drug-treatments on animals from organic or free-range farming. Botanicals are traditionally used against parasites in different cultures. Thus, this study aimed to evaluate tannin-rich extracts on *Ascaris suum*, a major concern in pig farming. The assay was evaluating in vitro tannin-rich plant extracts on *A.suum* larvae mortality. 5 single plants (1 rich in catechic tannins, 2 rich in gallotannins and 2 rich in ellagitannins respectively from *Schinopsis balansae*, *Syzygium aromaticum*, *Caesalpinia spinosa*, *Punica granatum*, *Terminalia catappa*) and 1 blend (*S.balansae*; *P.granatum*, *C.spinosa* combining these 3 tannin families) were prepared using 140g of dry plants/L solubilized into 50% DMSO using ultrasonic extraction during 30 minutes. Third-stage larvae of *A.suum* (L3) were isolated and suspended in culture media (RPMI1640 with L-glutamine, penicillin, and streptomycin). Larvae were added to flat-bottomed 96-well plates and cultured at 37°C, 5% CO₂ in air. The negative control was culture medium containing 1% DMSO. L3 were exposed to the diluted extracts for 24 hours, and live and dead larvae enumerated in order to calculate the mortality. This L3 exposure test was performed in triplicate with 2 independent repetitions. Results indicated that all samples provided an in vitro dose-dependent mortality on L3. *S. balansae* and *C. spinosa* expressed the highest activity with more than 50% mortality at 1:400 compared to other individual extracts achieving <10% mortality at this dilution. The blend of tannins provided an additional benefice to increase mortality at the 2 highest dilutions tested (1:1600) vs. individual extracts. This study confirms the potential of tannin-rich bioactives against nematodes with a variability of responses according to the type of tannin and their structure. The observed synergy in this study requires further investigation in order to explore the mode of action and optimize formula for in vivo test.

The European Partnership on Animal Health and Welfare and its strategy for communication and dissemination.

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Launched in 2024, the European Partnership on Animal Health and Welfare (EUP AH&W) is an ambitious 7-year programme aimed at enhancing animal health and welfare across Europe through collaborative research and the development of innovative solutions. The Partnership's scientific activities address challenges such as infectious animal diseases, antibiotic resistance, and sustainable farming practices. A key component of the EUP AH&W is its Work Package 5 (WP5) Communication, Education, Exploitation and Dissemination (CEED), which is dedicated to optimising the use of the Partnership's scientific outcomes by promoting knowledge exchange among stakeholders, including scientists, policymakers, farmers, and the general public, thus maximising the uptake and impact of the EUP AH&W-developed outcomes. WP5 CEED's strategy includes the use of diverse communication channels to ensure the wide accessibility of scientific knowledge such as the website and social media. In parallel, tailored dissemination to specific audiences is achieved through dedicated dissemination tools such as targeted reports and meetings. Education activities such as Summer Schools support the training of the next generation of animal health and welfare experts, and activities like Short Term Missions and workshops allow the technology and new knowledge produced by the EUP AH&W to reach the relevant partners, as well as fostering collaboration with external stakeholders. With 90 partner institutes, and co-funding through the EU Horizon Europe Framework Programme for Research and Innovation, the EUP AH&W is expected to have an impact at the scientific, economic/technological, and societal level, contributing to the advancement of animal health and welfare across Europe. The EUP AH&W IS fully aligned to the European Green Deal and its associated Farm to Fork strategy for a fair, healthy, and environmentally friendly food system. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

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Poster 22

Analysis of crossing of differentiated gradient angles by breeding goose

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The aim of this study is to develop an intelligent laying nest for breeding goose that uses AI, machine learning and modern measurement and control technology to enable complete parentage control of the offspring. The concept for the nest includes a structure for housing the technology under the construction. This requires the structure to be a certain height. Over a period of 4 times 7 days, a ramp construction with different heights and gradients was built between the barn and the run-out area: [1] 15cm and 32%, [2] 20cm and 44%, [3] 25cm and 58%, [4] 35cm and 98.0% were installed. During the test period, 717 crossings took place. The duration of the crossing and various observations of walking behavior were recorded and the individual crossings were then evaluated according to the traffic light principle. This was defined as follows: [Green] crosses the ramp without any problems; [Yellow] crosses the ramp but with slight difficulties; [Red] only crosses the ramp with great effort or breaks off and refuses to cross. It was found that the gradients of 32% [1] and 44% [2] can be crossed without difficulty. In contrast, more difficulties were encountered when crossing the 58% [3] incline (n=243; 39%). These were manifested by slipping on the ascents and descents with loss of balance. The 98% gradient [4] was a challenge for the animals. A total of 27 crossings (n=108; 25%) were aborted by the animals [Red]. If the slope was overcome, then often only with the support of the wings. The females tended to jump up and down the ramps (n=43; 67%). In contrast, the males tended to jump less (n=43; 33%), instead seeking support on the attached ledges of the ramp and pushing themselves up the ramp. After 3 days, the female animals in particular began to train successfully. There were no more abortions [red] and the number of problem-free crossings [green] increased significantly from 3% (n=34) to 40% (n=35). This could be due to the smaller body size and weight compared to the males. In summary, it can be concluded that an incline of up to 44% [2] can be overcome safely and adequately.

Transcriptome analysis of milk fat globules in cows: insights into gene expression patterns and β -casein genetic variants

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The A2 β -casein milk variant has attracted increasing scientific interest due to its potential health benefits. This has led to a growing trend in certain countries toward breeding A2A2 genotype cows to produce milk containing only A2 β -casein. However, it is unknown whether this selective breeding could result in differences in gene expression in the mammary gland, potentially affecting milk synthesis or composition. Therefore, first, we hypothesised that the transcriptome of the milk fat globules (MFG) accurately reflects the molecular profile of the mammary gland, supporting its use for transcriptomic studies, and second, that significant differences exist in gene expression profiles and key metabolic pathways involved in milk synthesis and composition between cows with different β -casein genotypes. MFG transcriptome from cows with A1A1 and A2A2 β -casein genotypes (n=14) was analysed using RNA-seq. The functional enrichment analysis of highly expressed genes in MFG revealed a strong representation of genes involved in protein synthesis, milk fat secretion and cellular energy metabolism, key processes essential for lactation. The genes encoding α -S1-casein (CSN1S1), β -lactoglobulin (PAEP/BLG), β -casein (CSN2), glycosylation-dependent cell adhesion molecule-1 (GLYCAM1), cytochrome c oxidase subunits I and III (COX1 and COX3), and κ -casein (CSN3) were found as the most abundant transcripts in MFG, with mean values of 90,401, 71,022, 53,579, 49,494, 38,551, 33,584, and 30,791 FPKM, respectively. This reinforces the suitability of MFG as a representative transcriptome source of the mammary gland. Differential expression analysis between the two genotypes revealed minimal differences, with only two genes identified as differentially expressed out of the 11,180 and 11,257 genes expressed in MFG (identified with DESeq2 and EdgeR packages, respectively). These findings suggest that selective breeding for the A2A2 β -casein genotype is unlikely to have significant transcriptional-level effects that would affect milk synthesis and composition, but other aspects, such as post-transcriptional mechanisms, should be further analysed.

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Theatre 2

Metabolic stress in laying hens and its immune-mediated resolution

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An outcome of intensive poultry husbandry in Israel, are the high energetic demands imposed upon layers selected to increase egg production. Consequently, energy deficiency is minimally tolerated and results in metabolic stress. We hypothesized that metabolic stress impedes homeostasis, and its resolution includes immune mediation. This hypothesis was based on our previous observations, in which homeostasis restoration was immune-mediated in other types of stress. Accordingly, our current goal was to examine the effect of metabolic stress on homeostasis in layers and the immune system's involvement in its restoration. We determined metabolic stress responses in 75-week-old Lomman layers subjected to a 72-hours food deprivation program compared to the regularly fed control group (n=50 per group). We observed a negative energy balance which resulted in extensive lipolysis and increased expression of a wide array of general stress-related genes (GCR, HSP-70 and IL-6) as well as energy stress-related genes (SIRT1). Additionally, we identified oxidative stress reagents, AGE, in the food-deprived layers, unique to metabolic stress. Furthermore, in vitro exposure of peripheral blood leukocytes from regularly fed layers to different dosages and exposure durations (dose & time dependency) of AGE, resulted in the production of pro-inflammatory cytokines (IL-6, IL-1 β) and oxidative reagents (lysozyme, transferrin) gene expression. We suggest that negative energy balance constitutes metabolic stress, and the pro-inflammatory immune response plays a major role in its resolution. Hence, understanding the mechanism by which the immune system assists in restoring homeostasis following the resolution of metabolic stress, would be beneficial to poultry husbandry and welfare.

How does stress due to water restriction and parasitic infection affect plasma hormone levels in high and low tolerance Rasa Aragonesa ewes fed with sainfoin?

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Cortisol, dehydroepiandrosterone (DHEA), and its sulphate (DHEA-S) have been used to assess the physiological response to stress in animals. In this study, we selected 20 Rasa Aragonesa ewes with high tolerance and 20 with low tolerance to water and heat stress (classified based on the neutrophil/lymphocyte ratio from a previous experiment). After a 2-week adaptation period, the trial lasted for 9 weeks. At the end of 0 week (basal condition) ewes were infected with *Haemochus contortus* L3 (10000/ewe) and half of them in each tolerance group were fed 400 g/d of a total mixed ration, while the other half received 400 g/d of sainfoin pellets. Both groups were also provided with 1kg/d of straw. A water restriction period (2-5 w) during which ewes experienced a 40% reduction in water available (calculated based on the intake in basal conditions) was conducted, followed by a post-restriction period (6-8 w) with water available ad libitum. Blood samples were collected weekly, and plasma cortisol, DHEA and DHEA-S were determined using ELISA tests. The number of adult parasites and larvae 4 collected at 8 w was not related to the hormone concentrations. Neither diet nor stress tolerance significantly affected plasma hormones levels, but time had a significant effect on cortisol and DHEA ($P < 0.05$). Cortisol levels decreased consistently over time from 9.6 ± 1.1 (0 w) to 2 ± 1.05 ng/ml (6-8 w), possibly due to acclimatization, whereas DHEA levels, however, increased during the restriction period (10.5 ± 0.94 ng/ml) compared to other periods (average 4.2 ± 0.94 ng/ml). The study suggests that short-term water restriction triggers an increase in DHEA, potentially as a physiological adaptation to stress.

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Theatre 4

Metabolic resilience in goats: Insights from nutritional challenge with metabolomic analysis

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The aim of this study was to investigate metabolic resilience through milk of ruminants, to understand mechanisms related to resilience and to identify associated milk biomarkers associated with resilience. This study was conducted on 70 Alpine goats, classified into resilient (R) and non-resilient (NR) groups, based on a previous clustering of responses of 13 targeted milk metabolites and one enzyme measured around a 2-day nutritional challenge, with access to straw only. A metabolomics analysis (LC-MS) was conducted on milk morning samples collected before (D0) and after (D2) the challenge. Metabolome change (D2 / D0) was analyzed using univariate (ANOVA) and multivariate (sPLS-DA) methods to identify differences between R and NR goats. Metabolites related to amino acid metabolism showed greater reduction in NR goats ($P\text{-value} - \text{FDR} < 0.05$). This identified metabolite changes was significantly and positively correlated with the reduction of NH_4^+ groups and glucose-6-phosphate. These findings support total amino acid concentration and specific amino acids as markers of resilience. Additionally, goats in this study were derived from divergent functional longevity lines, based on estimated breeding values (EBV) of their sire. A metabolite change was identified in both resilience-based and longevity-based comparisons. The EBV of sire show a strong correlation with the mean of this metabolite change of his daughters, weighted by the number of daughters ($r = -0.74$, $p < 0.001$). This metabolite change is associated with both nutritional resilience and longevity, suggesting its role in metabolic adaptation, and warranting further investigation as a potential biomarker for genetic selection in ruminants. Metabolites were identified using internal and external databases and will be validated by MS/MS analysis.

Metabolic indicators of short-term nutritional stress in goat milk

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This study aimed to identify metabolic indicators of nutritional stress in goat milk through both targeted and untargeted approaches. The experiment was conducted on 70 Alpine goats subjected to a 2-day nutritional challenge, with access to straw only. Milk samples were collected in the morning milking before the start (D0) and at end (D2) of the challenge. A targeted approach was used to quantify 13 milk metabolites, including β -hydroxybutyrate, cholesterol, choline, galactose, glucose-6-phosphate, glutamate, isocitrate, malate, free amino groups (NH₂), triacylglycerols (TAG), urate, and urea. Untargeted metabolomics approach was applied using high-resolution liquid chromatography-mass spectrometry (LC-MS) to identify additional metabolic changes. The selection of potential biomarkers for the untargeted approach was based on two criteria: metabolites showing the same direction of response (increase vs. decrease) in all the goats and a fold change greater than 1.5. Following metabolite selection, we assessed their ability to discriminate between D0 and D2 samples using an AUC-ROC analysis. Among the targeted metabolites, five metabolites showed AUC-ROC > 0.9 : cholesterol, choline, glutamate, malate, and TAG. The best predictive metabolite was choline, with an AUC-ROC of 0.997. In the untargeted analysis, 12 metabolites met the selection criteria, all displaying an AUC-ROC > 0.94. These findings highlight key metabolic shifts in response to nutritional stress and contribute to identify potential milk-based biomarkers for monitoring nutrition perturbation in dairy goats. Metabolites from metabolomic were identified using internal and external databases and will be validated by MS/MS analysis.

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Theatre 6

Changing the physiology of ruminants through genetic selection is a long-term solution for reducing methane emissions

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Domesticated ruminants are physiologically adapted to convert cellulose into human consumable protein and fat, filling an important gap in feeding the world. Unfortunately, methane (CH₄) is produced as a by-product of enteric fermentation and is a loss of potential energy for production. Over the past two decades, work has been conducted globally, which consistently showed genetic selection can reduce CH₄ production by approximately 1% per generation in sheep, beef, and dairy cattle. In Australia, these industries developed selection indexes that utilize variation in commonly recorded traits and the strong positive genetic correlation between feed intake and CH₄ production. These indexes were a first step in making long term physiological changes to cattle and sheep, which will result in animals that are productive but emit less CH₄. There is variation in CH₄ production between animals that have equal feed intakes or growth rates. The Australian sheep and beef industries both have ongoing large-scale projects to measure these differences in over 10,000 industry relevant animals. This is the next step for direct selection of animals that emit less CH₄. The changes direct selection will have on the physiology of ruminants is comparatively unknown at the moment. When CH₄ breeding values and genetic correlations with other traits is known, direct CH₄ can be included in selection indexes, this will ensure balanced selection, avoiding unfavorable changes to other traits such as reproduction or eating quality. Future projects in Australia and international collaborations intend to rapidly increase measuring of animals. As new technologies become available and methodologies improve, more measurements on physiological components of CH₄ production will be captured. Examples include; rumen volatile fatty acid profiles, and sequencing of the rumen microbiome population. These additional measurements are expected to be used as proxies for CH₄ which is expensive and difficult to collect, and provide a better understanding of changes selection for lower CH₄ emissions has on animal physiology.

Automated monitoring of beef cattle ingestive behaviour under heat stress

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Increased occurrence of heat waves is expected in Southern Europe as a consequence of current climate change trends. In fattening cattle, an early detection of changes in behaviour patterns can be useful to attenuate the effects of heat stress on their welfare and performance. The objective of this study was to establish thresholds for heat stress conditions leading to alterations in feeding behaviours in feedlot cattle. Fifteen young beef bulls (305 kg, 7.7 months) were finished indoors on the Pyrenees (950 m a.s.l.) on ad libitum concentrate and straw from July to October (132 d). Medria collar-attached accelerometers recorded physical activity continuously, providing the most dominant behaviour among 5 (ingestion, rumination, rest, over-activity and other) for each 5-min interval. Data from air temperature and relative humidity sensors placed outside the barn (three open sides, natural ventilation) allowed to calculate the temperature and humidity index (THI). The individual daily concentrate DM intake (DMI) was recorded in automatic feeding stations. Partition trees were constructed to estimate the threshold average and maximum daily THI that determined changes in activity patterns, and the daily time devoted to each activity was compared above and below the cutting point with a Mixed model considering the animal effect. Above an average daily THI threshold of 65, daily eating time decreased (185 vs 233 min/d at THI >65 vs <65, $p<0.001$) with a concomitant reduction of DMI (7.1 vs 7.7 kg/d, $p<0.001$), whereas rumination time increased (407 vs 447 min/d, $p<0.001$). Resting time decreased (479 vs 439 min/d) and time devoted to other activities increased (271 vs 219 min/d), and thus bulls spent longer standing up (729 vs 679 min/d at THI >65 vs <65, $p<0.001$) to facilitate thermoregulation. Similar values were obtained for comparisons above and below the cutting point for maximum daily THI (77), despite the fact that 9% of the recording days did not fall into the same category as using the average THI. Beef cattle modified their activity patterns in response to environmental conditions, even above thresholds considered as mild heat stress.

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Theatre 8

Diurnal influences on reticulorumen temperature and pH in fattening bulls measured by rumen sensors for early prediction of heat stress conditions

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Thermal changes in global climate influence feedlot cattle husbandry systems in economic perspective because it leads to stress situations that can have a negative impact on health and welfare conditions. The aim of this experiment was to find out whether reticulorumen temperature (RT) or rumen pH captured by a rumen sensor bolus system (smaXtec animal care GmbH, Graz, Austria) can be used as an early indicator for heat stress (HS) and how it is influenced by diurnal effects. Physiological data from 70 male feedlot cattle were investigated using THI from remote HOBO Onset climate sensors over a period of 190 days. Using time series analysis and seasonal ARIMA modelling, it was found that RT followed the same patterns throughout days with a THI > 75 as well as days with no HS. Same applies for rumen pH. Collective mean RT over the whole period was 39.49°C with a StdDev of 0.265°C, minimum mean at 38.31°C and maximum mean at 40.69°C. For collective mean pH the result was 6.31 with a StdDev of 0.244, minimum mean at 5.54 and its maximum mean at 6.89. Mean values were stable throughout winter and summer conditions and showed no significant differences in RT or rumen pH. Average residual of duration from maximum THI to maximum RT alternates from 4 – 17 hours under HS conditions and 5 – 15 hours without HS. Conclusively, HS has no visible short-term effects on RT or pH. Cattle are able to control their body temperature in a narrow range over varying climatic conditions. Apparently, RT and pH are rather dependent from diurnal rhythms and feeding cycles. Our thesis to use them as early indicators to predict HS could not have been confirmed. The project was funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE).

Long-term effect of postnatal photoperiod on muscle development and histone modifications in Japanese Black cattle

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Season of birth and early-life photoperiod has long-term effect on hormonal secretion and reproduction in mammals, including cattle and sheep. In our previous study using mice, postnatal photoperiod alters body weight gain, expression of photoperiod-imprinting genes in the muscle, and metabolomic profiles in adulthood (Uchiwa et al, *Physiol Rep*, 2016). It remains unclear whether early-life photoperiod has also long-term effect on muscle development in cattle. In this study, we analyzed the effect of postnatal photoperiod on muscle development and epigenetic markers in the muscle using Japanese Black cattle. Cattle were exposed to short-day conditions [SD, 10 hours light: 14 hours dark (10L14D)] or long-day conditions (LD, 16L8D) from birth to weaning at 3 months of age (mo). After weaning, cattle were raised under natural photoperiod. During photoperiodic treatments, the calves under SD and LD exhibited winter- and summer-patterns of coat, respectively, irrespective of actual seasons. The cross-sectional area of the longissimus thoracis muscle fibers at 4-10 mo was higher in LD group than SD group. At slaughter (more than 40 mo), total carcass weight tended to be higher in LD group than SD group. The expression of several photoperiod-imprinting genes in the muscle were sensitive to photoperiod in the cattle at least until 10 months old, but no significant effect was observed in DNA methylation and hydroxy-methylation rates of the promotor region. Next, we analyzed histone modifications in the muscle at slaughter. Among 31 sites of H3/H4 histone modifications, acetylation of H3K18 (H3K18ac) and H4K12ac were higher in LD group than SD group. Moreover, temporal analyses revealed that H3K18ac were already higher in LD group than SD group at 8 mo. ChIP-seq showed that H3K18ac enhanced some transcription factors, and comprehensive analysis suggested that pathway related insulin was enriched in LD group. These suggest that the postnatal photoperiod alters muscle development and physiology via specific histone modifications.

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Theatre 10

Influence of piglet birth weight on cell populations and gene expression of skeletal muscle, perirenal and subcutaneous adipose tissues

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In the last decades, the increase in litter size has been associated with a high proportion of piglets born with low birth weights (LBW; 20-30% depending on studies). At birth, these LBW piglets have fewer myofibres in muscle, lower adipose tissue mass and modified expression levels of myogenic and adipogenic genes in these tissues. At 6 months of age, these pigs have larger muscle fibres and decreased postnatal growth compared with animals born with average birth weight (MBW). Adipose and muscle tissues include heterogeneous cell populations: mature cells (adipocytes, muscle fibres) and mesenchymal stromal/stem cells (MSCs) involved in cell renewal or tissue expansion. Changes in lean and fat mass in response to nutritional or environmental factors are associated with differences in these tissues cell composition. Therefore, this study was undertaken to determine whether cellular and molecular features of muscle and adipose tissues differ between LBW and MBW piglets. Cross-bred piglets (n= 14; 6 males and 8 females) were selected from 7 litters according to their body weights at birth. MBW (1.58 ± 0.04 kg) and LBW (1.01 ± 0.09 kg) suckling piglets were slaughtered at 5 or 6 days of age. Organs were weighted and tissue samples were collected for cell phenotyping by flow cytometry and gene expression analysis by qPCR. Data were analyzed using R software by a two-way ANOVA with birth weight and sex as fixed effects. Although LBW piglets differed morphologically from MBW piglets, only slight changes were detected. Only CD90+ cells (stromal cells) were significantly over represented in perirenal adipose tissue of LBW piglets. The MYH1 gene in muscle and PPARγ in adipose tissues were down-regulated in LMW piglets while DLK1 was up-regulated in LBW piglets muscle in agreement with a lower adipose tissue development in these animals. Surprisingly, the proportion of myogenic CD56+ cells in muscle and of hematopoietic CD45+ cells in muscle and perirenal adipose tissue were significantly higher in females than males. In adipose tissues, lipogenic genes were overexpressed in females compared with males. Altogether, these results provide evidence that sex is an important factor to consider in research.

Describing adaptation to heat stress through untargeted metabolomics in pig genetic resources

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Climate change is significantly affecting pig production systems worldwide, pushing breeders to face a new challenge: pigs have to respond well to heat-derived stresses while maintaining good productivity. Local pig breeds are genetically and physiologically adapted to their native environments, making them valuable resources for the identification of novel biomarkers. In this study, we used untargeted metabolomics to explore the relationship between the metabolomes of four Italian breeds (Cinta Senese, Mora Romagnola, Italian Large and Italian Duroc) and two Polish breeds or populations (Złotnicka White and Złotnicka White x Złotnicka Spotted) and their response to high temperatures and related stress through the evaluation of the metabolomic profiles over the circadian rhythm of the animals. The study took place in summer, sampling 12 animals per breed at two time points: 1) in the late afternoon (the hottest part of the day) and 2) early in the morning (the coolest part of the day). For each breed, metabolomic profiles of about 700 molecules were compared through a machine learning approach (Random Forest). From each breed, about 30-50 heat-stress-related metabolites were identified and their discriminative power was confirmed with unsupervised analyses. What emerged was a heterogeneous composition of the heat-stress-related metabolome, with few molecules shared between two or more breeds. Nevertheless, this pilot study provided new insights into the biological processes involved in adaptation to heat stress conditions, deepening our understanding of the mechanisms underlying the circadian rhythm physiological processes in these pig breeds. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).

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Theatre 12

Proteomic analysis of blood monocytes from dairy cows that were supplemented ex vivo with cannabinoid receptor 2 ligand beta caryophyllene after inflammatory challenge

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Activating the cannabinoid receptor 2 (CNR2) in immune cells exerts mostly anti-inflammatory effects. We examined the effects of selective activation of CNR2 by full agonist, β -caryophyllene (BCP), on the proteome of monocytes following an inflammatory challenge ex vivo. Blood monocytes were collected from mid-lactation dairy cows, and were supplemented with BCP at 1 mM or controls. After 24 h, monocytes were challenged with lipopolysaccharide (LPS; 1 μ g/ml), and 6 h later proteins were extracted. We performed proteomic analysis of protein lysates from monocytes of controls and BCP-supplemented cells after LPS challenge (n = 4 from each treatment) using LC-MS/MS. A total of 3442 proteins were identified in monocytes; from which 113 proteins were differential by t-test in BCP vs. controls [$P \leq 0.05$ and fold change (FC) ± 1.5]. The protein abundance immune-related protein NF-kappa-B essential modulator (IKBKG) was lower in BCP-supplemented monocytes vs. controls ($P = 0.02$ and FC = -1.8), while the abundance of complement component 4 binding protein, alpha chain (C4BPA) was higher in BCP than in controls ($P = 0.03$ and FC = 1.8). The abundance of NAD-dependent protein deacetylase sirtuin-5, mitochondrial (SIRT-5) was lower in BCP than in controls ($P = 0.0006$ and FC = -6.3), and the abundance of the transient receptor potential cation channel subfamily M member 2 (TRPM2) was lower in BCP than in controls ($P = 0.005$ and FC = -4.9). The abundance of eukaryotic translation initiation factor 4C (EIF1AX) was higher in BCP vs. controls ($P = 0.002$ and FC = 2.2), while the abundances of signal transducer and activator of transcription 5B (STAT5B; $P = 0.02$ and FC = -3.5) and of prostaglandin E synthase 3 (PTGES3; $P = 0.01$ and FC = -1.5) were lower in BCP vs. controls. This is the first proteome dataset of monocytes from dairy cows. These results indicate that BCP supplementation may affect the response of bovine monocytes to inflammatory stimulus via alterations in the abundances of various immune, transcription and translation related proteins.

The knowledge of experts with key skills in livestock species resilience traits studies

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An animal's resilience is the ability to cope with short-term disturbances, including those caused by pathogens, through response and rapid recovery to its original state. The European Partnership on Animal Health and Welfare (EUPAHW) has been funded by the European Commission, involves 90 groups from 24 countries, and includes 17 three-year research projects among which is SOA16. The main goal of SOA16 is to combine resilience traits into selective breeding and create complete animal health management systems. Specific objectives are: (i) to find indicators of resilience, resistance, and robustness, and understand how they relate to health, welfare, and production traits; (ii) to study how genetics, feeding methods, and management practices affect resilience and health traits; (iii) to create new models to improve resilience at the population level and evaluate their usefulness in breeding programs. SOA16 consists of six major tasks, where Task 1 will set up a pan-European network of experts in genetics (breeding), feed additives (including pre-and probiotics), applied ethology, stress physiology, and immunology. This has been made possible through an online survey designed to identify researchers working in resilience-related fields. The primary results have been obtained among experts working on different internal projects of the Partnership. Thereafter the survey has been to be distributed outside the EUPAHW. The final expert network will be useful in strengthening long-term cooperation and information exchange for research regarding resilience traits in livestock species. Survey link for experts participation: <https://ec.europa.eu/eusurvey/runner/97cabefe-08cb-426d-a6cf-d7d87f585bf7>

Session 81

Poster 14

Effects of adding chenopodium formosanum seed extract on porcine oocyte

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In recent years, in vitro production has been widely utilized in animal breeding and reproduction fields. Previous studies have shown that reactive oxygen species (ROS) levels in oocytes developed in vitro are higher compared to those developed in vivo. This suggests that both in vitro maturation (IVM) and in vitro culture (IVC) may contribute to a decrease in oocyte development rates due to oxidative stress. Taiwanese *Chenopodium formosanum* (CF) seeds contain various antioxidant substances, including phenolic compounds, flavonoids, vitamin E, plant sterols, and saponins, which have demonstrated antioxidant and anti-inflammatory properties. Therefore, it is hypothesized that adding CF seed extract could reduce ROS production during the in vitro culture of porcine oocytes, thereby enhancing antioxidant effects and improving the efficiency of IVC. In this study, CF seeds were extracted using ultrapure water at 100°C for 10 minutes, and the extract was added to the IVC medium at concentrations of 0% (108 oocytes), 2.5% (116 oocytes) and 5% (112 oocytes). 3 replicates per treatment were conducted. The effects of the CF seed extract on the subsequent development of porcine oocytes were evaluated. The results showed that the cleavage rate in the 2.5% extract group ($86.9 \pm 3.4\%$) was significantly higher than that of the control group ($75.8 \pm 3.4\%$) ($P < 0.05$). Similarly, the cleavage rate in the 5% extract group ($88.8 \pm 3.7\%$) was also significantly higher than the control group ($75.8 \pm 3.4\%$) ($P < 0.05$). However, there was no significant difference in blastocyst rates between the groups. In conclusion, adding CF seed extract during the IVC period increased the cleavage rate of porcine embryos. This suggests that CF seed extract may improve the efficiency of in vitro pig embryo production.

Lactacisbacillus rhamnosus Peptidoglycan Suppresses TLR2/1-Mediated Inflammation in Bovine Endometrial Epithelial Cells

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Bacterial contamination and their products are implicated in bovine endometrial inflammation, contributing to reproductive failures. While *Staphylococcus aureus*-derived peptidoglycan (PGN-S) has been shown to induce inflammation and disrupt sperm-immune recognition in bovine endometrial epithelial cells (BEECs) via Toll-like receptor 2 (TLR2), the immunomodulatory effects of PGN from commensal lactic acid bacteria (LAB) in female reproductive tract, remains unclear. This study investigated the immunomodulatory effects of PGN derived from LAB (PGN-L) on PAM3CSK4- (PAM3: synthetic TLR2/1 agonist) induced uterine inflammation in vitro. PGN-L was extracted from *Lactacisbacillus rhamnosus* ATCC53103 and its composition was compared to commercially available PGN-S. BEECs were pretreated with PGN-L or PGN-S (0.01, 0.1, & 1 ng/mL) for 24 h before stimulation with PAM3 (100 ng/mL) for 3 h. Inflammatory gene expression (TNF, PTGES, CXCL8, IL1B) by RT-qPCR, protein expression (TNF and PTGES) by immunofluorescence, and PGE2 by ELISA were assessed. PGN-L shared the GlcNAc-MurNAc sugar backbone with PGN-S, but a characteristic modification could be found in PGN-L. Both PGN-L and PGN-S possessed a lysine residue at the stem of cross-bridge structure, linking one PGN chain to others. Further, the cross-bridge structure was composed of glycine residues and presumably modified D-aspartate in PGN-S and PGN-L, respectively. While PGN-S (1 ng/mL) and PAM3 significantly increased inflammatory markers, PGN-L alone did not induce inflammation. Importantly, PGN-L pretreatment decreased PAM3-induced inflammation and reduced PGE2 secretion. Computational modeling suggested that PGN-L acts as a TLR2/1 antagonist, while PGN-S and PAM3 promote TLR2/1 heterodimerization. These findings indicate that PGN-L suppresses PAM3-induced uterine inflammation possibly by modulating TLR2/1 signaling in BEECs. PGN-L needs further study as a therapeutic strategy for uterine inflammation, like endometritis, to improve bovine fertility.

Fasting-Induced Changes in Plasma Metabolites and Muscle Composition in Marbled Sleeper (*Oxyleotris marmorata*)

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Marbled sleeper (*Oxyleotris marmorata*) is an economically important freshwater fish in Southeast Asia. For marbled sleeper production, fingerlings are typically collected from the wild. Before adapting to farmed feed, the fish occasionally withstand periods of fasting. This study, therefore, investigates the effects of fasting on blood metabolites and muscle composition. The fish were collected from a river basin and transferred to a hatchery. Subsequently, they were adapted to the experimental culture conditions for two weeks. The fish were then subjected to a four-week fasting period. Blood metabolites and muscle composition were analyzed. Our results showed that plasma glucose, protein, and albumin levels decreased with increasing fasting duration ($P < 0.05$). In contrast, blood urea nitrogen increased with prolonged fasting ($P < 0.05$). There were no significant differences in plasma triglyceride and cholesterol levels ($P > 0.05$). In addition, lipid content in muscle appeared to decrease, while muscle water content increased during the fasting period, suggesting that marbled sleeper utilizes lipids as an energy source during feed deprivation. There were no significant differences in protein and ash contents in muscle. Taken together, fasting status modulated several blood metabolites and muscle components, indicating that marbled sleeper preferentially utilizes lipids as an energy source during feed restriction.

When ruminants eat insects: possibilities and constraints of a potentially sustainable practice

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While grazing, large mammalian herbivores, including farmed ruminants, incidentally ingest a large variety of plant-dwelling arthropods belonging to different taxonomic and functional groups. Most of them are insects. Even though the image of ruminants eating insects while grazing or browsing seems plausible and “natural” to our minds, its scientifically driven proof is a recent matter of fact. This direct trophic interaction is so common and frequent that some authors have lately highlighted that it may be appropriate to consider these obligate herbivores as omnivores. So, why shouldn't we consider the possibility to include insect-derived products into the diet of farmed ruminants? A first constrain, which regards insect proteins, is associated to safety concerns and regulatory compliance. Like any other protein from animal origin, insect proteins cannot be fed to ruminants in Europe (EU). This ban is aimed at preventing the potential risk of Bovine Spongiform Encephalopathy, which is the only animal prion disease that has been demonstrated to be zoonotic. While recent research has shown that insect species used as feed (i.e., black soldier fly larvae) have the potential to serve as mechanical vectors for prion diseases, stakeholders should be aware that insects lack the prion protein gene, therefore being unable of prions propagation. Moreover, current EU regulations ensure the safety of insect rearing substrates, so that it is unlikely that prions would be introduced in the food chain. Another major constraint is linked to neophobia, which exerts a considerable influence on consumers' attitudes, perceptions, and purchasing decisions. Even if surveys have suggested that most people have a positive attitude towards the use of insects as animal feed, the acceptance level depends on the farmed animal species to which dietary insects are destined, with the lowest acceptance being reported for ruminants. Despite these challenges, in the last five years research on insect-fed ruminants has risen and started unravelling related effects in terms of feed digestibility, production performance, metabolism and quality of derived products (milk and meat), showing promising opportunities for more sustainable ruminant farming practices.

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Theatre 2

Dietary Inclusion of Black Soldier Fly (*Hermetia illucens*) Oil Alters Serum Metabolome in Dairy CowsG. Liu¹, M. Renna², L. Rastello², M. Coppa³, A. Kenez¹

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Hermetia illucens oil (HIO) could be a sustainable alternative to conventional dietary energy sources, such as hydrogenated palm fat (HPF), in ruminant nutrition. However, the physiological effects of dietary HIO inclusion in ruminants have not yet been evaluated. We hypothesized that replacing HPF with HIO in diets destined for early lactating dairy cows can initiate alterations in their metabolism. Twenty-six Valdostana Red Pied cows (parity = 3 ± 1.9 ; days in milk = 68 ± 27.5 ; body weight = 556 ± 49.5 kg; milk yield = 22.0 ± 3.64 kg/d) were randomly allocated to one of two experimental diets (ad libitum hay and 5.4 kg/head×day concentrate) containing either 3% HPF or HIO (DM basis) as the main energy source. The experimental period spanned 50 days, with serum samples collected from the jugular vein at the beginning (day 0) and end of the trial (day 50) for metabolomic analysis using liquid chromatography-mass spectrometry. A total of 8,061 serum features were detected, with 689 metabolites annotated. Principal component analysis (PCA) revealed a separation between day 0 and day 50 in both HPF and HIO groups, indicating marked temporal shifts. We found significant changes ($P < 0.05$) in 160 and 144 metabolites in HIO and HPF, respectively, with 87 and 71 metabolites being unique to either group. KEGG pathway analysis revealed that these distinctive metabolites were mainly related to amino acid and lipid metabolism pathways. Specifically, L-proline, L-arginine, and D-serine were upregulated in HIO but unchanged in HPF, whereas malic acid was upregulated in HPF but downregulated in HIO. In lipid metabolism, traumatic acid increased and stearidonic acid decreased in HIO, with no changes in HPF. We did not detect adverse metabolic effects associated with the tested HIO inclusion level. Our findings suggest that HIO can replace HPF to some extent in dairy cow diets, maintaining metabolic balance while offering a more sustainable alternative.

Replacing palm distillate fatty acids by *Hermetia illucens* oil in the diet of lactating sheep: effects on performance and milk fatty acids

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The use of insect oils as a replacement for conventional energy sources in ruminant diets (e.g., palm distillate fatty acids; PFA) is of great interest due to the increasing concern about the environmental impact of the animal feeding industry. *Hermetia illucens* oil (HIO) has a fatty acid (FA) profile similar to that of PFA (rich in medium-chain saturated FA) but with a predominance of 12:0 instead of 16:0. This may affect the ruminal environment (due to the antimicrobial effect of 12:0) and the mammary lipid metabolism, which could be reflected in milk FA profile. On this basis, a study was conducted to evaluate the effects of replacing PFA with HIO in the diet of lactating ewes. To this aim, 14 dairy Assaf ewes were used in a replicated 2 × 2 change-over design with 2 periods of 25 d each and 2 experimental diets: total mixed ration (forage:concentrate 50:50) containing 2% DM of either PFA or HIO. On the last 3 days of each period, individual milk yield and DM intake were recorded, and individual milk samples were collected for chemical composition and detailed FA analysis. Data were analysed with the MIXED procedure of SAS 9.4. No differences were found in animal performance. HIO increased milk percentages of 12:0, 14:0, but caused a larger decrease in 16:0. Besides, HIO promoted greater milk percentages of 18:0, most cis and trans 18:1 (e.g., c9-18:1 and t11-18:1) and c9t11 conjugated linoleic acid, while it reduced 18:2n-6 and 18:3n-3. No effect was observed on the t10/t11 18:1 ratio. Overall, replacing PFA by HIO at the tested dose did not impair ewes' performance. In addition, it increased the ruminal biohydrogenation (BH) extent of dietary PUFA without altering metabolic pathways, favouring milk accumulation of potentially health-promoting BH intermediates, but also 12:0 and 14:0. Acknowledgements: CSI020P23 and FEDER.

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Theatre 4

Do consumers like milk and butter obtained from dairy cows fed black soldier fly oil?

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Black soldier fly oil (*Hermetia illucens*; HIO) could be used as a sustainable lipid source, alternative to hydrogenated palm fat (HPF), in dairy cow diets. This study aims to assess consumers' perception and acceptance of milk and butter obtained from HIO- and HPF-fed cows. Two equivalent groups of 13 Valdostana Red Pied cows were fed for 50 d ad libitum hay, and isonitrogenous and isoenergetic concentrates containing either HPF or HIO (3% as fed; 162 g/head×day). At the end of the trial, bulk milk was collected and separately processed for each group. Pasteurized whole milk and butter were evaluated by 106 untrained assessors, using a 9-point hedonic scale (1 = "dislike extremely" to 9 = "like extremely") to rate overall liking of the tested products. A 5-point Just-About-Right (JAR) scale was used for color, odor, taste, and creaminess (milk) or texture (butter) appropriateness. The Mann-Whitney U test and Penalty Analysis (PA) allowed identifying differences between groups and key attributes influencing liking, respectively. Both milks were appreciated, with HIO milk being liked more than HPF one (6.6 vs. 6.1, respectively; P<0.05). Conversely, consumers liked HPF butter only, while HIO butter received a "neither like nor dislike" score (6.6 vs. 5.3, respectively; P<0.001). PA showed that HIO butter was penalized for having a "too strong" taste (63% of answers), which caused a mean drop from the liking score of 2.80 (P<0.001). Differences in the fatty acid (FA) composition of HIO and HPF (C12:0 and C16:0 being the most abundant individual FA, respectively) affected milk FA profile. Indeed, HIO milk showed higher proportions of C4:0 to C14:0 FA, which confer strong and undesirable aroma in dairy products, and the fat concentration in butter accentuated the perception of such milk fat changes. Supported by Hi-Ga project; Green Era Hub on Agri Food and Biotechnology

Black soldier fly oil replaces soybean oil and beef tallow; their comparative efficacy on reproductive hormones, connexin expression, and performance in laying hens

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The increasing demand for sustainable and energy-dense feed in poultry nutrition has prompted the exploration of alternative dietary fat sources. The objective of this study was to evaluate how black soldier fly oil (BSFO) at varying levels could serve as a replacement for soybean oil (SBO) or beef tallow (TA), their effect on connexin expression, reproductive hormone, cytokine levels, and the performance of laying hen. A 14-week experiment (2 weeks adaptation, and 12 weeks trial) was conducted with 288 Hy-Line Brown layers aged 24 weeks allocated to 6 treatments, 6 replicates, and 8 birds per replicate in a completely randomized design. The 12-week trial was categorized into 3 phases (26-29 weeks, 30-33 weeks, and 34-37 weeks) subjected to six dietary treatments as diet+2% BSFO (BSF2), diet+4% BSFO (BSF4), diet+2% SBO (SOY2), diet+4% SBO (SOY4), diet+2% TA (TA2), and diet+4% TA (TA4). The SBO treatments showed higher hen-day egg production during 30-33 weeks ($p=0.015$), and 34-37 weeks ($p=0.009$) comparable to BSFO treatments and TA2 which outperformed TA4. Egg mass was higher ($p=0.030$) in the SBO treatments comparable to BSFO during 34-37 weeks. The Haugh unit was higher ($p=0.016$) in the BSFO treatments which is comparable to SBO during 26-29 weeks. Eggshell thickness was higher ($p=0.043$) in the BSFO treatments comparable to SBO treatments and TA4 during 26-29 weeks. Eggshell hardness was higher in BSFO and SBO treatments during 26-29 weeks ($p=0.045$), and 34-37 weeks ($p=0.023$). Moreover, SBO and BSFO treatments significantly reduced serum tumor necrosis factor- α levels and increased the expression of gap junction protein $\alpha 1$ /connexin 43 and gap junction protein $\alpha 4$ /connexin 39 in the oviduct. The study concludes that BSFO can replace SBO and TA in poultry diets, supporting laying performance and egg quality while mitigating inflammation. The findings highlight the potential of BSFO as a sustainable and functional ingredient in poultry nutrition. Keywords: gap junction; *Hermetia illucens*; insect; oil

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Theatre 6

Larval meal of the black soldier fly (*Hermetia illucens*) is suitable for feeding the white leg shrimp (*Litopenaeus vannamei*) under practical conditions in a modern recirculation system

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Black soldier fly larval (BSFL) meal has proven suitable to substitute fish meal (FM) in diets for various aquaculture species. Counter to extensive research in farmed fish, only few studies examined the suitability of BSFL meal as a protein component in shrimp diets. We present a feeding trial using the Pacific white leg shrimp (*Litopenaeus vannamei*), conducted under practical indoor farm conditions. Feeding efficiency of a FM-based control diet was compared to an isonitrogenous, -lipidic and -energetic test diet in which 10% of the FM were replaced by BSFL meal in a recirculation system with 2 tanks. Both diets were provided as extruded pellets that were adjusted to shrimp size. Each dietary treatment included 12.5k pre-adapted postlarval shrimps, and tanks were swapped between each of 4 consecutive runs, which on average lasted 97 days. Shrimp growth rates were measured at least every other week (>3 samples of >20 individuals). At harvest, final weights, mortality and feed conversion were assessed. Shrimp performance was on a comparatively high level, with no treatment effect on mortality ($P=0.23$), and positive effects of the BSFL meal on performance for feed conversion ($P=0.11$), specific growth rate ($P=0.04$) and harvest weight ($P<0.01$). Shrimp protein content (4 pooled individuals/replicate) did not differ between treatments ($P=0.15$), yet profiles of some amino acids did. This work shows that BSFL meal at the tested inclusion rate is an alternative protein source in shrimp production in recirculating systems without compromising production performance.

Use of *Acheta domesticus* meal in diets for piglets during the post-weaning phase

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The use of insect meals in pig nutrition is less studied than in poultry or fish. *Hermetia illucens* and *Tenebrio molitor* show no adverse effects on growth, while research on *Acheta domesticus* focuses mainly on human nutrition due to its high-quality protein. Enzymatic hydrolysis may enhance macronutrient availability, supporting piglet growth during the critical post-weaning phase, improving digestion and sustainability in pig farming. This study is the first to evaluate AD meal inclusion in piglet diets. Over 48 days of trial, 48 mixed-sex piglets (4 replicates/treatment, 4 piglets/box) were assigned to three isonitrogenous and isoenergetic diets: control (C), 5% untreated AD meal (UAD), and 5% hydrolyzed AD meal (HAD). Acid-insoluble ash (1%) was used to determine apparent total tract digestibility coefficients (ATTDC %) for dry matter, crude protein, ether extract (EE), and gross energy. Growth parameters – body weight (initial and final [FBW]), average daily gain, feed intake, and feed conversion ratio – were recorded at mid and end points. Fecal samples, collected during the last four days, were freeze-dried and analyzed for ATTDC calculations. Data were analyzed using IBM SPSS (One-way ANOVA, $P \leq 0.05$). The growth performance showed a statistical trend for FBW of piglets fed AD-based diets, which tended to be higher than C ($P=0.057$). A slight effect on EE digestibility ($P=0.044$) showed UAD (79.91%) tending to be less digestible than HAD and C (84.26–84.24%). AD meal is a viable protein alternative for piglets, ensuring growth and nutrient digestibility. The study was supported by the projects Harnessing the potential of cricket based-fortified food on gut health (CRICKFOOD Prot. 2022SL8PKP) funded by the European Union–NextGenerationEU, NRRP Missione 4 “Istruzione e Ricerca”-Componente C2, Investimento 1.1 “Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN).

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Live *Tenebrio molitor* larvae as feed ingredient for native chickens: a multi-perspective view on egg quality

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Live insect larvae have been identified as environmental enrichment for native chickens, but very limited data are available about their use as feed ingredient. This study aimed to investigate the effects of live *Tenebrio molitor* (TM) larvae on egg quality of Bionda Piemontese native hens. A total of 198 forty-week-old Bionda Piemontese birds were allotted to 3 isonitrogenous and isoenergetic diets for 84 days (6 pens/diet, 11 birds/pen [1 cockerel and 10 hens]): C (control, consisting of a core feed [90%] and a feed mix [10%, mimicking the larval nutritional profile]), TM5 and TM10 (containing 5% and 10% of live TM larvae calculated in dry matter [DM] on the daily feed intake of the birds as partial or complete replacement of the feed mix, respectively). Egg physico-chemical quality and malondialdehyde (MDA) concentration (10 eggs/replicate), as well as sensory characteristics of boiled eggs ($n=63$ panelists), were assessed. Data were analyzed by IBM SPSS software ($P \leq 0.05$). Shells from TM-fed hens showed lower lightness and higher yellowness and chroma than C ($P < 0.05$), with the TM5 groups being also characterized by lower hue when compared to C ($P < 0.01$). Dietary live TM larvae inclusion did not influence shell ratio ($P > 0.05$), but surface area and index were higher in TM10 group than C ($P < 0.01$). Albumen ratio and Haugh unit decreased in hens fed TM diets ($P < 0.05$), whereas yolk ratio tended to increase ($P = 0.087$). Whole eggs from TM10-fed hens displayed lower DM and gross energy contents when compared to C ($P < 0.05$), tending to be characterized by reduced crude protein as well ($P = 0.052$). Dietary live TM larvae inclusion neither influenced MDA concentration nor sensory scores ($P > 0.05$). In conclusion, live TM larvae may partially impair egg nutritional profile, but can lead to the production of darker eggs with more resistant shell and similar oxidative stability and consumer appreciation. Research supported by PRIMA (ADVAGROMED project).

Pilot-scale refinement and application of industrially produced black soldier fly larvae lipids

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Black soldier fly larvae (BSFL, *Hermetia illucens*) are rich in lipids, which serve as energy reserves for metamorphosis and reproduction. These larvae biosynthesize saturated fatty acids, predominantly lauric acid (40-50%), a compound with antimicrobial properties similar to those found in coconut and palm oil. There is currently a strong interest across industries in exploring ways to replace palm oil because socio-economic and environmental concerns. Despite the well-understood potential of BSFL lipids, there is substantial room for their application in feed and technical products, often requiring refinement beyond raw lipids extracted through dry or wet rendering. This study explored the refinement of BSFL lipids at two-hundred-liter scale with lipids from an industrial BSFL facility, including processes such as filtration, degumming, deacidification (with NaOH), and bleaching (two bleaching agents). Lipid quality was assessed before and after refinement using key indicators like peroxide and acid numbers. Stability tests were conducted across temperatures (0-170°C) and time (0-96 hours) and compared to benchmarks. Additionally, novel applications of refined BSFL lipids were investigated as frying oil and bird feed uses. The results showed that the BSFL lipids higher acid numbers (1-5) than common plant-based lipids (<0.4). Yet, refinement significantly improved quality parameters, such as reducing peroxide and acid numbers and enhancing color from yellow to white opening new applications. Refined lipids demonstrated better performance compared to non-refined fat, with lower foamability, and lower increases in peroxide and acid numbers (43% and 41%) and higher stability as frying oil than sunflower oil (167% and -42%). Furthermore, BSFL lipids successfully replaced beef tallow in bird feed, although formulation adjustments were necessary to match current quality benchmarks. The best formulation was used in industrial pilot production. This study highlights that refining insect lipids opens new opportunities for the application of BSFL lipids in various industries.

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Theatre 10

Beyond Authorized Insect Species: Unveiling the Potential of Native Coastal Insects for Sustainable Aquafeed

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The use of insect-derived ingredients in aquafeed has gained significant attention due to their high nutritional value and sustainable production potential. However, current regulations primarily authorize terrestrial insect species such as, the black soldier fly (*Hermetia illucens*). Beyond concerns regarding the sustainability of producing non-native species in a European context, a major limitation of these terrestrial insects for aquaculture is their low content of n-3 polyunsaturated fatty acids (PUFAs), which are essential for the growth and health of marine organisms. This study explores the potential of native coastal insect species as alternative protein and lipid sources for marine aquafeeds. By utilizing species naturally occurring in coastal ecosystems, this approach supports native biodiversity and reduces the ecological risks associated with the introduction of non-native species for mass production. We analyzed the nutritional composition of these insects and evaluated their suitability for feeding marine species. Preliminary findings suggest that some coastal species may provide essential fatty acids beneficial for fish and shrimp diets. By broadening the range of insect species considered for aquafeed, this study not only contributes to the diversification of sustainable feed ingredients but also highlights the ecological and economic potential of utilizing native biodiversity. Future research should focus on scalable production methods and regulatory pathways to integrate novel, locally adapted insect species into aquafeed production while ensuring ecosystem integrity.

The fluctuation in functionality of frass as fertilizer

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The use of insect frass as fertilizer has been assessed in multiple publications. However, the conclusions of those studies are variable. In this study, the potential effect of the feeding substrate on the potency of the frass was assessed. The first step was *Hermetia illucens* (BSF) larvae on ten different feed substrates, the frass was collected and chemically analyzed. Then, each frass 'type' was mixed, according to the analysis, with peat to ensure an equal nitrogen (N) addition and water was added. Based on a pilot trial, a nitrogen addition of 210 mg N/plant was used. Four pots (replicates) were filled with four kg of this mixture and 15 seeds of *Triticum aestivum* were added. Several controls were also set-up in an identical way: a negative control without fertilizer, two organic fertilizers (at 210mg N/plant) and two positive mineral controls (low: 70 mg N/plant and high: 210 mg N/plant). For the latter, two concentrations were chosen as it is known that animal manure has never the same N availability as mineral fertilizer. The seed germination was determined after two weeks, thereafter the wheat density was decreased to 8 plants/pot. Water was replenished twice a week by weighing the pots and refilling them to the original weight. The amount of evaporation was noted as proxy for plant growth. The plants were also photographed multiple times to determine the leaf area. At harvest, both the above ground and belowground biomass were determined. Finally, the composition of the plants and soil are assessed to construct a mass balance. The results indicate a large variation in the chemical composition of the frass with N concentrations between 1.5 and 5.2% (and similar ranges for K and P). Surprisingly, even though all pots received the same amount of nitrogen, major differences in fertilization capacity of frass were still observed. Certain types performed barely better than the negative control while other types rivaled or outperformed the low mineral control. Furthermore, this seems to be positively correlated with the original N concentration of the frass. This indicates that the variation in literature in the use of frass as fertilizer can, at least partially, be explained by differences in the substrate they received and should be taken into account in future studies.

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Poster 12

Innovation in piglet nutrition – use of black soldier fly meal

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Insects are an alternative nutritional protein source, resulting from a sustainable production system with efficient conversion rates. The use of insect meal is an opportunity to reduce the pressure on raw materials for animal feed and "Food vs Feed" competition, leveraging a new sector with great potential. This study is being conducted under the Mobilizing Agenda InsectEra (AAC nº02/C05-i01/2022). The study aim is to evaluate the added value of defatted larvae BSF meal in piglets feed, considering zootechnical performance. The first phase of the trial was conducted in a commercial farm, from day 8 to 28 of age. The litters were selected to be homogeneous within and between treatments (age, weight). At 28 days of age (weaning) animals were transported to the UTAD facilities (phase 2). In both phases animals were allocated to three groups considering different BSF meal incorporation rates: T1P1=control – 0%; T2P1 – 5% and T3P1 – 10%; T1P2=control – 0%; T2P2 – 2,5% and T3P2 – 5% (iso-nitrogenous and/or isoenergetic diets). In the 1st phase 18 litters were selected (6 replicates/treatment; 6 animals/replicate), from which 72 piglet males were selected for the 2nd phase. Piglets' average weight was 3.2 kg at 8 days of age. The 2nd phase of the trial (28 to 70 days of age of piglets), was divided in two: • from 28 to 42 days (pre-starter diet). Each treatment had 8 replicates for consumption (measurements by pen; 3 animals/pen) and 24 replicates for growth (measurements by animal; 24 animals) • from 42 to 70 days (starter diet). Each treatment had 8 replicates for consumption (pens) and 16 replicates for growth (animals), as 8 animals/treatment were slaughtered at 42 days of age. The measurements were: Individual weight; ADWG; ADFI; FCR; mortality and morbidity. ADWG was the only difference between treatments being significantly higher in T2P1 than in T1P1 (289g/d vs. 246g/d).

Study on the Growth and Nutritional Composition of the Black Soldier Fly (*Hermetia illucens*) Fed with Korean melon waste (*Cucumis melo* var. *makuwa*)

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Agricultural by-products are generated throughout the production, processing, and distribution stages of the food supply chain. Among them, fruit and vegetable by-products including low-grade or discarded produce are often discarded at the farm level or lost during distribution. As a sustainable strategy to recycle and valorize these organic wastes, the black soldier fly (*Hermetia illucens*) has garnered attention due to its capacity to biodegrade a wide range of organic substrates and its potential as a high-value livestock feed ingredient. In this study, black soldier fly larvae were reared on discarded Korean melon (*Cucumis melo* var. *makuwa*) to evaluate their growth performance and nutritional composition. When 4 day-old larvae were fed the Korean melon waste-based diet, their development period was extended by 22 days, and their average body weight was reduced by approximately 40% compared to larvae fed calf feed (control). Despite the reduced growth performance, larvae fed on melon waste exhibited a high substrate reduction rate of 80.5%, although their bioconversion efficiency was relatively low at 11.8%. In contrast, the control group showed a lower substrate reduction rate (62.1%) but a higher bioconversion efficiency (19%). These results suggest that the high moisture content and semi-liquid characteristics of melon by-products may facilitate substrate degradation but provide insufficient nutrients, thereby limiting larval biomass conversion. Nutritional analysis revealed that dried larvae reared on Korean melon waste contained approximately 36.8% crude protein and 37.7% crude fat on a dry matter basis, indicating their potential as a feed resource. However, supplementation of the Korean melon waste-based diet with nutrient-rich additives such as food waste or yeast may be necessary to support optimal larval growth and improve feed quality.

Effect of diet supplementation with chitin and two types of chitosan from *tenebrio molitor* on in vitro ruminal fermentation

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The rise of the insect farming industry results in an increasing availability of new products. Chitin and chitosan obtained from the exoskeleton of crustaceans have shown potential to modulate ruminal fermentation; however, the literature concerning insect chitin and chitosan is scarce. Therefore, an in vitro trial was designed to study the effects of the inclusion of chitin and chitosan of *Tenebrio molitor* in the diet of lactating ewes on ruminal fermentation. Four treatments consisting on a total mixed ration (forage:concentrate 50:50) without supplementation (Control) or supplemented with 2% chitin, 2% high-weight chitosan (HW-chi; 100-150 kDa), or 2% low-weight chitosan (LW-chi; <40 kDa) of *T. molitor* were incubated in 4 different runs (days) for 20 h with ruminal inoculum from 3 cannulated sheep. The gas production (GP), the disappearances of DM (DMD) and neutral detergent fibre (NDFD) and the in vitro true substrate digestibility (ivTSD) were measured. Lactic acid and ammonia concentrations and volatile fatty acid (VFA) production were also determined. The latter was used to estimate the production of CH₄. Data were subjected to ANOVA using the MIXED procedure of SAS (v9.4). The supplementation with both types of chitosans led to lower GP compared to Control or chitin ($P < 0.01$). Lower DMD and NDFD values were obtained in LW-chi than in other treatments ($P < 0.05$), whereas ivTSD was decreased with all supplements vs. the Control treatment ($P < 0.001$), with the greatest reduction observed in LW-chi. Compared to the Control, all supplements promoted a higher percentage of acetic acid and both chitosans reduced the percentage of butyric acid ($P < 0.01$). Individual and total VFA productions were similar among treatments, as were lactic acid and ammonia concentrations and CH₄ production estimates ($P > 0.10$). Overall, the dose of 2% of chitin and chitosans seems to negatively affect in vitro ruminal fermentation. However, further research is needed to investigate whether lower doses or adaptation of the microbiota (in vivo) could give more promising results. Acknowledgements: Projects PID2023-146979OB-I00, CSI020P23, FEDER and Tebrio Group S.L.

Conservation of *Hermetia illucens* larvae via co-ensiling for use as protein feedT. Hartinger^{1,2}, K. Schedle³, R. Resch⁴, K. Hartinger¹, R. Khiaosa-Ard¹¹ Vetmeduni Vienna, Veterinaerplatz 1, 1210 Vienna, Austria, ² University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany, ³ BOKU University, Muthgasse 11, 1190 Vienna, Austria, ⁴ AREC Raumberg-Gumpenstein, Altirdning 11, 8952 Irdning-Donnersbachtal, Austria

Soybean meal is the most used protein feed for poultry in Europe. However, there is a strong import dependency and insect larvae may constitute an alternative protein source. After rearing, the larvae are typically dried to obtain a storable feed, but this is a highly energy-intensive process. Hence, ensiling of larvae could be a suitable substitute for drying, which has been only marginally considered. We investigated the conservation of *Hermetia illucens* larvae when ensiled alone [CON] or with 15% or 25% inclusion of crushed wheat [WC15 and WC25] or wheat middlings [WM15 and WM25], respectively. All treatments were ensiled for 90 days and subsequently analyzed for gaseous weight loss, pH, crude protein, ash, ammonia-N, acetic acid, butyric acid, and ethanol. Data were analyzed using a generalized linear model including co-substrate, inclusion rate, and their interaction as effects. The results showed that the gaseous weight losses were highest in CON (6.83%), followed by WC15 (6.29%) and WC25 (5.59%), and lowest in WM15 (5.18%) and WM25 (4.63%; $P=0.02$). Similarly, silage pH was higher in CON (pH 5.31) compared to other treatments (pH 4.25-4.44; $P<0.01$), while inclusion rate had no effect. Co-ensiling reduced the crude protein concentration ($P=0.03$) compared to CON, with a greater reduction at 25% than at 15% inclusion rate ($P<0.01$). Ammonia-N levels ranged from 0.7 to 1.8% of total N and rose with higher inclusion rate ($P=0.01$), while there were no differences between co-substrates. In conclusion, co-ensiling of *Hermetia illucens* larvae allowed a successful conservation, with 15% co-substrate inclusion being sufficient. The use of wheat middlings as co-substrate was as suitable as cracked wheat and may therefore be preferred due to mitigating feed-food competition. Follow-up feeding trials should be conducted in future research.

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Poster 16

Introducing LIFE4Waste Growth: Biostimulating organic fertilizers from insect frass

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Insect frass is the largest product of black soldier fly larvae (BSFL) farming on organic byproducts. Consequently, increasing its market value could be a key driver for the profitability of this emerging technology generating bio-based products for the bioeconomy. We introduce the new four-year LIFE project – Waste4Growth (2025-2029). LIFE is the key instrument of the EU to support disruptive high-risk projects with potential for dramatic positive environmental impact. Today, insect frass is predominantly used for biogas production or as low-quality organic fertilizer. LIFE Waste4Growth aims to increase the value of insect frass by converting insect frass into biostimulating organic fertilizers and applying them in agriculture. Returning insect frass as biostimulating organic fertilizers into agriculture is superior to current uses in biogas plants because it preserves its unique properties to improve environmental quality. The project is being implemented by private and academic stakeholders with farmer participation in Crete/Greece, Saxony/Germany, and Rhineland-Palatinate/Germany due to agricultural importance and challenges like poor soil and climate-change-related drought benefiting from biostimulating fertilizer application. The poster will outline the key aims and activities of the project including demonstrating the biostimulating effects of insect frass and developing a scalable production process to produce at least one liquid and one solid biostimulating organic fertilizer. Product functionality is being demonstrated with potato, maize, sugar beet, grapevine and olives in greenhouse and open field trials. Applications of the products with farmers is expected to reduce the crop land area with soil quality issues, climate-change related crop vulnerability to drought, greenhouse gas emissions by substitution of synthetic fertilizers and create green jobs. The project works towards a circular and climate-resilient EU, aligned with the Green Deal, Farm to Fork and Soil Protection Strategy.

Hermetia illucens pupal exuviae and extracted chitin as functional feed for laying hens: application of artificial senses for evaluating the organoleptic quality of eggs

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The growing demand for chitin, due to its properties and applications, has encouraged the search for sustainable alternatives to the traditional commercial source (crustaceans). Bioconverter insects, such as *Hermetia illucens*, are ideal candidates because chitin derived from pupal exuviae is a byproduct of insect farms, which primarily serve feed applications. This study aimed to assess the organoleptic characteristics of eggs produced by laying hens fed pupal exuviae and chitin functionalized feeds derived from *Hermetia illucens* larvae reared on agricultural waste substrates, using an artificial sensory platform composed of an E-Eye and an E-Tongue. These artificial senses replicate human sensory abilities, enabling the perception and interpretation of sight and taste through advanced sensors, similar to the human experience. A total of 72 ISA Brown laying hens were randomly assigned to three groups (six replicates with four hens per replicate): group CT (fed a basal diet), group CH (basal diet with 1g of chitin per head per day), and group EX (basal diet with 2.5g of exuviae per head per day). The trial lasted 70 days, and at the end of the study, 24 eggs were sampled to evaluate their organoleptic characteristics (yolk color, raw yolk, albumen and whole egg taste). These characteristics were assessed using the E-Eye (RGB color code) and E-Tongue (with seven potentiometric sensors). Data were analyzed with discriminant function analysis (DFA) to assess the differences between groups. The results from the E-Eye revealed a significant change in yolk color in the CH group, especially highlighted by color code 1872. Regarding taste, clear distinctions were observed among the three groups for both yolk, albumen, and whole egg. Notably, the CH group differed more significantly from the CT group in taste. While these results are preliminary, they are important for understanding the effects of novel feed sources on the organoleptic properties of eggs, which could influence consumer acceptance. Funded by European Union–Next Generation EU, PNRR-Mission 4, Component 2, Project ID: J53D23018560001.

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Theatre 1

Using data from sensors and other precision farming technologies to enhance the sustainability of dairy cattle breeding programs

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The adoption of sensor technologies and precision farming tools is revolutionizing the dairy industry by enabling real-time monitoring of animal health, welfare, and productivity. These technologies provide high-frequency, large-scale, and objective phenotypic data that offer significant opportunities to enhance genetic improvement programs. In this invited talk, we will explore the potential of sensor-derived data for optimizing genomic evaluations, addressing key challenges, opportunities, and implementation strategies. Dairy farms increasingly utilize automated systems, such as accelerometer-based behavior monitors, robotic milking systems, in-line milk bio-marker sensors, and wearable devices. Integration of these data streams into breeding programs remains limited due to fragmentation across sensor brands, lack of standardization, and challenges related to data accessibility, ownership, and governance. To fully leverage digital technologies in dairy breeding, data harmonization and integration are essential. Establishing standardized data collection protocols, robust quality control, and novel trait definitions are critical. Therefore, we will discuss the definition and the genetic background of various sensor-based traits and the feasibility of incorporating such traits into dairy genetic evaluations. However, several hurdles must be addressed, including data access and portability challenges, interoperability across sensor systems, and the necessity for collaboration among farmers, researchers, breeding organizations, and technology providers. We will outline a roadmap for effectively utilizing sensor-derived data in genetic evaluations and advocate for centralized data infrastructures, transparent data-sharing agreements, and global standards established by organizations such as ICAR. Overcoming these challenges will enable dairy breeding programs to harness precision dairy farming technologies, leading to enhanced production efficiency, improved animal health and welfare, and sustainable genetic advancements in the dairy industry.

Dairy cow resilience: data driven detection and quantification of perturbations using daily milk weights
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Resilience is an animal's capacity to recover after a perturbation or maintain specific functions under stress. US dairy herds group cows into pens based on factors such as parity, lactation stage, and milk production. By coupling daily milk yield data with day-to-day pen location information, we can detect management and environmental perturbations and subsequently measure the responses of individual cows to these stressors over a particular period. Our dataset included 62,580,945 daily milk weights and pen locations of 227,633 cows in parity 1, 2 and/or 3 from 204 herds representing 30 US states from 2018 to 2024. Individual lactation curves were fitted using polynomial quantile regression with a 0.5 quantile to derive expected lactation curves. Perturbation periods were identified based on residuals between mean expected and mean observed daily milk weights at the pen level. The initial dataset was stratified and considered 40 combinations that included severity levels from $\geq 3\%$ to $\geq 7\%$ and durations ranging from ≥ 3 days to ≥ 14 days. Delta milk yield (ΔMY) was measured as the change in a cow's mean daily milk production relative to her mean expected daily milk yield during an identified perturbation period. The statistical model used to estimate variance components and heritabilities for ΔMY included calving age, days in milk, parity and herd-year-season as fixed effects, along with event, additive genetic and permanent environmental effect as random effects. Our findings suggest that animals differ in their response to perturbations at the pen level, and this measure of resilience using daily milk data is heritable.

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Theatre 3

Analysing lactation curves to define resilience indicator traits in Austrian Fleckvieh

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In dairy cattle, various stressors can adversely affect reproductive and productive performance. Resilience, which is the ability of cows to cope with and recover from such challenges, is crucial for ensuring their well-being while maintaining production and reproductive capabilities. Recent research indicates that fluctuations in milk yield (MY) during lactation can be used to define resilience traits, with minimal variations showing better resilience of a cow. In this study, we defined two resilience indicators: log-transformed variance ($\ln(\sigma^2)$) and lag-1 autocorrelation (rlag) of the daily deviations in MY. Automatic milking system (AMS) data used for analysis comprised 8,615,511 daily milk records from 20,723 Fleckvieh cows over multiple lactations. The indicators were calculated from three fitted lactation curves, including the Wilmink curve and curves based on quadratic and cubic splines. Average daily MY was included as an additional trait to determine the correlations between the resilience indicators with MY. Genetic analyses were conducted using multivariate analysis in WOMBAT to estimate heritability (h^2) and genetic correlations between the traits. The estimated h^2 for $\ln(\sigma^2)$ ranged between 0.14 to 0.20, while 0.05 to 0.08 for rlag considering the different lactation curves. Moderate genetic correlations (0.31 to 0.39) were observed between the resilience indicators. High genetic correlations were estimated between average daily MY and $\ln(\sigma^2)$ (0.71 – 0.79), but the correlations between average daily MY and rlag (0.03 – 0.19) were low. The higher h^2 observed for $\ln(\sigma^2)$ suggests it may be a robust indicator of resilience in lactating Fleckvieh cows. Estimating correlations between the resilience indicators with traits in the selection index is recommended for further validation.

Heritabilities for sensor-derived traits and their genetic relationship with early lactation ketosis

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The increasing use of sensor systems monitoring dairy cow behaviour and physiology awakened interest to explore their potential for phenotyping in dairy cattle breeding. Focus is put on the development of new phenotypes or proxies for complex and low heritable traits such as functional traits. Ketosis in early lactation is prevalent on most dairy farms, but low frequencies and heritabilities of clinical diagnoses and high workload of routine testing for β -hydroxybutyrate (BHB) complicate an improvement through breeding. Within the D4Dairy project we examined proxies for early lactation ketosis from sensor data on commercial farms equipped with smaXtec (measuring activity and rumen temperature) and Lely (measuring activity, rumination and eating time) sensor systems. Blood BHB tests on days 7 and 14 after calving were used as reference with values >1.2 mmol/L indicating a state of ketosis (1) or healthy otherwise (0). Sensor variables were defined as daily sums (activity), means (temperature, rumination, eating time) and standard deviations (SD; all) at the day of testing and as means and regression slopes over 30 days postpartum. Heritabilities (h^2) were 0.09 for ketosis, 0.15-0.22 for activity, <0.01 -0.16 for temperature, 0.09-0.15 for rumination and 0.12-0.37 for eating time traits on the test day. Genetic correlations significantly different from zero were found for activity traits (-0.73 to -0.79). Means over 30 days showed h^2 of 0.16-0.50 for activity, 0.21 for temperature, 0.18-0.44 for rumination and 0.27-0.51 for eating time, while for slope-based traits h^2 significantly differing from zero were 0.06 (activity) and 0.10 (eating time). Activity traits were genetically correlated to ketosis (-0.63 to -0.99) as was eating time (0.42-0.56). Results suggest that traits defined from sensor data are heritable and genetic correlations suggest that activity and eating time may serve as auxiliary traits for early lactation ketosis.

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Theatre 5

Advancing metabolic health: Genomic evaluation of energy status indicator traits in Finnish dairy cattle

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Integrating feed efficiency traits into dairy cattle breeding requires accounting for early lactation energy status to support cow health. Energy status indicator (ESI) traits, like blood metabolites or milk fatty acids (FA) predicted using mid-IR reflectance spectroscopy (MIR), are of particular interest. This study aimed to enhance the evaluation of ESI traits through single-step genomic BLUP prediction. Data were collected from Finnish Red Dairy Cattle, Finncattle, and Holstein cows with test-day records from 8 to 60 days in milk. Milk samples were analysed using MIR and calibration equations for blood plasma non-esterified FA (NEFA) and beta-hydroxybutyrate (BHB). The dataset, starting from 2016, included 0.61 million ESI records from 0.28 million cows, with pedigree data covering 0.65 million animals. Among these, 63934 cows were genotyped since 2009. Genetic analysis used multiple-breed, multiple-trait linear mixed models distinguishing NEFA and BHB in first and later lactations as separate traits. The genomic models were developed using VanRaden method 1 for the genomic relationship matrix, with a 30% residual polygenic proportion and solved using the ssGTaBLUP method. Validation involved linear regression of full and reduced data breeding values (BV) and comparison of pedigree-based and genomic BVs. Heritability estimates for NEFA and BHB were higher in first lactation (0.13 and 0.23) than in later lactations (0.09 and 0.18). A slight, favorable decline in genetic trends for both traits indicates that current breeding goals do not adversely affect early lactation energy status. The genomic model improved predictive ability for ESI traits by 21.5 percentage points in cows and 16.3 in bulls, with R^2 ranging from 0.63 to 0.75 in cows and 0.42 to 0.58 in bulls. Additionally, b_1 values were closer to unity in cows (0.95–0.99) and improved in bulls (0.90–0.93), indicating reduced overprediction. Thus, genomic prediction enhances the accuracy and unbiasedness of BVs for ESI traits, making their inclusion in breeding programs both feasible and essential for supporting cow metabolic health while improving feed efficiency.

Estimation of variance components for selected metabolic traits in Fleckvieh Cattle

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Following extensive research on metabolic traits across multiple projects such as “Efficient Cow”, “FoKUHs” and “D4Dairy”, we aim to implement a genomic evaluation for metabolic stability within our populations. To achieve this, variance components were estimated using all available data from previous research projects and routine data recording. The phenotypic traits included in our REML analyses were fat-protein ratio (FPR), the mid-infrared-based predictor KetoMIR, hand-held β -hydroxybutyrate (BHB) tests using milk or blood (ketotest), and clinical ketosis diagnoses from farms with reliable health data recording. As phenotype collection was primarily conducted on farms with herd genotyping, we incorporated genotype information into single-step genomic REML analyses to compare results with conventional REML estimates. Analyses were performed using WOM-BAT software, which supports single-step GREML and utilizes multi-threaded computing to handle large datasets efficiently. The estimated heritabilities for FPR, KetoMIR, ketotest, and ketosis diagnoses were 0.11, 0.17, 0.035, and 0.015, respectively, aligning well with literature values. Correlations between KetoMIR and ketotest were moderate (0.55–0.60), while correlations between FPR and ketosis diagnoses (0.15–0.20), between KetoMIR and ketosis diagnoses (0.45–0.50) and between ketotest and ketosis diagnoses (0.80–0.90) indicate that both are highly informative auxiliary traits for metabolic status. Together with additional traits such as milk fever and losses due to metabolic disorders, these findings offer valuable insights for the genetic evaluation of metabolic stability.

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Genome-wide association study on differential somatic cells in Italian Simmental

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Somatic cell count (SCC) is the most adopted indicator of udder health in dairy animals. A high value of SCC is usually associated with subclinical mastitis and, in turn, with a lower quality of milk and a worsening of milk coagulation properties. SCC does not discriminate between different type of cells. Differential somatic cell count (DSCC), that represents the combined proportion of polymorphonuclear leukocytes and lymphocytes, has been proposed as a more precise indicator of mastitis in cows and sheep. DSCC. In this study, variance components estimation and a genome-wide association study (GWAS) on DSCC recorded in Italian Simmental cows were carried out. A dataset with 362,094 test-day records from 36,633 cows, farmed in 846 farms with calvings from 2020 to 2023 were used. Data were analyzed with a repeatability animal model that included parity as cross-classified fixed effect, days in milk as fixed covariate, and herd-test-day, animal, permanent environment, and residual as random effects. EBVs were then used as input for a Bayesian GWAS through BayesR using four classes of effects' size (0.0, 0.0001, 0.001, 0.01). Genomic breeding values (GEBV) were back-solved to get SNP variance and p-value using blupf90 suite. Heritability and repeatability were 0.15 ± 0.01 and 0.34 ± 0.001 , respectively. From the Bayesian GWAS, a total of 240 markers had a non-zero posterior probability larger than 50%. One SNP located on chromosome 14 (rs41634571) showed 81% of probability to be in the largest effect class. Close to this SNP, six coding genes were mapped (SNX16, CHMP4C, ZFAND1, IMPA1, SLC10A5, and ENPP2). From the back-solving of GEBV, two markers had a p-value greater than Bonferroni corrected threshold using blocks. The SNP were located at the beginning of chromosome 20, where some QTL significantly enriched for maintenance efficiency and maturity rate were found. Results of the present study showed that DSCC is a heritable trait in Italian Simmental and some candidate genomic regions associated with this trait were found.

Identification of functional SNPs and INDELs within lncRNA associated with mastitis in Holstein dairy cows
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Bovine mastitis is a common disease in lactating dairy cows, causing reduced milk yield, discarded milk, and early culling. Despite extensive research, it remains a major challenge for dairy producers. Advances in high-throughput transcriptomic and genomic sequencing provide new opportunities to explore its underlying biological mechanisms. In this study we used RNA-Sequencing (RNA-Seq) to analyze somatic cells (SC) RNA from milk samples of six cows, each with both healthy (n=6) and mastitic (n=6) quarters, classified by a somatic cell count >100,000 cells/mL. The objective was to identify novel differentially expressed (DE) long non-coding RNAs (lncRNAs) with potential regulatory roles (FDR < 0.05; |FC| > 2). A total of 94 lncRNA were DE between healthy and mastitic samples; however, only five were deemed functionally relevant based on involvement in immune-related metabolic pathways, and proximity to key immune candidate gene network nodes. To further investigate genetic variation, whole-genome sequencing (WGS) was performed on an independent population of seven healthy and seven mastitic Holstein cows. This analysis aimed to identify fixed variants—single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs)—uniquely present in either group within DE lncRNAs. Preliminary WGS analysis identified 86 and 73 unique, fixed INDELs (present in 100% of animals) in mastitic and healthy cows, respectively, along with 611 fixed SNPs in mastitic cows and 190 SNPs in healthy cows. Next steps will focus on determining the precise location of these variants and assessing whether they impact lncRNA expression or function. This study highlights the potential of multi-OMICs integration in advancing our understanding of genetic resistance to mastitis in Holstein dairy cattle. Keywords: Holstein dairy cow, INDEL, long non-coding RNA, mastitis, RNA-Sequencing, SNP, whole genome sequencing

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Theatre 9

Functional genetic variants associated with subclinical intra-mammary infection in Holstein cattle

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Beyond the well-established transcriptome analysis for gene expression, RNA-Sequencing technology could also be valuable for identifying functional genetic variants (FVs) (including SNPs and INDELs) located within associated candidate genes linked to traits such as mastitis resistance or susceptibility. MGISEq RNA-Sequencing was conducted on milk somatic cells collected from Holstein cows with no history of mastitis (n=9; Neg) and with subclinical intramammary infection (sIMI) due to *Streptococcus agalactiae* (n=11; Sa+) or *Prototheca* spp. (n=11; P+). Raw sequencing data were aligned to the *Bos taurus* ARS-UCD1.3 reference genome using CLC Genomic Workbench (Qiagen) to generate unique alignments for each condition and identify group-specific FVs within the transcriptome. CLC workbench was also used to perform variant discovery analysis and to determine whether the variants lead to amino acid changes and fall within potential splice sites. The Variant Effect Predictor tool (Ensembl) was employed to predict their functional consequences. Results revealed 306,440 FVs uniquely fixed in the Neg group (253,190 SNPs, 28,308 insertions, 23,617 deletions), 264,132 FVs in the P+ (21,4745 SNPs, 25,581 insertions, 22,496 deletions), and 246,777 FVs in the Sa+ (201,984 SNPs, 22,819 insertions, 20,795 deletions). Among these, 273, 252, and 185 FVs unique for Neg, P+, and Sa+, respectively, were predicted to fall within potential splice sites. Next steps involve functional analysis and the identification and enrichment of quantitative trait loci regions and classes which co-localize with the detected FVs using GALLO package (R studio). Final results may provide valuable insights to better understand the genetic architecture of sIMI resistance/susceptibility. This may contribute to improve genetic selection for udder health in dairy cattle, providing economic benefits and enhancing animal welfare. Acknowledgment: Agritech National Research Center, funded by the European Union Next-Generation EU (Piano Nazionale di Ripresa e Resilienza (PNRR)—Missione 4, Componente 2, Investimento 1.4—D.D.1032 17/06/2022, CN00000022)

Novel lncRNA in milk somatic cells of dairy cows with subclinical mastitis caused by *Streptococcus agalactiae* and *Prototheca* spp

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Regulatory elements including long non-coding RNAs (lncRNAs) make up the majority of the transcriptome. Despite that, the functional understanding of their role in bovine mastitis remains limited. This study aimed to analyze lncRNA expression in milk somatic cells from healthy cows and those affected by subclinical mastitis caused by *Streptococcus agalactiae* and *Prototheca* spp. Three experimental groups were defined based on bacteriological analyses of 188 animals from one farm: healthy (H; n=15), *Streptococcus agalactiae* infected (Sa+; n=10), and *Prototheca* spp. infected (P+; n=9). RNA sequencing was performed using the MGISEq platform with a paired-end approach. After aligning reads with Hisat2, Cufflinks was used to reconstruct the transcriptome. The resulting GTF files were merged into a unified transcriptome and compared to the reference annotation using Cuffcompare. Differential expression (DE) analysis was assessed with DESeq2 (log fold-change >|1| and FDR < 0.05). Candidate lncRNAs and their interaction partners were predicted using FEELnc. When comparing the mastitis samples (regardless of the pathogen) with the H ones, the DE analysis identified 347 lncRNAs (251 up-regulated and 96 down-regulated, respectively). One of the most up-regulated lncRNA, TCONS_00189579, had as predicted target the *SELL* gene, involved in leucocyte migration. TCONS_00311488 was downregulated in mastitic animals and targeted to the *PDE4* gene, encoding for phosphodiesterase type 4 enzymes involved in the modulation of inflammatory processes. Moreover, this study identified 216 novel lncRNAs potentially involved in mastitis development, requiring further validation for their role in immune regulation and udder health improvement in dairy cattle. This project was part of 2024DAFNAE1DOR-00172 funded by UNIPD. This study was also carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

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Theatre 11

Single-step genomic evaluation of calf health traits in Holstein breed

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Improving calf health while enhancing animal health standards is a major objective of the dairy industry. Respiratory issues (RESP) and diarrhea (DIAR) are the two most common calf diseases on Canadian farms. Genetic selection is one potential solution to help reduce the incidence of these diseases. A single-step genomic evaluation for calf traits in Holstein breed has been developed by Lactanet Canada, with official implementation in August 2025. Lactanet Canada plans to include calf traits into both national selection indexes, Lifetime Performance Index (LPI) and Pro\$, beginning in April 2026. The evaluation uses producer-recorded calf disease data, which after quality control, included 316,932 Holstein calf disease binary records for RESP and 130,027 records for DIAR from 1,217 Canadian dairy herds between 2007 and 2024. The prevalence for RESP was 19.5%, while DIAR was 21.1%. A two-trait Single-Step genomic evaluation with a linear animal model was conducted using MiX99 software. The model factors included a fixed year-season effect, and random herd-year-season, animal, and residuals. The genomic reference population, consisting of genotyped animals in the 4-generation pedigree, included 107,254 animals. Heritabilities of 0.054 for RESP and 0.044 for DIAR, with a genetic correlation of 0.526 between the traits were previously estimated in the same population by MC-EC-REML. A total of 2,974 sires achieved official status based on having daughters in at least 5 herds and having an estimated breeding value (EBV) reliability $\geq 70\%$. Over time, no genetic trend in sires was observed, highlighting the need for direct selection on calf disease traits. EBV proof correlations between calf disease traits and other evaluated traits were very weak ($< \pm 0.15$), further emphasizing the need for direct selection on calf disease traits. For inclusion in national evaluations, Lactanet Canada is proposing an index weighting of 30% on RESP and 70% on DIAR, driven by economics, mortality rates, and the role of DIAR as a major precursor to RESP.

Exploring the genetic basis of calf health and vitality traits in Holstein Friesians

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Healthy and robust calves are the foundation of a successful dairy farm, as they will develop into productive dairy cows. The welfare and health of dairy calves during the rearing period are major animal welfare concerns that have been criticised in the past. Moreover, the health and development of a calf can significantly influence future performance as dairy cow, impacting both, the profitability of the dairy farm and the direct costs associated with sick and underdeveloped calves. Despite their importance, calf health and vitality traits are not yet adequately considered in the breeding program of the Holstein Friesian breed and their genetic basis remains largely unknown. Therefore, this study aims to investigate the genetic basis of these traits in Holstein Friesian calves as well as to establish the new trait complex “calf vitality” to be incorporated into future breeding programs. The data set comprises 1,767 female Holstein Friesian calves born between Sep. 2023 and Dec. 2024 on one farm in eastern Germany. The phenotypic data of these calves include information about the birth weight, calving ease as well as the weight development, recorded repeatedly (six measurements) from birth to first insemination. Additionally, health events (pneumonia, navel infection, diarrhea) as well as animal deaths and cause of death were documented. Furthermore, all calves were genotyped after birth using a bovine 50 K SNP chip. Genome-wide association studies (GWAS) and the estimation of genetic parameters are currently conducted for the studied traits. The results of this study will contribute to the development of the new trait complex “calf vitality” with the goal of establishing a genomic breeding value for this trait. A better understanding of the genetic basis of calf vitality and the development of a new breeding value will not only improve calf health and welfare, but also contribute to more efficient and sustainable dairy farms. This work is funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number 281C809A21).

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The Relationship Between Telomere Length, Health, Welfare and Production Performance in Holstein Cattle

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Ensuring animal health and welfare in intensive production systems presents a major challenge in cattle breeding, requiring long-term monitoring and complex data collection. Identifying heritable, easily measurable traits linked to health and welfare could enhance herd management and genetic selection. Telomeres, repetitive DNA sequences at chromosome ends, serve as indicators of cellular aging and stress. In humans, telomere length (TL) is associated with various diseases, yet research in livestock remains limited. This study aimed to assess TL in Holstein calves in relation to hormonal, nutritional, and productive traits, as well as the effects of individual versus group rearing. TL was measured in calf and maternal white blood cells using qPCR (T/S ratio) and validated against the Southern blot method. A significant correlation ($R^2=0.3$, $p<0.01$) was found between maternal and offspring TL at birth ($N=118$). Telomeres shortened in early life, followed by elongation, returning to birth values by eight months. Individually reared calves exhibited longer telomeres at four months ($P=0.04$). Hair cortisol, an indicator of chronic stress, was initially similar between rearing systems but significantly higher in group-reared calves at two months ($P<0.05$), likely due to social stressors. By four months, cortisol levels declined in group-reared calves but increased in individually reared calves following their transition to group housing. A negative correlation was observed between cortisol levels at birth and TL at two months in individually reared calves ($R^2=0.22$, $P=0.017$). Additionally, TL at birth was influenced by maternal factors, including TL, age, and genetic value for dystocia ($P<0.0001$). In conclusion, TL holds considerable potential as a biomarker for health status, welfare, and production attributes in cattle.

Understanding barriers and motivators to sharing hoof health data

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Lameness affects dairy cattle worldwide, imparting substantial impacts on the welfare of the individual cow, and on the sustainability of a dairy herd. Our recent research has highlighted the impact that genetics can have on an individual's susceptibility to the lesions that cause lameness. However, UK genetic evaluations for lameness are reliant on the hoof health records submitted by farmers voluntarily, either through their milk recording organisation, or through the newly established national database of foot trimming records called the UK Hoof Health Registry. Our study aimed to understand the barriers and motivators to farmers sharing their hoof health data with the UK Hoof Health Registry, to better understand how to encourage data sharing to improve genetic evaluations. A qualitative study was conducted using a purposive (non-random) sampling strategy which aimed to include more hesitant voices. We advertised a series of focus groups for farmers, hoof trimmers, and veterinarians. Focus groups were conducted online using a semi-structured interview script. All focus groups were audio recorded and transcribed verbatim using a professional transcription service. Transcriptions were thematically analysed using an inductive approach. We recruited ten farmers, four hoof trimmers, and three vets to the focus groups. The recruitment process proved challenging despite a range of strategies being deployed to encourage potential participants to attend. Thematic analysis has highlighted social barriers including awareness of data ownership, and concerns around the visibility of sensitive animal health data. Wanting to contribute to improving dairy cow health, and the reputation of the research institute operating the database were cited as some of the motivators driving farmers to share hoof health records. Our results have provided important new insight into the mindsets of different stakeholders and supported the dissemination and uptake of the UK Hoof Health Registry project. We have identified a range of social barriers and motivators which can influence farmers, foot trimmers and vets' perceptions and behaviours in relation to sharing hoof health records.

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Poster 15

Genetic correlations of Resilience Indicators with Production, Fitness and Welfare Traits in Austrian Fleckvieh Cattle

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Breeding for resilience in livestock can potentially lead to improvement in herd productivity, better fitness and welfare thereby significantly easing management and lowering cost of production. Resilience measures the ability of an individual to cope with short-term disturbances and quickly return to normal performance. The correlations of the resilience traits with diverse production, fitness and conformation traits present a best-bet scenario for breeding for improving the traits. Genetic analysis was undertaken with 2 resilience indicators; log transformed variance of milk yields (LnVar) and lag-1 autocorrelation (rauto) from milk yield (MY) data collected across multiple lactations of Austrian Fleckvieh cattle. Low LnVar and lag-1 autocorrelation indicates low variability in daily MY and quick recovery from a disturbance and therefore indicator for good resilience. The MY data was collected across multiple lactations using Automatic Milking Systems (AMS), comprising of 8,615,511 daily MY records of 20,723 Austrian Fleckvieh cows. Correlations of estimated breeding values (EBV) of -LnVar and -rauto, with EBV for production, fitness and conformation traits from routine genetic evaluation were analysed. The resilience indicators were scaled so that high values are desirable. The LnVar resulted into negative correlated response with most of the production traits and positive correlated response with fitness and health traits. Meanwhile, lag-1 autocorrelation had negligible correlations with almost all other traits, suggesting that LnVar is a better predictor of resilience than rauto. Selecting for the resilience indicator LnVar can lead to improvement in fitness and welfare outcomes in dairy cattle breeding programs.

Genetic relationships between milk lactose content, lactosaemia, udder health and metabolic status in dairy cows

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Mammary inflammation in dairy cows has an adverse effect on the mammary epithelium, with an increase in lactosaemia (blood lactose, BL) and a decrease in milk lactose (ML) content. On the other hand, the high glucose demand for lactose synthesis during lactation can lead to metabolic disorders. The objectives of the present study are to assess the genetic link between lactose contents and the udder health and metabolic status of dairy cows. We investigated genetic associations between ML, BL, and indicators of udder health (Somatic Cell Score, SCS) and metabolic status (non-esterified fatty acids (NEFAs), beta-hydroxybutyrate (BHB), glucose, and urea) at different stages of lactation. The study is based on 3197 test-day records collected from 662 Holstein, Normande and crossbred cows raised at the INRAE experimental farm of Le Pin (doi: 10.15454/1.5483257052131956E12). We estimated heritability and genetic correlations by applying multi-trait animal mixed models based on test-day records, including a random permanent environmental effect and non-genetic fixed effects. Our findings reveal moderate heritability estimates for blood metabolites (0.12, 0.14, 0.17, 0.19, and 0.25, for urea, BL, BHB, NEFAs, and glucose, respectively) and for ML (0.28). Genetic correlations between blood metabolites ranged from -0.62 (BL and glucose) to 0.28 (BL and BHB), while genetic correlations between ML and blood metabolites varied from -0.40 (with BL) to 0.43 (with glucose). ML and BL, negatively correlated, presented a negative (-0.24) and positive (0.61) genetic correlation with SCS, respectively. Standard errors varied from 0.04 to 0.07 for heritability estimates and from 0.18 to 0.26 for genetic correlations. These results support the hypothesis of a genetic link between lactose metabolism and cow health, suggesting that milk and blood lactose may serve as valuable indicators of udder health and metabolic status, with potential applications for genetic selection and management practices in dairy herds.

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Transcriptome analysis for heat and cold stress exposure in Romanian Spotted breed

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Thermal stress, during exposure to both heat (HS) and cold (CS) challenges, impacts productivity, reproductive efficiency, health and welfare in cattle. The aim of this study was to determine the differentially expressed genes (DEGs) and their functional pathways during HS and CS exposure in Romanian Spotted breed. A transcriptome analysis was performed on cows under thermoneutral conditions (TN; 13°C), heat stress (HS; 36°C) and cold stress (CS; -7°C) exposure. RNA-seq technique was used for transcriptome gene expression profile in blood samples of 7 Romanian Spotted multiparous lactating cows. The differential expression analysis was performed using the edgeR R package and the KEGG pathway enrichment analysis of differential gene sets, using ClusterProfiler software. Compared to TN, 1582 (996 up-regulated and 586 down-regulated) and 411 (89 up-regulated and 322 down-regulated) significantly DEGs ($p \leq 0.05$) were identified for HS and CS, respectively. The enrichment analysis of the DEGs of cows exposed to HS compared to TN highlighted the biological processes and pathways including MAPK signaling pathway, chemokine signaling pathway, platelet activation, necroptosis, cytosolic DNA-sensing pathway, viral protein interaction with cytokine and cytokine receptor, apoptosis and NOD-like receptor signaling pathway. The most significant KEGG pathways of cows exposed to CS vs. TN were related to focal adhesion, ECM-receptor interaction, protein digestion and absorption, calcium signaling pathway, relaxin signaling pathway and cardiac muscle contraction. The obtained results provide new insights regarding a series of molecular mechanisms underlying the thermal response of cows under heat and cold stress.

Genome-Wide Association Studies for Milking Reactivity in Romanian Spotted Cattle

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Cattle behaviour during milking is a key trait affecting animal welfare and farm efficiency. Highly reactive cows exhibit excessive movement or resistance during milking, impacting milk yield and overall herd management. Behavioural traits in cattle were shown to have a genetic component, however, their underlying mechanisms remain poorly understood. This study aimed to identify genetic markers associated with milking reactivity in Romanian Spotted dual-purpose cattle breed using a genome-wide association study (GWAS). The experiment was conducted on a number of 185 cows, between 1st and 3rd parities, milked twice per day in a 'herring-bone' 2×14 parlour. Milking reactivity was scored on a 5-point scale, where lower values indicated calmness and higher values indicated nervousness. The Axiom Bovine v3 SNP-chip was used to genotype 88 SNP markers belonging to 24 candidate genes, which were previously found to be associated with behavioural traits. Principal component analysis (PCA) was performed to assess population structure and GWAS was conducted using a mixed linear model, adjusting for parity and the first 10 principal components. Five significant SNPs were associated with milking reactivity, located within the USH2A, ADAMTS7, TBC1D2B and ZMAT4 genes, previously identified as candidate genes associated with milking temperament. These findings highlight the polygenic nature of milking reactivity, with genes involved in sensory perception, motor control and intracellular signalling influencing this trait. Understanding these genetic factors could support breeding strategies aimed at improving milkability and stress resilience in dual-purpose cattle. Future studies should incorporate whole-genome sequencing to refine genetic associations and validate these results in larger populations.

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Poster 19

Assessing the Impact of Pharmacological Treatments on profiling Resilience Indicators in Dairy Cows Using PLF Data

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Resilience in dairy cattle is a key factor influencing both productivity and health. One potential determinant of resilience is the occurrence of health issues, that can be identified by pharmacological treatments, which may alter cows' responses to perturbations and their ability to recover. This study aimed to assess whether resilience patterns differ between treated and non-treated cows by analyzing milk production and rumination patterns using modeling and perturbation detection methods. The study involved 754 Holstein cows across multiple lactations on a commercial farm in Italy. Data were collected from automated milking systems and sensor-based rumination monitors, while health treatments were officially recorded in the electronic health register. Expected lactation curves were estimated using quantile regression to establish unperturbed trajectories. Deviations from these curves were analyzed to identify perturbations, which were quantified in terms of magnitude, duration, and recovery speed. Statistical analyses used linear mixed models, by treatment status, i.e. treated vs. non-treated as the main factor. Preliminary findings suggest that treated cows experienced longer and more severe perturbations in milk production, with a slower recovery than non-treated cows. Notably, perturbations in milk production frequently coincided with a reduction in rumination time, suggesting interdependence between these two physiological parameters. Statistical analyses revealed evidence in perturbation magnitude between treated and non-treated cows. These results highlight that resilience indicators associated with specific treatments could serve as a tool for monitoring the immunocompetence of dairy cows. Future research could integrate genomic data to identify genetic determinants of resilience and immunocompetence, potentially informing breeding strategies for healthier and more robust animals.

Susceptibility of cows to Reproductive and Metabolic Diseases in Slovakia

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Metabolic and reproductive diseases are common in dairy cows, often resulting in early culling, decreased reproduction, and reduced milk production. This research aims to evaluate factors influencing metabolic and reproductive diseases in dairy cows, estimate their genetic parameters, and validate estimated breeding values. Veterinary records from 2017 to 2023 were analysed. Two datasets were used to estimate genetic parameters and breeding values: dataset I with 9,446 cows across 19,680 lactations between 2017 and 2023, and dataset II (validation dataset) with 3,446 currently living cows on 4,038 lactations that calved between 2022 and 2023. Multiparous have an increased risk of both metabolic and reproductive diseases. Metabolic diseases were significantly influenced by herd and seasonal effects, indicating that their occurrence is more dependent on environmental and management factors compared to reproductive diseases, but heritability was generally low for all diseases. Higher relative breeding values (RBVs) for reproductive diseases were observed in dataset I (ranging from 110.39 to 149.43) compared to II, whereas the latter exhibited higher values for metabolic diseases (ranging from 99.84 to 156.47). The model adopted proved to be reliable, with significant correlations between the RBV from dataset I and II (validation dataset), i.e. 0.97 for metabolic diseases and 0.98 for reproductive disorders. By including health traits in genetic evaluation in Slovakia it is possible to reduce prevalence on farms, produce healthier animals which stay longer in the herds, and improve farms profitability. This research was funded by DS-FR-22-0016 and APVV-20-0161.

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Theatre 1

Evaluation of enzymatically hydrolysed hemoglobin (Peptein) in dairy cows diet

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A total of 8 primiparous and 12 multiparous Holstein cows (660 ± 15.9 kg BW; 30.8 ± 1.55 kg/d milk; 174 ± 35.3 DIM; mean $\pm$ SD) were enrolled in a cross-over experimental design of 2 periods of 28 days to assess the effect of an enzymatically hydrolysed protein supplement in the diet of dairy cows. In each experimental period, the groups received either of two diets: PEP diet supplemented with 150 g/d of Peptein or USR diet with 50 g/d of urea slow release added to the ration to achieve similar total N content in the final TMR (14.7% CP). Animals were milked twice daily and were weighed after milking. Individual feed intake was recorded with electronic feeders. After the 21-days of adaptation, a 5-day period of methane concentrations measurements using a laser methane detector was done. Furthermore, a 3-day collection period started for liquid rumen samples 3 h after the morning feeding for microbiota sequencing, VFA analysis, and pH. Besides urine spot samples were obtained and composited for further determination of creatinine, urea, and purine derivatives (PD, allantoin and uric acid) concentrations to estimate rumen microbial protein synthesis using the PD concentration index (PDC index). Finally, milk and blood samples were obtained for milk composition analysis and urea and hepatic enzymes determination. Data were analysed with a mixed-effects model. Energy-corrected milk was half a kg greater ($P < 0.05$) in PEP than in USR, and this benefited feed efficiency in PEP diet cows (1.41 vs 1.37 ± 0.020 , respectively). Milk protein (3.79 vs 3.75 ± 0.069) and solids non-fat (9.37 vs 9.33 ± 0.077) were greater ($P < 0.05$) in PEP than in USR fed cows, but neither rumen nor blood analysis differed between treatments. The number of eructation events in the PEP group was numerically lower (7.4 vs 7 , respectively) than in USR cows that may help reducing methane production. Cows fed the PEP diet had a greater value of PDC index compared with USR fed cow (535 vs 479 ± 16.1 , respectively). Enzymatically hydrolysed haemoglobin is a rumen degradable protein source that improves milk protein quality and feed efficiency, which it may be explained by an increase of microbial protein synthesis.

Forage Value as Production Factor: a new tool for estimating forages productive and economic value
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The Forage Value as Production Factor (FVPF), is proposed as a tool for forages evaluation, representing the maximum forage economic value when considering the milk production cost as equivalent to the milk market price. Starting from the forage Net Energy for Lactation at production levels of intake (fNELp; Mcal/kg) based on NRC 2001 equation, the FVPF is obtained by the ratio between the fNELp and the milk Net Energy content adjusted by the proportion of feeds cost over the total milk production costs and by the dietary forage proportion multiplied by the milk price. In the present study, these values were assumed to be 0.805 Mcal/kg, 60%, 60% and 0.53 €/kg respectively. The study was conducted on a total of 86 forage samples, 37 alfa-alfa hays (AAH) and 49 mixed hays (MH), collected from farms distributed in the Emilia-Romagna region. The samples were analyzed for DM, CP, EE, NDF, ADF, ash, ADL, NDICP, ADICP content as well as NDF digestibility at 48 hours. The fNELp, and consequently the FVPF, was calculated both from in vitro (NELV for FVPFV calculaton) or estimated digestibility (NELSt for FVPFSt calculation). Forages market prices (MPx) were obtained from the AGER (Bologna, Italy) bulletin during the sampling period. Comparisons between the FVPFV and the FVPFSt were performed using the univariate procedure of the GLM and the Pearson's correlation. Differences were observed between FVPFSt and FVPFV values with averages of 25.19 ± 1.72 and 31.56 ± 2.02 €/100 kg ($p < 0.001$), and 23.15 ± 2.00 and 27.12 ± 2.84 €/100 kg ($p < 0.001$) for AAH and MH respectively. FVPFSt and FVPFV showed a correlation of $r = 0.80$ ($p < 0.001$). The market prices were on average and 21.47 ± 3.10 and 16.48 ± 4.55 €/100kg for AAH and MH respectively. Both FVPFV and FVPFSt values exceeded the market price of 32.16 and 52.52% on average for AAH and MH, indicating a good profitability of forages as production factors. The evaluation of the FVPF encourage the production of higher-quality forages and promote their optimal allocation within the farm. This work has been produced with the co-financing of the Ministry of University and Research within the framework of the PNC.

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Theatre 3

Assessment of rumen solubility of global feed grade magnesium sources using acid titration and rumen simulation

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Magnesium (Mg) is an essential nutrient for ruminants and is involved in many biological processes as an enzymatic co-factor. Maintenance of normal Mg concentration in ruminant plasma is nearly totally dependent on absorption of dietary Mg. Supplemental Mg solubility and bioavailability when fed to ruminants has been estimated to range from 10-56% and 12-27%, respectively. However there are few reports on the evaluation of Mg sources commercially available to determine the accuracy of these solubility estimates. This study has been conducted to assess the composition, particle size and solubility of Mg sources from different geographical locations in the US, Australia, Asia and Europe. The solubility of the Mg sources was evaluated using 3 different approaches; HCl based titration, acetic acid titration and rumen simulation using rumen fluid. For the rumen simulation each vessel contained a standard dairy diet to which 40 ml of fresh rumen fluid from a Friesian cow was added in addition to 80 ml of phosphate buffer which was subsequently incubated at 39°C for 12 h. Samples were taken from each vessel after 4h, 8h and 12h with solubilised Mg, pH, methane and VFA production measured. Samples used in this study ranged from 8.0-61.9%w/w Mg. The HCl titration showed significant variation in the volume of HCl required for sample dissolution, ranging from 2.7 mL to 104.7 mL. This variance correlated with the extent of sample solubilisation, which varied between 9.8%w/w and 92.6%w/w. Further analysis determined the median particle size (Dx50) ranged from 20.0 µm to 151.3 µm, while the Dx95 ranged from 62.2 µm to 303.3 µm. Results of the acetic acid titration gave a further insight into the sample groups solubility showing lower levels of solubility than the HCl titration. When compared with the two previous methods the rumen simulation showed the lowest solubility of the three methods. The results of this study provide a greater understanding of the variability of Mg solubility from feed grade Mg sources under different rumen simulation models.

The effect of hydroxy-Zn as a trace mineral source on the performance, carcass characteristics and wool quality of finishing lambs

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This study evaluated the effect of zinc (Zn) hydroxychloride and Zn-sulphate on finishing lamb performance and wool quality. Thirty-six Dohne Merino ram lambs (28.65 ± 2.83 kg) were randomly assigned to three dietary treatments ($n=12$ lambs per treatment; $n=1$ lamb per replicate): CON (basal diet with no supplemental Zn; 50.62 mg Zn/kg DM); ZnS (basal diet supplemented with Zn-sulphate; 91.40 mg Zn/kg DM); ZnH (basal diet supplemented with Zn-hydroxychloride; 84.37 mg Zn/kg DM). Zinc source inclusion was formulated to supplement an additional 55 mg/kg of Zn to the basal diet: treatments ZnS and ZnH. The production study lasted for 60 days, including a 12-day adaptation period. A fleece wool sample was evaluated for fibre diameter, staple length, and fibre consistency at the start and at day 59 of the study. All animals were slaughtered at the end of the production study, carcass characteristics recorded and statistical significance assessed using analysis of covariance (ANCOVA). Post hoc analysis was performed on performance data and carcass characteristics with a Bonferroni adjustment for initial start weight, as well as final wool quality similarly adjusted for start wool quality. Significance was declared at the 5% probability level. Zinc source did not affect finishing lamb dry matter intake. Metabolisable energy utilized for live weight gain was less efficient for lambs supplemented with ZnS ($P=0.001$) compared to the CON and ZnH treatments. ZnH significantly improved cold carcass mass ($P=0.041$) and carcass length ($P=0.010$) compared to the CON and ZnS groups. No significant differences were found for wool quality parameters. Improved cold carcass mass as affected by Zn-hydroxychloride supplementation of finishing lambs is promising since it is used as a critical indicator of meat yield and overall carcass value post-chilling. Further research is required to explore the long-term effects of Zn-hydroxychloride supplementation on wool quality.

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Theatre 5

Comparative analysis of precision complementation and standard complementation in dairy cattle

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The individual allocation of concentrates in lactating dairy cows is commonly based on milk production and lactation stage. Individual monitoring devices open up the possibility of a more precise estimation of cow's requirements, and thus of precise complementation. The objective was to compare it with two standard feeding methods: a total mixed ration (TMR), and a partial mixed ration (PMR) where production concentrate was allocated based on milk production. During the first 7 weeks (wks) ('challenge'), 69 Holstein dairy cows were fed ad libitum with a unique balanced basis diet and supplemented individually with 5 kg/d/cow of production concentrate for 3.5 wks and 1 kg/d/cow for the following 3.5 wks. For each cow, the response to the concentrate's reduction was estimated as the change in her milk yield and the change in her basis diet intake, measured by individual weighing bins. This characterization was used to balance the treatments as it is the basis of the precision feeding strategy: the more the cow loses milk yield and/or the less she increases basis diet intake, the more concentrates she will get. Each cow was then assigned to one out of three treatments for 12.5 wks: the first two were based on the same basis diet, supplemented with production concentrate that was allocated either according to their milk yield (PMR) or to their response profile, and the third was based on a TMR. The three groups were fed with the same overall amount of production concentrates. The results showed that, on average, the three strategies had similar average performance. On an individual basis, precision feeding was only interesting for cows that were able to maintain their production and/or increase their basis diet intake during the challenge period ($N = 36$ cows out of 69). In fact, they performed similarly to the cows in the other two treatments, while consuming less concentrate, and this way increased the economical return. Interest for precision complementation appears to be limited to the aforementioned cows, with no benefit to be broadened to the whole herd, as precision feeding performed similarly to TMR or PMR. Further research could focus on how to characterize those cows on farm.

Low-level DON and ZEA contamination in animal feed in Lombardy

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Fungal contamination of animal feed is a challenge to the animal production system. Fungi growing on animal feed produce mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA). Mycotoxin contamination causes feed refusal and decreased productivity in farm animals. However, there are limited studies on mycotoxin contamination of animal feed, particularly in the context of Italy. Therefore, we aimed to study the presence and concentrations of DON and ZEA in animal feed over 10 years using enzyme-linked immunosorbent assay. A total of 19,523 feed matrices were sampled by the Regional Breeders Association of Lombardy, Italy, between January 2014 and December 2023. The feed matrices sampled were cotton seed, cereals (wheat, wheat bran, barley, maize), alfalfa hay, maize silage, compound feed, and total mixed rations. Here, we show that DON was detected in 84.3% of samples with the maximum concentration of 64.4 µg/kg, whereas ZEA was detected in 76.2% of samples with the maximum concentration of 55.0 µg/kg. However, these values are within the safe limits recommended by the European Union for farm animals, which is 900 µg/kg for DON and 100 µg/kg for ZEA. As expected, maize was one of the most contaminated feed matrices for both DON and ZEA. There was also variability in feed matrices over the years. For instance, DON and ZEA concentrations in maize peaked in 2014 and 2015, followed by consistently low values. The difference in DON and ZEA contamination across the years could be due to the influence of climatic factors such as precipitation, temperature, and humidity during the cultivation or storage phase. Hence, our next step is to study the association of climatic variables with the DON and ZEA levels in the feed matrices. In conclusion, DON and ZEA contamination is prevalent in animal feed matrices in Lombardy, but not above the threshold. Monitoring the mycotoxin concentrations at the individual feed ingredient is one of the mitigations strategies to combat mycotoxin contamination.

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Theatre 7

Effects of a phytogenic feed additive on chewing behavior, ruminal variables, and immune response of cows

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This study evaluated the effects of a phytogenic feed additive (PHY) on chewing behavior, rumen pH and microbiome, and immune response during transition period. Primiparous Holstein cows were supplemented (Control, n = 8; PHY, n = 8) from two weeks before parturition until eight weeks postpartum. Cows were fed close-up and lactation diets with 32 to 60% (DM basis), respectively. Rumen microbiome was evaluated through 16S amplicon sequencing. Immune response was tested in four cows of each group by stimulating isolated lymphocytes with concanavalin A (10 µM) or lipopolysaccharides (1 mg/mL). Results showed that PHY increased rumination post-calving ($P < 0.05$) and minimum rumen pH ($P = 0.05$), however, it did not affect the rumen volatile fatty acid profile. The rumen microbial diversity was not affected, but with PHY the genus *Pseudomonas* increased, and mitigated the genera *Bacteroides* and *Turicibacter* ($P < 0.05$). In addition, PHY increased immune response capacity one week before parturition ($P < 0.01$) as indicated by higher proliferative response measured by cellular incorporation of 5-ethynyl-2'-deoxyuridine. Furthermore, leading to 38% higher relative cell count of lymphocytes after stimulation. Overall, supplementation of transition cows with PHY seems to enhance rumination behavior and mitigate the drop of ruminal pH, as well as immune response capacity before calving, suggesting a preparatory adaptation of the mucosal immune system to upcoming physiological and microbial challenges associated with parturition.

Supplementing Fattening Pigs With a Precision Biotic Can Reduce Stress Response and Agonistic Behavior

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This study evaluated the potential of a glycan-based precision biotic (PB) supplementation to modulate the gut-microbiota-brain axis and reduce the stress response and agonistic behavior in fattening pigs. It was conducted on a commercial farm with 600 pigs ((Landrace x Yorkshire) x Pietrain; half entire males (20.1±2.89 kg) and females (17.4±3.51kg)) with docked tails and blocked by sex in 28 pens randomly assigned to a control diet (CG) or supplemented with the PB (500 mg/kg; TG) during the 3-phases of the fattening cycle (28, 70 and 26 days). Animals were subjected to two challenges: 1) mixing animals on day 28, keeping treatments; 2) high stocking density from late phase 2 and through phase 3 (0.7 m²/pig; 95-106 kg). Performance, cortisol and chromogranin A (CgA) measurements in saliva, along with lesions and behaviour records (scan sampling) were conducted. No performance differences were observed but stress response changes were registered. Compared to the CG, TG pigs showed a lower cortisol level at the end of phase 1 (1.8 vs. 2.4 pg/mL; p=0.045) and at the end of the cycle (3.6 vs. 5.2 pg/mL, p=0.007) together to a reduction in CgA (350 vs. 475 ng/mL, p=0.032). After mixing, TG showed a significant increase in animals without lesions in the front area (11.6 vs. 6.3%; p=0.036) and a reduction in negative social behaviors (p=0.052), and an increase in positive social behaviors (p=0.002). Regarding stress from high stock density, fewer lesions in the front and middle areas were observed in TG at the end of phase 2 (p<0.05), together to a higher number of animals without lesions (69.4 vs. 54.8% front, p=0.014; 88.4 vs. 74.4% posterior area, p=0.003). Moreover, a decrease in negative and an increase in positive social behaviors was also observed in Phase 2 and 3 of TG (p<0.005). A treatment x sex interaction was observed, with TG males having a higher percentage of animals without lesions in the front area (74.4 vs. 52.6% in Phase 3, p=0.005). In conclusion, this glycan-based precision biotic was shown to reduce agonistic behaviors and regulate the response to stress in fattening pigs, particularly in the final stages of the production cycle.

Session 84

Theatre 9

Excretions and performance of fattening pigs with strongly reduced trace mineral level in feed

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The essential trace minerals zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) are part of various enzymes. At the same time, they are among the finite sources. Not metabolized Zn and Cu is excreted via feces accumulates in the soil and interfere with plant growth, and water quality. The aim of the study was to reduce trace minerals under practical conditions in fattening pigs, comparing different sources, focusing on performance and carcass quality. 756 pigs (DanBreed x Duroc, Ø30.4 kg), were divided into three groups (Con, TG1, TG2) just differing in mineral content (Zn, Cu, Mn, Fe) and source. Con: standard level inorganic minerals TG1: reduced level inorganic minerals, TG2: reduced level (like TG1) organic minerals (E.C.O.Trace®, Biochem Zusatzstoffe, Germany). During fattening period, pigs were weighed three times and feed intake was documented penwise. Mortality and individual medical treatments were documented. Liver and feces samples (Trial end: penwise over three days) were analyzed for trace mineral content. Carcass classification was documented at slaughterhouse. Statistical analysis was performed with the software SPSS (Ver. 29) by suitable testing procedures. Differences at p<0.05 were considered as significant. Zootechnical performance showed no differences between all groups (DWG: Ø1136 g/day; FCR: 2.45, mortality Ø1.72%). Analysis of feces revealed a significant reduction in Zn, Cu, Mn and Fe for TG1 & 2 compared to Con (p<0.05). The analysis of the liver showed no differences between the groups. The carcass classification TG2 showed clear numerical but statistically not significant improvements in favor of the best slaughter class S. A strong reduction in trace minerals was possible in both trial groups with a significant reduction in minerals' excretion and without negative effect on performance. In terms of carcass quality, TG2 performed numerically better. Overall, as inorganic trace minerals have a lower bioavailability, it is safer to carry out the reduction by using organically sources as this guarantees a sufficient supply in times of higher demand, e.g. during infections or other stress situations or varying feed quality.

Effect of lignans and tannins on performance, organ profile and intestinal histology of heat stressed rats

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Heat stress is a challenge for livestock, affecting performance, health, and welfare. Lignans and tannins, found in wood and bark, are recognized for their antioxidant, anti-inflammatory, and antimicrobial properties. The aim of this study was to evaluate the effects of a lignan and tannin-rich complementary feed (LT) on performance, organ profiles and intestinal histology under normal and heat stress conditions in a rat model. We hypothesised that LT could prevent the negative effects of heat stress and positively support the animal performance and physiology compared to the unstressed control. A total of 48 male Sprague Dawley rats (6 weeks old, approval no.: GZ 2022-0.151.441) were arranged in a 3×2 factorial study design, fed a semi-synthetic diet with increasing concentrations of LT (NC: 0 g/kg; LT1: 0.75 g/kg; LT2 1.5 g/kg) and exposed to two continuous ambient temperature levels (23°C vs. 32°C) for three weeks. The effects of diet, temperature and their interaction were assessed using the SAS mixed procedure with Tukey Kramer as post-hoc test. Heat stress reduced average daily gain (ADG) and average daily feed intake (ADFI), while increasing feed conversion ratio (FCR). Specifically, LT2 prevented this reduction in ADG in week 1, with higher ADG than the heat stressed control ($P < 0.10$). In contrast, LT1 showed higher ADG than LT2 in week 3. Heat stress reduced gut length and relative liver and kidney weights, whereas LT2 reduced relative kidney weight compared to LT1. Histomorphometry showed that heat stress adversely affected gut morphology, with thinner villi and crypts in jejunum and reduced crypt depth and goblet cell number/crypt in the colon. However, no dietary effect on gut histology was observed. In conclusion, heat stress affected animal physiology at several levels. The LT showed selected potential benefits in mitigating the adverse effects of heat stress on performance in rats, however, with minor effects on organs.

Session 84

Theatre 11

Geometry and nutrition: an unlikely but long-lasting marriage that can help drive nutrition research to new dimensions

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Since the 1960s, geometry has played a pivotal role in shaping nutritional research, providing powerful visual and analytical tools to understand how organisms regulate their diets. Over the decades, multiple geometrical approaches have emerged, each contributing unique insights into nutrient balancing, dietary trade-offs, and metabolic constraints. In this talk, I will first review the history and evolution of these methodologies, highlighting key breakthroughs that have driven the field forward. This includes developments that have formalised the application of geometry to nutrition in high-dimensions, enabling scientists and industries to address the complexity of nutrition experimentally. I will then demonstrate how my research is pushing the boundaries of nutritional geometry through new methodological and analytical developments. Specifically, I will present cutting-edge approaches that apply geometric theorems and computational analytics to extract deeper insights into animal nutrition. Using insects as model systems, I will illustrate how these methods offer practical applications for insect mass-rearing—optimising artificial diets for sustainable food production—while also shedding light on fundamental insect physiology. By integrating mathematical precision with biological complexity, this work opens new dimensions in nutritional research, paving the way for interdisciplinary innovations in ecology, agriculture, and beyond.

Effects of supplementation with a clay mineral-based product on immune response capacity of transition dairy cows

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Supplementation of dairy rations with clay minerals has been suggested to be effective for preventing endotoxins from entering the bloodstream and causing inflammation. However, there is scarce information on how clay minerals affect the immune response capacity of primiparous cows during the transition period, a critical time for cows, often undergoing immune suppression state. The objective of this study was to investigate the effects of supplementing a clay mineral-based product (CM) on the immune response capacity of cows. Primiparous transition Holstein cows were supplemented (Control, n = 4; EB, n = 4) with CM starting two weeks before parturition and ended eight weeks postpartum. Dietary concentrate was increased from 32 to 60% (DM basis) over the first week of lactation. The immune response capacity of cows was assessed by first isolating lymphocytes from blood using density gradient centrifugation, followed by stimulation with concanavalin A or lipopolysaccharides (LPS). The cellular response capacity was measured through cell proliferation assessed by incorporation of 5-ethynyl-2'-deoxyuridine using a flow cytometer. We found that the immune response capacity of cows tended to be higher with CM one week before parturition ($P = 0.06$). Additionally, four weeks postpartum, CM supplementation enhanced immune response capacity when challenged with LPS as indicated by higher cellular proliferative response ($P < 0.05$), suggesting improved immune cell health in cows. This effect was also reflected in the highest lymphocyte counts observed in the CM-supplemented group four weeks after calving. Taken together, our results show that the supplementation of transition cows with CM led to an enhanced immune response capacity, suggesting potential for mitigating the immune suppression of cows around parturition.

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Poster 13

How does transition from an indoor feeding system to part-time grazing affect dairy cow performance?

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We studied how transition from indoor feeding to part-time grazing (PTG) in spring affects metabolism and milk production of dairy cows in northern Sweden. The experiment followed a randomized complete block design with 44 lactating Nordic Red dairy cows, including a 14-day covariate and a 28-day treatment period. During the covariate, cows were fed a TMR indoors ad libitum, consisting of grass silage and concentrate (60:40 forage-to-concentrate; FC). Treatments were: indoor feeding (CON), PTG with ad libitum indoor feeding (G1), PTG with restricted indoor feeding (80% of DM intake during the covariate period; G2), and PTG with ad libitum indoor feeding at a 50:50 FC ratio (G3). Cows grazed for 12 h overnight with herbage allowance >20 kg DM/cow/day. Milk and blood samples were collected at the end of covariate and at d14 and d28 of the treatment period (repeated measures). Treatment comparisons were made using the Tukey-Kramer test. Indoor DM intake was lower ($P < 0.05$) in G1–G3 than in CON (12.1–12.6 vs. 24.3 kg/d), and increased (Pinteraction<0.01) over time in G1–G3, while remained stable for CON. Milk yield was lower ($P < 0.01$) in G1–G3 (28.9–29.6 vs. 32.9 kg/d) than in CON, and decreased ($P < 0.01$) over time, regardless of treatment (Pinteraction=0.17). Milk protein, fat, and lactose contents were unaffected ($P \geq 0.10$), whereas milk urea N was higher ($P < 0.05$) in G1–G3 (10.9–11.9 vs. 9.71 mg/dL). Plasma NEFA levels were higher ($P < 0.01$) in G1–G3 than in CON (337–397 vs. 137 μ Eq/L), while plasma insulin levels were lower (0.22–0.24 vs. 0.46 μ g/L). At d14, blood urea was higher in G1–G3, but similar to CON at d28 (Pinteraction<0.01), decreasing from d14 to d28 in G1–G3 ($P < 0.01$). In conclusion, cows experienced nutritional and metabolic challenges during the transition to PTG, leading to lower productivity.

Feed efficiency in dairy cows: Characterization, relationship to chemical composition and feed value of diets and prediction

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Feed costs remain the primary expense in dairy production, thus improving feed efficiency (FE) can improve farm profitability. The objectives of this study were three-folded: 1) to evaluate factors related to FE during lactation, 2) to assess the relationship between FE and chemical composition and feed value of diets, and 3) to predict FE based on chemical composition and feed value of rations. Data from 54 feeding trials conducted at Schothorst Feed Research from 1984-2010 were used and FE was defined as kg of fat and protein corrected milk (FPCM) output per kg of dry matter intake (DMI). Orthogonal polynomial contrasts were used to test whether a linear, quadratic or cubic regressions better explains or predicts the effect of lactation cycle (or days in milk (DIM)) on FE, and correlations between variables were assessed. Finally, FE were regressed against chemical composition and feed values. FE decreased with increasing stage in lactation during first 100 DIM, seemed to stabilize between 100-200 DIM, and decreased again from 201 DIM. Dataset was reduced to include cows between 100-200 DIM for the second objective, avoiding masked effects of body mobilization on FE. There was a minor impact of lactation number on FE (1.48, 1.48, and 1.52 for cows with 1, 2, or >3 lactations, respectively). FE was correlated to milk yield, FPCM, BW and to all diet parameters ($P < 0.05$) except crude ash, total fermentable organic matter, and rapid fermentable carbohydrates ($P > 0.17$). Regression coefficients using chemical composition and feed values of diets were very low and did not deliver reliable equations for FE prediction. In conclusion, FE is variable in a dairy cows' productive life as it is highly affected by stage of lactation and it seems to be better explained by a cubic equation, and lactation number was of importance when looking at the association of DIM and FE. Also, cows in mid-lactation seem to better reflect the animals' ability to transform dietary nutrients into milk, as cows in early lactation are likely using energy from body mobilization instead. Recommendations and reliable equations to predict FE could not be delivered, but in general, increasing nutrients and energy concentration improve dairy cows' FE.

Session 84

Poster 15

Impact of dry matter estimates on animal production

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Maize silage is widely recognized as the primary forage source for dairy cattle and is routinely analysed to formulate balanced mixed rations. Despite standardized sampling protocols taught to veterinary practitioners and livestock consultants, variability in analytical results can occur when identical silage samples are sent to different laboratories. A recent case highlighted this issue, where two analyses conducted from samples taken one day apart had nearly identical chemical compositions but differed in dry matter (DM) content. This study investigated the effects of sampling day and sampling depth on maize silage DM measurements, along with the influence of the material used to transport and store the sample (specifically, the type of plastic glove used) and the duration of refrigerated storage prior to analysis. Two maize samples were taken by different vet practitioners for each condition (Date $\times$ Depth), and each sample was duplicated for subsequent analyses. Intra-sample variability was less than 1% for every sample. Intra-condition variability ranged between 1% and 20% (mean: 6%). Dry matter values were highly variable (mean $\pm$ SD : $40.3 \pm 3.6\%$). Both sampling day and depth significantly affected DM content. The variation associated with sampling day could be attributed to differences in weather conditions, as shown by variations in temperature and humidity at the time of sampling, as well as differences among the individuals performing the sampling. Additionally, a simulation was conducted to assess the economic consequences of DM estimation errors on milk production. The results indicated that overestimation of DM led to reduced milk yield due to inadequate nutrient intake but could also induce imbalances between dietary energy and protein, while underestimation resulted in excessive feed refusals. Furthermore, humidification of the forage may alter its conservation characteristics (i.e., its pH may no longer be low enough to maintain proper preservation). Ultimately, all these issues can decrease profitability.

Decades of change : the evolving composition and energy values of major feed ingredients

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Feed ingredients play a crucial role in animal nutrition, and their composition and energy values can vary over time due to changes in cultivation, processing techniques, and environmental conditions. This study aims to analyze the evolution of the composition and energy values of six major feed ingredients – wheat, barley, maize grain, rapeseed meal, soybean meal, and sunflower meal – over several decades to assess long-term trends in nutrient composition and provide valuable insights for feed formulation and animal nutrition management. The study was conducted using a large dataset of feed ingredient samples collected by the Association Française de Zootechnie, with data going back to the 1970s. Parameters included crude protein, total mineral content, mineral concentrations (P, Ca, Mg, K, Fe, Cu, Mn, Zn), gross energy (all obtained by chemical analysis), and UFL (Forage Unit for Milk, net energy) calculated using the INRAE system. The statistical approach involved determining quartiles, mean values, and outlier detection using interquartile range methods. Linear regression models were used to identify significant variations over the last 30 years for feed ingredients used in France. Maize grain and barley grain showed significant downward trends for crude protein, total mineral content, phosphorus and gross energy, with also a decrease in UFL for maize. The composition of wheat grain and soybean meal did not exhibit such trends though annual variations can be important for wheat protein. Rapeseed meal and sunflower meal, whose compositions were stable for decades, saw an increase in protein content and UFL in the past 10 and 15 years respectively, due to varietal changes for rapeseed and to dehulling being more common in France for sunflower. Results regarding other parameters were inconclusive due to a lack of data and/or high variability. The evolution of the composition of certain major feed ingredients shows significant shifts in nutrient values over several decades, which underscores the need for continuous monitoring and adaptation in feed formulation, notably in the context of changes introduced by agroecological practices.

Session 84

Poster 17

Effect of supplementing a protected form of potassium carbonate in diets of dairy cows in heat stress conditions on milk production and mineral absorption

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When submitted to heat stress, dairy cows increase loss of minerals/electrolytes through urination and sweating and decrease intake. Hence, it is recommended to increase dietary mineral supply, especially potassium (K), but also magnesium (Mg), as high levels of K can interfere negatively on Mg absorption. The aim of this study was to investigate the benefits of using a protected, slow-release form of K supply, on milk production parameters and magnesium absorption in heat stress conditions. The study was conducted in an Italian farm on multiparous Holstein cows, using a randomized block design including a CONTROL group (TMR based on corn and wheat silage, alfalfa hay and concentrate, + 150 g/cow/day of unprotected K carbonate, n=135) and a TEST group (same TMR + 180 g/cow/day of SIPASSIUM, K carbonate protected through a fat coating, n=144). The trial period started the 19th of July and lasted 60 days. Individual milk yield was recorded daily, as well as group feed intake, by weighing feed offers and refusals. Every 15 d, individual milk samples were collected and analyzed for fat and protein contents for all cows, and urine samples were collected from 30 cows to determine magnesium excretion. Average THI was above 75 most of the days. Milk production was greater for the TEST group during the 2nd month (+0.86 kg/cow/day, P<0.01). Milk fat content was also greater for the TEST group at 45 d (+2.3 g/kg, P<0.01). A higher concentration in milk lactose was also observed throughout the study for the TEST group (44.4 vs 43.6 g/kg, P<0.01). No significant differences were observed for milk protein content. Urine Mg excretion was greater (P<0.05) in TEST than in CONTROL cows, especially after 45 d of exposure to treatments (4.1 vs 4.4 g/d, P<0.05). These results suggest specific benefits of using a coated form of potassium carbonate in the diets of cows submitted to heat stress on milk production and milk fat content, but also on Mg digestibility, as the higher urinary Mg excretion observed for the TEST group could reflect a lower negative interaction between Mg and K when K is supplied in a slow-release form.

Impact of Different Plant-Based Ingredients on Dairy Cow Performance

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Some plant-based ingredients have demonstrated an influence on rumen fermentation, leading to enhanced milk production and improved feed efficiency in ruminants. Appellation d'origine protégée (AOP) is the French term for a European Union certification aimed at preserving the origin designations of agricultural products. This kind of production adheres to a strict set of guidelines, particularly in terms of nutritional standards. In this way, the utilization of plant-based products must align with these guidelines. This study aimed to evaluate the impact of Turboviv and Turboviv AOP (CCPA Group, Janzé, France), a blend of based-plants ingredients designed to meet specific nutritional guidelines (Turboviv AOP) or not (Turboviv), on the performance of dairy cows. Seventy-two Holstein dairy cows were divided into three homogeneous groups: Control (without supplementation), Tur (supplementation with Turboviv), and TurAOP (supplementation with Turboviv AOP). The cows were fed with maize-silage-based diet. Key parameters measured included daily milk production, milk quality (fat, protein, lactose, and urea content). Statistical analysis was performed using a mixed model with repeated measures. Results indicated that both Tur and TurAOP increased daily milk production (+2 kg/day, $p < 0.001$) and energy-corrected milk (ECM; +1.1 kg/day for Tur and +1.5 kg/day for TurAOP, $p = 0.002$). While no significant differences were observed in milk fat and urea content between groups, both milk protein production and total solids production (kg fat + kg protein + kg lactose) were higher for Tur and TurAOP ($p < 0.001$). Observations of individual dry matter intake (DMI) estimated from the group level, without statistical analysis, showed similar values, averaging 26.9, 27, and 26.6 kg/day per cow for Control, Tur, and TurAOP, respectively. In the same approach, the supplemented cows showed similar feed efficiency (milk production/DMI) of 1.38 and 1.40 (Tur and TurAOP, respectively) versus 1.31 for Control group. In conclusion, both Turboviv and Turboviv AOP improved the performance of high-producing dairy cows.

Session 85

Theatre 1

Structural variant imputation and association with dairy traits in a large cattle population

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To date, most studies that fine-map putative causal variants in cattle use imputed sequence that includes small variants (up to 50bp long: SNP & INDEL) but excludes Structural Variants (SV, ≥ 50 bp long). SV are generally excluded because the imputation reference populations were generated with short-read sequence (e.g. 1000 Bull Genomes project) and this technology is not reliable for SV discovery. Long-read sequencing is a better approach to discover SV and a collaboration has been initiated to build a long-read sequence reference population (Bovine Long Read Consortium). However, it will take time to generate a large SV imputation reference population, therefore, the objectives of this study were to: 1. leverage a set of SV discovered in available long-read sequence to build an SV imputation reference population of 515 cattle with short-read sequence, 2. impute the SV (as well as SNP & INDEL) into 67,000 cattle with high density SNP genotypes and dairy phenotypes, 3. test the effect of the imputed sequence SV, SNP and INDEL on milk and fertility traits using a standard GWAS approach (fitting single variants at a time) as well as BayesR models (fitting all variants jointly). The linkage disequilibrium between SV and SNP was consistently slightly lower than between SNP and SNP but decayed at a similar rate with distance between variants. The empirical accuracy of imputation from high density SNP genotypes to sequence was a little lower for SV than for SNP, but after filtering (Beagle software $DR2 > 0.7$), 35,507 SV were retained for the study. The fine-mapping analyses identified previously documented candidate genes confirming the accuracy of the imputed data and importantly, several SV were found to show strong associations for some traits. In conclusion, this study demonstrates that while we work to build a long-read reference population for imputation, in the interim we can jointly leverage existing long- and short-read databases to study the impact of SV on traits of interest.

Identifying reciprocal translocations in pigs through offspring genotypes

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Reciprocal translocations (RTs) are chromosomal rearrangements where DNA segments are exchanged between two non-homologous chromosomes. RTs are one of the most common chromosomal abnormalities in both humans and livestock, with an estimated prevalence of 0.47% in pigs. Due to no genetic information being lost, individuals carrying an RT are often not directly affected. However, RTs can lead to reduced reproductive performance, in pigs often observed through a reduction in litter size. Consequently, RTs can lead to severe economic losses especially in the case of influential boars. A recent study managed to successfully detect RTs using offspring genotype data to search for abnormal linkage disequilibrium (LD) patterns (Jourdain et al., 2023). Approximately half of an RT-carriers' offspring will inherit a balanced copy of the RT, increasing the LD between the two chromosomes involved in the RT. In this study, we developed a method based on this same principle to identify RTs in pigs using only offspring genotypes. Our dataset consisted of offspring genotypes (50K) from seven boars carrying an RT, previously identified by karyotyping. Additionally, we performed Nanopore long read sequencing on one of the RT-positive boars, allowing us to identify the exact breakpoints of the RT. We used PLINK to perform extensive LD analysis on the offspring populations and compared the results to reference values of offspring populations from 100 RT-negative boars. We found that we can confidently detect RTs when at least 30 genotyped offspring are available. In addition to developing this method, we inspected the impact of RTs on reproductive performance using data from 16 RT-positive boars with a total of 320 litters, which we compared to 200+ RT-negative animals. Each of the 16 RT-positive boars had a reduced litter size of 15% to 42%, with a total average reduction of 35%. To conclude, this study presents a method to screen animals for RTs without the use of cytogenetic testing, provided offspring genotype data is available. In addition, we highlight the importance of identifying RTs in breeding populations by showing the significant impact they have on fertility.

Session 85

Theatre 3

Integrating copy number variants to improve the resolution of genome-wide association analyses for milk production traits in Croatian Istrian sheep

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Genome-wide association studies (GWAS) help to characterise the genetic architecture of traits. GWAS often use the most common type of genomic variants, single nucleotide polymorphisms (SNP) because they are easy and affordable to identify and genotype. Copy number variants (CNV) are structural variants that are deletions or insertions in the genome larger than 50 base pairs in size. CNV are particularly interesting in the GWAS context, as they can affect gene structure, expression, and regulation, potentially capturing genetic variation missed by SNP. While more challenging to detect and genotype, CNV can be identified from SNP arrays (using total signal intensity and allelic intensity ratio data) or whole-genome sequence (WGS) data. We propose using SNP and CNV in GWAS to dissect the genetics of milk production traits (milk, fat and protein yields, and somatic cell score) in Istrian sheep. The transnational Istrian sheep breed is a good example of a locally adapted sheep breed, crucial for the livelihood of extensive small-scale dairy farmers, and a reservoir of genetic diversity with unique characteristics needed to meet future challenges for livestock production systems. We have used pedigree data for registered Croatian Istrian sheep, 41,170 test day milk records, 50K SNP array data for 1,265 animals, and short-read WGS data (~30x coverage) for 15 key animals. We have identified CNV in genotyped and sequenced animals, imputed SNP and CNV to include in the GWAS and compared the results with standard GWAS methods. We have done functional gene annotation in regions of interest by leveraging available information from published public datasets. Local breeds, like Istrian sheep, are important for maintaining species-level genetic diversity, to be able to respond to future challenges, and understanding the genetic architecture of their traits through GWAS will contribute to this.

Genome-wide association studies explain introgression pattern of Red Holstein in Fleckvieh cattle

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Between the 1970s and the 1990s, crossbreeding with some Red Holstein (RH) sires was used in the German Fleckvieh (FV) population to improve milk yield and udder traits while maintaining breed-specific characteristics. RH bulls and their male offspring were introduced in different phases, incorporating RH-derived chromosomes and fragments into the FV genome, which were then positively or negatively selected according to the breeding goal. However, the specific genomic regions within FV that exhibit a particularly high RH proportion, potentially indicating interesting genes, remained unknown. To address this knowledge gap, this study examined a total of 58,849 haplotyped and imputed genotypes, comprising 50 pure FV animals (born before Dec. 31, 1985), 65 pure RH animals (born before Dec. 31, 1995), and 58,734 FV animals with an initially unknown proportion of RH ancestry (born after Jan. 1, 2010). Using the hap-IBD method (Zhou et al., 2020), which detects identical-by-descent haplotype segments in phased genotype data, we generated a genome-wide map with the frequency of RH haplotypes for all SNP positions and identified genomic regions that exhibit an above-expectation concordance with pure RH. Considering the average observed RH proportion in our FV population of around 4.4%, we identified three chromosomal regions with a significantly higher frequency of RH haplotypes than expected (Chr. 8: 54.2-112.3 Mb, up to 20.38% RH haplotypes per position; Chr. 20: 58.3-64.2 Mb, up to 12.71% RH haplotypes per position; Chr. 25: 21.4-38.7 Mb, up to 17.56% RH haplotypes per position). To identify the underlying causal factors, genome-wide association studies were conducted using routinely estimated breeding values for milk performance traits, udder conformation traits, reproduction traits and meat production traits supporting the observed introgression pattern.

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Theatre 5

Geometrical Linkage Disequilibrium for QTL and Gene Mapping

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The concept of geometrical linkage disequilibrium (LD) defines the disequilibrium as the angle between two vectors constructed with elements of the deviations of AA, Aa, and aa genotypes with respect to the population mean. An angle of 0° represents maximum LD, while 90° indicates orthogonality (no LD). We have extended this concept to QTL and gene mapping, where geometrical disequilibrium is modeled as the angle between vectors of phenotypic performance and SNP genotypes. Let P be a matrix made of column vectors of phenotypic performance divided by its norm, and H a matrix with column vectors of genotype effects divided by its norm. A simple multiplication of matrices by $\Phi = P'H$ can speedily compute thousands of dot products between performance and SNPs genotypes. We tested this model with a Monte Carlo simulation of egg weight and sequences for 20 individuals of Castellana Negra chicken. We computed Φ using 25,210 SNPs on chromosome 22 (H) in addition to one SNP at an intermediate frequency at which phenotypic performance was added or subtracted depending on homozygous genotype status (P). The simulation experiment was replicated 1000 times and for either one or two phenotypic standard deviations. The average angular LD over replicates for a QTL effect of one phenotypic standard deviation was 81.00° (SD = 2.79) for the simulated SNP with the QTL effect, and the average for the 25,210 SNPs was 86.9° (SD = 0.61). When simulating a gene effect of two phenotypic standard deviations, the average angular LD was 72.48° (SD = 2.72) for the simulated SNP with the QTL effect, and was 84.9° (SD = 0.64) for the 25,210 SNPs. Formulae for the expected angular disequilibrium were developed and had values of 81.02° and 72.46° for gene effects of one and two phenotypic standard deviations, respectively. Our results demonstrate that mapping QTLs and genes to genomes with a large number of SNPs can be performed very efficiently using vector space algebra. We also demonstrated algebraically that the dot product of two vectors of genotypic values of two SNPs is $\arccos(\sqrt{r^2})$ where r^2 is the standard measure of LD between the two SNPs. Formulae for the expected angular disequilibrium between a snp and performance was extended to deal with incomplete linkage disequilibrium.

Integrative Data Fusion Approach Reveals the Genetic Architecture of Mastitis: Identification of Causal SNPs and Key Immune Molecules Using the 1000 Bull Genomes Dataset

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Mastitis stands out as a major production disease, causing substantial economic losses due to reduced milk production and increased mortality, especially in Holstein cattle. Despite extensive research on mastitis and molecular studies aimed at identifying candidate genes associated with the disease, a critical need remains for meta-analyses to collectively identify potential causal SNPs within differentially expressed genes (DEGs) triggered by different pathogens. To study the complex interplay of biological processes orchestrated by causal SNPs, genes, and downstream effectors during mastitis progression, we analyzed four RNA-seq datasets corresponding to mastitis caused by textit{Escherichia coli}, *Streptococcus agalactiae*, *Staphylococcus aureus*, and naturally occurring mastitis. Our analysis identified 2,325, 3,204, 2,334, and 2,184 DEGs in each dataset, respectively, with 237 overlapping genes, the majority of which were downregulated. Using the common genes' start and end sites, we extracted variant data from 5,116 Taurus cattle within the 1000 Bull Genomes Project, which contained 424,359 variants, and annotated them using SnpEff analysis. Computational analysis identified 99 SNPs across 73 DEGs with a high impact on gene function, playing a significant role in immune cell activation associated with mastitis. These SNP-bearing DEGs were analyzed using the TRANSPATH database, executed by GeneXplain, highlighting ISG56, MX1, IRF3, and Biotin as major downstream effectors intricately linked to IFN-I response activation, antiviral defense, immune cell function, and inflammatory regulation, suggesting an important role in mastitis resistance. The presence of causal SNPs in these immune-regulating genes may impair their function, increasing susceptibility to mastitis. Furthermore, the allelic distribution of the causal SNPs in the DEGs was analyzed in Holstein cattle compared to non-Holstein breeds to assess their population-level distribution and potential role in mastitis susceptibility.

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Theatre 7

Detecting genetic load: A GWAS model accounting for identity by descent

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Inbreeding depression is due to the expression of genetic load through inbreeding and is one of the main threats in livestock as it can lead to reduced fitness and performances. While many strategies to cope with inbreeding depression are based on the avoidance of inbreeding, only few strategies focus on the genetic load itself since it is difficult to estimate it. The primary basis for genetic load is the segregation, at low frequencies, of deleterious and recessive mutations in the populations, making them difficult to detect in the genome. Thus, the goal of this study was to design a model to detect regions of the genome involved in the genetic load. We set a GWAS model considering additive and dominance effects as well as pedigree-based inbreeding to account for inbreeding depression effect. Moreover, runs of homozygosity (RoH) located along the genome were added as a covariable in the GWAS model. While very few studies included RoH into GWAS models, even less have discussed the interpretation of considering this covariable in models and what is the added value of doing so. Using simulation, we aimed to better decipher which statistical contrasts should be computed to detect genetic load and their meaning. We assumed that RoH gave access to HBD (homozygosity by descent), thus helping to get rid of weak linkage disequilibrium between causative and targeted SNP, and to cope with low frequency mutations. We applied this model to production (milk yield, fat and protein content) and health (Somatic Cell Score) traits in heifers from 5 local breeds located in France, the Netherlands and Norway. We identified several genomic regions putatively involved in genetic load in at least one breed. Finally, we discuss these regions relative to those detected using other approaches. Keywords: Inbreeding depression ; GWAS ; RoH ; Dominance

A weighted genomic gametic model for an accurate estimation of parent-of-origin effects

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Gametic models incorporate parent-of-origin effects in genetic evaluations. It allows to consider the effects of imprinting. Genomic evaluations can also consider imprinting if they are based on genomic gametic relationship matrices, but these methods assume a priori equal weights to all SNP markers. Weighted genomic BLUP improves accuracy by addressing this limitation in standard GBLUP models. Nevertheless, an analogous methodology has not been described for genomic gametic models. Therefore, the aim of this research is to develop a bivariate weighted genomic gametic model and test it with the birth weight trait the Rubia Gallega beef cattle population. The data set consist of 4,743 records (42.25 Kg. $\pm$ 6.37). All individuals were genotyped with the Axiom_BovMDv3 DNA array, which provided 45,291 informative SNP markers after standard filtering. Subsequently, haplotype phasing was performed using the FImpute software. Next, the gametic relationship matrices were calculated and variance components were estimated using BLUPF90+. Two models were compared, the standard genomic gametic model and the proposed weighted genomic gametic model. The results showed a better fit for the weighted model (AIC: -6463.23 vs -6434.36). Likewise, the correlation between both gametic effects increased from 0.66 to 0.81 and the maternal and paternal gametic variances also increased (7.7 to 9.5 and 3.8 to 5.4, respectively). These results suggest that the differential weighting of genomic imprinting effects is feasible in gametic evaluations and may improve the estimation and prediction of genetic parameters.

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Differences in crossover count and genetic shuffling in two pig lines with contrasting selection histories

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The objective of animal breeding is to select the best animals to reproduce, and thereby to achieve genetic gain for different breeding goal traits in the upcoming generations. This is only possible for heritable traits, and the rate of genetic gain becomes higher when genetic variance increases. One way to increase genetic variance is to increase the number of crossovers (CO). CO take place during meiotic recombination and enable DNA exchange between homologous chromosomes, thereby creating novel allele combinations and releasing genetic variance. Besides CO number, the release of genetic variance is affected by intrachromosomal genetic shuffling, which estimates the likelihood of allele shuffling between two loci during meiosis, and strongly depends on the position of CO. In this study, we aimed to estimate and compare autosomal CO number and intrachromosomal shuffling between two pig lines: a sow line and a boar line. The sow line was mainly selected for reproduction traits, and the boar line for production traits. Results showed variation in CO number between sexes, with females having higher CO number than males in both lines. In addition, the sow line showed on average one more CO than the boar line (23.9 and 22.4 in females, and 18.1 and 17.4 in males), suggesting that CO count might have increased due to selection for fertility traits. Also, CO distribution across the genome differed between top and bottom 10% of individuals with high and low CO, near the center and the extremes of some chromosomes. Regarding intrachromosomal genetic shuffling, high recombinant individuals showed ~50% greater shuffling values than low recombinant individuals. In conclusion, we detected differences in the number and distribution of CO, as well as in intrachromosomal genetic recombination between sexes and both pig lines. This provides useful insights about the potential to select pigs for CO number for increasing genetic variation.

Signatures of selection using ROH island in a commercial rainbow trout population

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Population management within breeding programs, along with the evolutionary history of species, could leave signatures of selection across the genome. One of the most commonly used method for detecting these selection signatures is the identification of ROH islands (ROHi). Runs of homozygosity (ROH) are long genomic segments comprising only homozygous genotypes. While they can be used to detect and control inbreeding depression for productive traits, a high prevalence of a ROH within a population could also correspond to a selection signature. In this study, we analyzed 6,267 rainbow trouts (*Oncorhynchus mykiss*) from two lines of a commercial population selected for different objectives: line A, 3,806 individuals selected for better reproduction performance, and line B, 2,461 individuals selected for increasing weight. The aim of this study was to identify genetic differences between the lines as well as shared selection signatures from their common origin. All individuals were genotyped using medium-density SNP arrays, covering a total of 49,671 SNPs. ROHs were identified using the consecutiveRUNS method from the detectRUNS package. Our results revealed that, in both lines, over 20% of ROHs exceeded 16 Mb in length, likely reflecting recent selection pressures. The inbreeding coefficient (Froh) was similar between lines, with values of 0.33 for line A and 0.35 for line B. Both lines shared ROHi with a z-score lower than 0.001 on chromosomes 2 and 14. However, line A exhibited a unique ROHi on chromosome 3, while line B had a distinct ROHi on chromosome 13, highlighting both their shared genetic background and the different selection objectives applied to each lineage.

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Evaluating Selection History Models in Animal Breeding: A Comparative Study of BayesS and Ghat

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Selecting on breeding goal traits may cause indirect selection on traits not included in the breeding goal. Understanding the history of selection on these traits is crucial to monitor changes caused by indirect selection. Crossover count (CC) is a trait that might be under indirect selection in pig breeding programs, because it is expected to be linked to fertility. The final goal of our project is to determine whether selection on a breeding goal including production and reproduction traits in pig breeding lines has resulted in indirect selection on CC. Two models, BayesS and Ghat, are developed to assess the selection history of traits. BayesS estimates a parameter (S) that reflects the relationship between allelic effect size and minor allele frequency. This model has primarily been used in human data and is effective for traits influenced by a few genes. Estimating effects of rare alleles is more difficult in populations of limited size as seen in breeding populations, which may pose a challenge for BayesS. Ghat calculates the expected genetic change of a trait based on allele frequency changes and estimated additive marker effects. Ghat is developed for breeding populations under selection for complex traits influenced by many genes. However, its performance may be affected by increased linkage disequilibrium caused by inbreeding. The aim of this study is to evaluate the performance of BayesS and Ghat in an animal breeding context, focusing on detecting indirect selection for a trait with low heritability in pig populations. Both models will be applied to a simulated pig breeding program under either phenotypic or random selection, with varying heritabilities (0.05, 0.1, 0.3) and number of generations under selection (1, 5, 10, 15, 20). Initial results reveal that Ghat was best able to detect selection over 15 or 20 generations. Selection was there detected in 100% of the replicates for $h^2=0.3$, 70% for $h^2=0.1$, and 32% for $h^2=0.05$. No selection was detected for random selection. We expect to present the final results at the time of the conference, which provides insights into the strengths and limitations of both approaches and their potential in animal breeding.

Differences in Genomic and Conventional Inbreeding Trends – Reasons and Implications

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Using data from dual-purpose Fleckvieh cattle we compared inbreeding trends and estimates of inbreeding-effective population sizes calculated from genomic data to those using a conventional pedigree-based approach. Genomic inbreeding coefficients, when calculated in a way to reflect the true level of homozygosity of an individual at genomic markers, result in considerably steeper inbreeding trends per year at a population level when compared to the pedigree-based estimates. By repeating the calculation of genomic inbreeding trends using different subsets of SNP, each exhibiting a specific pattern of allele-frequency changes over time, we illustrate how allele-frequency changes caused by artificial selection are shaping genomic inbreeding trends. This demonstrates that the validity of the overall estimate using all markers depends on the representativity of the distribution of markers across the genome. It therefore represents some kind of ‘average’ of effects caused by genomic regions approaching intermediate frequencies, those departing from intermediate frequencies and regions that show no directional trend at all (‘neutral’), respectively. As expected, genomic inbreeding trends based on subsets of ‘neutral’ markers alone, show the closest agreement to the pedigree-based estimates. This highlights the difference in the fundamental theoretical conception of the definition of inbreeding between conventional and genomic approaches. Genomic estimates therefore can provide the basis to monitor both the distribution and number of neutral sites in the genome as well as the continuous changes produced by artificial selection at others. As a conclusion we argue in favor of the development and use of genomic approaches capable of reflecting the complexity of various processes on the genome level, whenever the available genotyped animals sufficiently represent the breeding population, spatially as well as temporally.

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Analysis of inbreeding depression and genetic purging on litter size traits of Pannon Ka Rabbits using animal models

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The objective of this study was to evaluate the effect of inbreeding on the number of kits born alive (NBA) and number of kits born dead (NBD) and the possibility of genetic purging based on the reproductive performance of the Pannon Ka rabbit breed. The kindling data consisted of 21748 records collected from 5343 does between 1995 and 2024. Wright, Ballou, Kalinowski and Kalinowski new inbreeding coefficients were calculated both for the does and for the litters using a pedigree extended with dummy litter identities using the GRAIN software. The extended pedigree file contained 30841 animals. The effects of inbreeding on these traits were evaluated by two-trait repeatability animal model applying the REML and BLUP procedures using the VCE6 software. Two different models were used. In the first model the included factors were Wright inbreeding coefficients of the litter (FL) and the doe (FD), Ballou inbreeding coefficient of the litter (FBAL), parity, year-month of kindling, permanent environmental effects and additive genetic effects. In the second model FL and FD were replaced by the Kalinowski new inbreeding coefficient of the litter and of the doe (NEWL, NEWD) while FBAL was replaced by the Kalinowski inbreeding coefficient of the litter (FKAL). The other factors remained the same as before. The estimated heritability values were low both for NBA (0.05 ± 0.01 ; 0.06 ± 0.01) and for NBD (0.03 ± 0.01 ; 0.04 ± 0.01) based on both models. The genetic correlation coefficient between NBA and NBD was not significantly different from zero in either case (-0.02 ± 0.15 ; -0.04 ± 0.14). The effect of inbreeding differed greatly between the F/NEW and BAL/KAL coefficients. Ten percent increase of FL, FD, NEWL and NEWD decreased NBA (-0.23 , -0.07 , -0.34 , -0.41) and increased NBD (0.02 , 0.05 , 0.18 , 0.06) showing inbreeding depression. On the contrary 10% increase of FBAL and FKAL mostly induced favourable changes both for NBA (0.03 , 0.04), and for NBD (0.06 , -0.03) which signals the possibility of genetic purging.

Combining selection strategies to efficiently manage genetic diversity and maximize short- and long-term genetic gain

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Over the years, a multitude of selection strategies for the management of genetic diversity have been proposed. In a previous study, we compared various approaches using stochastic simulation of a generic breeding program with the software MoBPS. Strategies considered include optimum genetic contribution selection, weighting SNP effects based on their allele frequency, avoiding mating between related individuals, introducing average kinship as a trait in the selection index, and altering prior mean and variances in the genomic prediction model. Building on this, we assessed which selection strategies complement each other and subsequently employed an evolutionary algorithm (MoPBSopti) to efficiently combine strategies to determine the ideal weighting of each approach and further optimize breeding program design. Notably, while altering prior mean and variance to emphasize rare variants had limited benefits in isolation, it proved highly complementary when combined with other strategies. Furthermore, the impact of key design characteristics, such as the proportion of phenotyped or genotyped animals and particularly selection candidates, on the choice of ideal weighting was critically assessed. The combination of selection strategies for diversity management allows for the selection of fewer animals as the finally selected individuals were less related. The suggested optima of the evolutionary algorithm with a target function that included both genetic gain and diversity resulted in a 5.1% increase in genetic gain and a 37% reduction in inbreeding rates in the baseline breeding scheme. This underscores the superiority of integrating multiple selection strategies for diversity management over singular approaches. As such methods can be implemented at no additional cost beyond computation, their adoption is critical for the long-term sustainability and success of breeding programs.

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Poster 15

Assessing cheese bacterial taxonomy and resistance genes using whole genome sequencing

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INTRODUCTION: Whole genome sequencing (WGS) has emerged as a powerful tool in microbial taxonomy and functional genomics, enabling comprehensive characterization of bacterial species (Loman & Pallen, 2015). **OBJECTIVE:** WGS analysis of seven bacterial or yeast isolates from cheese has been conducted, to elucidate their taxonomic classification and investigate their antibiotic resistance genes. **MATERIALS AND METHODS:** Genomic DNA from cheese isolated strains was extracted and sequenced using high-throughput sequencing technology in an Illumina iSeq 100. Subsequent analysis involved taxonomic assignment, de novo assembly and genome annotation using bioinformatic tools available in Galaxy. Furthermore, genomic data were used to predict various genes. Through comparative genomic analysis and functional annotation, antibiotic resistance genes and other pathways of biosynthesis and degradation of interest, have been researched. **RESULTS AND DISCUSSION:** Taxonomic classification has revealed the precise phylogenetic placement of each isolate within their respective bacterial and yeast species. Among the seven isolates, two from a Culture Collection bank have been accurately identified, while the remaining five comprise one yeast (*Yarrowia lipolytica*), three distinct species of *Corynebacterium*, and *Enterococcus faecalis*. This analysis validates the methodology and the identifications of isolates since the known bacteria have been correctly identified. Gene-associated analyses have revealed different antibiotic resistances in some bacteria, such as Streptomycin and Chloramphenicol in a *Corynebacterium*, or Lincomycin and Clindamycin resistance in *Enterococcus faecalis*. Additionally, the potential capacity of these bacterial strains to synthesize or degrade compounds with an impact on both food safety and human health has been researched. **CONCLUSION:** Overall, our study demonstrates the utility of WGS in accurately assigning taxonomic identities to bacterial isolates and evaluating their antibiotic resistance. This comprehensive genomic approach provides a foundation for further research on the genes implicated in food microbiology and public health.

Histamine and tyramine concentrations in commercial cheeses and their relationship to bacterial population
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INTRODUCTION: EFSA recommends modifying food processing to decrease the risk of histamine and tyramine presence that are produced by bacteria in fermented foods (EFSA, 2011) because of related health problems to consumers and even more to those with histamine intolerance and with MAOI treatment. **OBJECTIVE:** To assess the concentration of biogenic amines in commercial cheeses and to establish their bacterial profile. **MATERIALS AND METHODS:** 77 cheeses (4 categories) were sampled at retail level in Basque region. Biogenic amines were analysed (Redruello et al., 2013) and two pool of hard cheese samples were made to 16S metagenomic analysis by iSeq100 (Illumina): very high (HCBA) and very low concentration (LCBA) of histamine and tyramine. **RESULTS AND DISCUSSION:** No significant differences were found in concentration of histamine, but tyramine was higher in blue cheese (average of 1168,44ppm, $p < 0.05$) probably because its proteolytic activity. The high variability of results hampered to show more differences between categories, but among hard cheeses there were the samples with highest concentration of histamine (30,31 mg/30g) and tyramine (111,94 mg/30g). In both LCBA and HCBA cheeses, the *Lactococcus lactis* whose use in cheese factories is usual as acidifying ferment was the dominant bacteria, with higher relative abundance (RA) in LCBA than in HCBA samples (87% vs 21%). Hard cheese with HCBA shown higher bacterial biodiversity, so LCBA samples shown low presence of other minor bacteria (<5% of RA) whilst HCBA samples presented *Brevibacterium* spp. (8%), *Lactobacillus* spp. (7%) and *Leuconostoc* spp. (5%) among others (<5% of RA). **CONCLUSION:** Despite the high variability in the concentration of biogenic amines of commercial cheeses, the results shown a health risk when eating a portion of blue and hard cheeses in case of people with sensitivity to histamine and tyramine. Differences between cheese with HCBA and LCBA can be explained by their bacterial biodiversity.

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Poster 17

Sequence-based GWAS identify genomic regions associated with resistance to gastrointestinal strongyles in Holstein heifers

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Gastrointestinal strongyles (GIS) are a raising concern for cattle health and productivity due to increasing resistance to anthelmintics and their ecological impacts. Enhancing genetic resistance represents a sustainable strategy to mitigate parasite burdens. As part of the GIVERNI project, we conducted a sequence level genome-wide association study (GWAS) to identify genomic regions associated with GIS resistance. A total of 1,736 Holstein heifers were genotyped using a 50K SNP array and phenotyped after their first grazing season. Four resistance traits were analyzed: serum immunoglobulin G Elisa test (IgG), Ostertagia oocysts count per gram of feces (OPG), log-transformed OPG, and serum pepsinogen levels (Pepsi). The 50k genotypes were imputed to whole-genome sequence using Run 9 of the 1000 Bull Genomes Project. GWAS were performed using a linear mixed model, with performance data adjusted for non-genetic effects. Heritabilities ranged from 0.07 (OPG) to 0.30 (Pepsi). Several significant QTLs were detected, highlighting candidate genes involved in immune response and gastrointestinal function. A major association was identified for IgG in a region implicated in enteric nervous system development. For OPG, a key signal appeared within an area involved in extracellular matrix composition, while Pepsi was associated with a region that plays a crucial role in gastric mucosal integrity. Post-GWAS analyses are ongoing to refine candidate genes. These findings advance knowledge on parasite resistance genetics and support genomic selection for resilient cattle. The GIVERNI project is funded by APIS-GENE.

Population Genomics of Peruvian and European Alpacas: Insights into Diversity, Structure, and Selection

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Alpacas (*Vicugna pacos*) are domesticated camelids native to Peru, with a smaller founder population in Europe since their introduction in the 1970s. Selective breeding practices have enhanced alpacas' environmental adaptability and improved fleece quality. The founder population with a restricted gene pool may elevate their risk of inbreeding. This study investigates genetic diversity and selection signatures in alpacas from German-speaking regions (GSR) and Peru using genome-wide SNP data. Of 330 Huacaya alpacas genotyped with an Affymetrix 76K SNP array, 258 animals (113 GSR, 145 Peru) and 49,099 markers passed quality control. Genetic diversity parameters, including heterozygosity, runs of homozygosity (ROH), and genomic inbreeding coefficient (FROH), were analyzed alongside selection signature analyses using FST and XPEHH. Principal component analysis (PCA) revealed distinct clustering of alpacas from GSR and Peru, reflecting clear genetic differentiation driven by geographic isolation and breeding practices. Both populations maintain similar genetic diversity levels (Ho, He, MAF) and low recent inbreeding levels (FROH: 0.0285 for GSR, 0.0226 for Peru). Effective population sizes (Ne) remain robust (210 for GSR, 260 for Peru at 5 generations ago). Selection signatures analyses identified key loci in ASIP and KITLG for coat color, along with candidate genes (FGF5, RIC8B, BSN, DAG1) associated with environmental adaptation and production traits. Both alpaca populations show a low risk of diversity loss, reflecting effective breeding practices; however, integrating pedigree and genomic data is recommended to prevent future inbreeding.

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Poster 19

Metadata Challenges: An explorative analysis of the 1000 Bull Genomes Project

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The availability and amount of genomic data in the agricultural sciences has increased tremendously in the past decade. This growth raises questions about the integrity of metadata in externally curated datasets, as it is crucial for the reusability of the datasets. The consequences of misassignments in the metadata range from the reduction of sample sizes to potential analysis impairments by the unintentional analysis of the wrong samples. We use a data-driven approach to demonstrate how large-scale genomic data can be employed to address these challenges, using the 1000 Bull Genomes Project as an example. By randomly sampling 50,000 genomic positions across all autosomes, we efficiently employ the available genomic information to examine the assigned subspecies and breeds through principal component analysis followed by an analysis of the ancestral populations and genetic distances. Following our analysis framework, we identified misassignments at both subspecies and breed levels, affecting single individuals and, in more complex cases, groups of entire breeds. Such complex cases result from transpositions or insertions of letters or words, or the use of different languages, making it particularly difficult for automated approaches to detect such misassignments. Through our investigation, we identified subspecies misassignments for 1% of the individuals. Further, the breed information was initially missing for 6% of the individuals, which we reduced to 3%. Moreover, we identified 23 breed-pairs with highly similar names, 5 of which actually represented individuals from one breed. Apart from these duplicate assignments, we found one case where several breeds were recorded under a single breed name. Our exploratory approach highlights the need to critically re-evaluate the metadata of (publicly) available genomic data and provides an exemplary framework for addressing these challenges.

Estimating genetic diversity parameters with traditional and novel metrics based on the metafounders methodology

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During the last decade, the concept of metafounders has been developed to overcome the unrealistic assumption of unrelated base populations. Metafounders are used in genomic evaluations to estimate relationships within and across populations. However, so far, relationships based on gamma from the metafounders theory have not been compared to traditional genetic diversity parameters in real populations. Therefore, the objective of this study is to use gamma to estimate genetic diversity parameters and compare them with estimates based on traditional parameters. We will focus on different metrics used to estimate genetic distance, i.e., fixation index (Fst) and Nei's minimum genetic distance (D), but also on population inbreeding coefficients based on homozygosity (Fhom), drift (Fdrift) or average heterozygosity (Fb), and apply them to a real population. To make the comparison between traditional and gamma estimated parameters, we will use the following formulas: for fixation index, $Fst = 1 - H_{within}/H_{between}$ and $Fst = (\gamma b/8 + \gamma b'/8 - \gamma bb'/4)/(1/2 - \gamma bb'/4)$; for Nei's Minimum genetic distance, $D = 1/n \sum (pb - pb')^2$ and $D = \gamma b/8 + \gamma b'/8 - \gamma bb'/4$; for inbreeding coefficient based on homozygosity, $Fhom = 1 - 1/n \sum ((2pb(t)qb(t))/(2pb(0)qb(0)))$ and $Fhom = \gamma b/2$, for inbreeding coefficient based on drift, $Fdrift = 1/n \sum ((pb(t) - pb(0))^2 / (pb(0)qb(0)))$ and $Fdrift = \gamma b/2$; for inbreeding coefficient based on average heterozygosity, $Fb = 1 - 4/n \sum (2pbqb)$ and $Fb = \gamma b - 1$, with H_{within} the within population heterozygosity, $H_{between}$ the between population heterozygosity, γb and $\gamma b'$ the within population gamma estimates, $\gamma bb'$ the across-populations gamma estimate, n the number of SNP, pb and pb' the allelic frequencies in the different populations, $pb(t)$ and $pb(0)$ the current and initial allelic frequencies (AF), respectively, and $q = 1 - p$. The definition of equations based on gamma estimates supposes AF of base population of 0.5. Considering that initial heterozygosity in populations is probably lower than this, we might expect some differences in the traditionally and gamma computed genetic diversity parameters, except if they are more robust to changes in AF than expected.

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Detection of deletion impacting fertility, stillbirths and juvenile mortality in the European population of the Holstein breed by leveraging genotype datasets

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Copy number variations (CNV) can severely affect animal phenotypes depending on their size, and gene content of the region concerned. Artificial insemination and the wide use of elite sires under genomic selection scheme can participate to spreading such genetic defect. At the same time, the range of genomic tools and the large genomic datasets produced by the routine genomic evaluation of reproducing animals provide opportunities to detect these variations. Here, we leveraged fluorescence (log(R)ratio) and B Allele Frequency data from animals genotyped on Illumina EuroG MD arrays, with the aim of linking CNVs with fitness issues. By doing so, we discovered a 2.86 Mb deletion on BTA9 segregating only in the progeny of a German Holstein bull in Europe. The deletion was confirmed by HiFi sequencing of the presumed founder bull and 3 of his offspring as well as by screening for sire-offspring Mendelian inheritance errors method in a broader population ($n=1,688$). This defect causes increased stillbirth rates (doubled to tripled compared to the German and French average populations, 5.3 and 6.8%, respectively) and juvenile mortality (two-times increased mortality, 11.1 and 13.0% in the precited national populations), as well as decreased fertilizing ability (reduced NRR in male fertility). The defect is also supposed to cause delayed growth, subfertility and decreased milk production in the affected female progeny. This study highlights the opportunity to use genotyping data to screen selection scheme candidates early in their life to appropriately manage each case and potentially eliminate them from breeding schemes.

Genetic variability in five dog breeds analyzed using 22 genetic markers in a single PCR multiplex

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Dogs, nowadays, carry out several important functions, as pets or as animals performing different tasks in live-stock or disaster management. Over the years, breeders have selected these animals for the characteristics best suited to the function to which they are to be devoted, which has resulted in a large number of breeds. Globally, the species maintains high levels of genetic variability and low inbreeding, but within each breed the genetic situation is very different. In addition, some indigenous breeds are in serious danger of extinction. In this study, we propose a multiplex PCR including a set of 21 microsatellite markers: AHT121, AHT137, AHT130, AHT171, AHT260, AHTk211, AHTk253, CXX279, FH2054, FH2848, INRA21, INU005, INU030, INU055, REN105LO3, REN162C04, REN169D01, REN169O18, REN247M23, REN54P11 and REN64E19 and the sex marker Amelogenin, included in the ISAG dog panel, to be co-amplified simultaneously in a multiplex PCR. Modifications to the usual primers were required to achieve a final configuration allowing all markers to be analyzed together in a single PCR reaction. Proposed microsatellites were all well-amplified in dog biological samples and all the microsatellites used in this study were amplified and were polymorphic in blood samples of more than 80 domestic dog breeds around the world, obtaining an average number of 9.95 alleles per marker, mean heterozygosity values of 0.7986 and a PEG greater than 99.9999%. When Cavalier King Charles Spaniel, German Shepherd, Golden Retriever, Labrador Retriever and Rottweiler breeds are researched separately, the average number of alleles per marker, in the samples analyzed, falls to 2.90, 3.45, 3.05, 5.00 and 2.95; the mean Heterozygosity values are reduced to 0.5033, 0.5734, 0.5045, 0.6405, and 0.5136 and those of PEG to 99.8977, 99.9789, 99.9247, 99.9990, and 99.8993, respectively. This data can help to manage breeding programmes for the most endangered breeds.

Designing a breeding programme for brine shrimp (*Artemia franciscana*)

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Brine shrimp, members of the *Artemia* genus, have exceptional nutritional value and can be used as live food for early larval stages in aquaculture species. Most *Artemia* in the market are currently sourced from natural ecosystems, however there is growing interest in farming brine shrimp to establish a sustainable and reliable supply. Despite this, studies on breeding and quantitative genetics remain limited in number. The goal of this study was to initiate the development of a commercially orientated genetic breeding programme for *Artemia franciscana*. We began defining phenotypic traits of interest and collected various life cycle and genomic parameters on *Artemia franciscana*, to use as an input for stochastic modelling of different breeding programme scenarios. To this end, we have simulated breeding programmes based on various types of selection, population sizes, and phenotyping strategies, with particular focus on technical feasibility to implement a realistic large-scale breeding programme. Furthermore, we tested multi-trait selection by modulating various levels of heritability and genetic correlations between two traits mimicking naupliar length and pre-reproductive period, as well as their respective weights in the selection index. We have compared breeding programmes in terms of efficacy to generate desired gains and maintain genetic variation. Preliminary results showcase mass selection, based on either performance at the individual or family level, as the most promising strategies at this early testing stage. In that sense, individual selection was more effective in preserving genetic variation and driving the genetic mean of both traits toward the desired direction. On the other hand, family-based selection might be more appealing for small-scale and controlled breeding programs, but should be set-up with caution, as it can lead to faster decay of genetic variation. This work set the foundations for the development of large-scale breeding programmes for *Artemia franciscana* and other similar species, thus contributing to more sustainable aquaculture.

Identification of developmental stages of bovine embryos using high-throughput transcriptome sequencing
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Background: The assessment of embryo developmental stages can be conducted using various methods. Light microscopy allows for the observation of cell morphology, while immunofluorescence staining enables the identification of stage-specific proteins. Additionally, genetic studies, such as gene expression analysis, provide insights into the molecular markers characteristic of each developmental phase. For this purpose, we adapt the technique of transcriptome sequencing of cattle single cell (embryos) and deconvolution, using bioinformatics methods. The obtained results across the human embryonic reference. **Methods and Results:** We applied deconvolution methodology on 32 cattle embryos, for which transcriptomic scRNAseq data were sequenced using Illumina SBS technology, and for which the expression levels across all genes were identified. The developmental stage for each embryo was rated using light microscopy. We used scRNAseq workflow based on QC (fastp, fastqc), alignment and estimating gene expression levels (STAR + RSEM) which was extended on addition of the MuSiC deconvolution algorithm optimized for single-nuclei reference data and DeTREM packages. In addition, the single-cell reference data based on Yan et al. (2013) study was used. **Conclusions:** Due to the deconvolution methods, most embryos were assigned to the late blast for MuSiC and DeTREM methods. The obtained results were more unambiguous for MuSiC than for DeTREM. **Acknowledgement:** The research work was financially supported by the Preludium BIS2 National Science Centre project number: UMO-2020/39/O/NZ9/00621, entitled Transcriptome and epigenome analysis for oocyte maturation and developmental competence of preimplantation embryos in Polish HF cattle.

Genetic correlations between slaughter loss and other economically important traits measured on live pigs in DanBred Yorkshire

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Pig breeding programs focus on improving production efficiency, but some economically important traits, such as slaughter loss, receive less attention. Slaughter loss, defined as the difference between live weight and carcass weight, affects profitability but can only be measured post-mortem. Currently, slaughter loss is evaluated using a single-trait model, which does not account for genetic correlations with other traits. Since breeding candidates lack direct records, selection relies on pedigree information and records from relatives. Identifying genetic correlations with traits measured in live animals could improve selection accuracy for slaughter loss and increase the amount of data available for breeding value estimation. We hypothesize that slaughter loss has significant genetic correlations with other economically important traits, supporting the use of a multi-trait model for genetic evaluation. The model for slaughter loss included season (a combination of physical unit, year, and season) as a fixed effect, pen as a random effect, live weight as a covariate, and a random animal genetic effect based on pedigree relationships. To estimate genetic correlations, we used multivariate models incorporating average daily gain (30-100 kg), feed efficiency conversion ratio, and lean meat percentage (estimated using ultrasound backfat measurements). The estimated heritability of slaughter loss was 0.31. Statistically significant genetic correlations were found between slaughter loss and average daily gain (-0.16, favorable), and with lean meat percentage (0.14, unfavorable). The genetic correlation with the feed efficiency conversion ratio was not significantly different from 0. These findings confirm that significant genetic correlations exist between slaughter loss and both average daily gain and meat percentage. These results support the implementation of a multi-trait model for the genetic evaluation of slaughter loss, which would allow for more accurate selection by incorporating information from live animals.

Opposite inbreeding load for reproductive traits in lines of mice divergently selected population

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A divergent selection experiment on the birth weight environmental variability in mice showed that genetic control of variability is achievable through selection. Homogeneity has been shown to enhance litter size, embryo survival and fertility, among others, suggesting improvements in robustness and welfare. Nevertheless, related matings for the selection lead to an increase in inbreeding, which has usually been associated with reduction in fitness and loss of performance in traits under selection. Some studies have shown that inbreeding has not only a extensive range of negative effects but also neutral or even positive effects in livestock. Thus, the objective of this study was to analyse the inbreeding depression load (IL) in three reproductive traits (number of parturitions-NP, litter size-LS, and fertility-Fert) in a divergently selected mice population. Female mice that acted as breeder and with both parents known were selected (2829) and the pedigree included 4117 records (1070 males and 3047 females). Partial inbreeding coefficients were estimated for all animals in the population. The genetic model included the line (2 levels) and generation (33 levels) as fixed effects and female age as a covariate. Direct additive genetic and IL variances were estimated using own software. Heritabilities ranged from 0.03 (Fert) to 0.10 (TC) and IL variance was equivalent to 0.90 times the phenotypic variance in all cases. Even when both lines have a similar inbreeding level (around 20%), breeding values for IL showed clear differences between lines and, in all traits, similar divergent trends as the phenotype. The line selected for low environmental variability for birth weight also showed IL that improved their descendants' phenotype, reaffirming that inbreeding depression not only depends on the inbreeding level, but also on the common ancestors and the partial inbreeding coefficient they transmit to their inbred descendants. Understanding these genetic values is crucial to make more informed selections based on the effects of specific lineages.

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Poster 27

Identification of recessive genetic defects in the Abondance regional cattle breed using routine genotyping and life history information

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Cattle breeds are genetically small populations prone to recurrent outbreaks of recessive defects, only a fraction of which are detected by on-field observatories and characterized by positional cloning due to lack of specific signs or under-reporting. Here, we present the detection of the first two genetic defects described in a regional cattle breed from the French Alps, the Abondance, using reverse genetic strategies based on life history information for over a hundred thousand animals, 30 000 genotypes collected for genomic evaluation since 2015 and whole genome sequences of 24 influential ancestors. In a first step, a search for homozygous haplotype deficiency identified an haplotype on BTA19 with 142 homozygotes expected versus 0 observed, which was strongly associated with a frameshift variant in the UTP6 gene. Analysis of several fertility traits, large scale genotyping using a custom SNP array and procreation of 7-day embryos from at-risk mating confirmed that this recessive variant was causative and responsible for early embryonic loss. In a second step, we investigated postnatal mortality and searched for haplotype enrichment/depletion in heifers that died of natural causes in comparison with females that completed at least one lactation. One haplotype on BTA4 had an enrichment of +300% of observed versus expected homozygotes in the case group and a complete absence of observed homozygotes in the controls. The best candidate variant for this locus affects a splice donor site in SLC25A13, a gene previously associated in human with citrullinemia type II metabolic disease. Recruitment and clinical examination of homozygous animals is underway. These findings highlight the power of reverse genetics approaches and pave the way for improved genetic management strategies to reduce mortality and enhance herd health in Abondance cattle. This project was funded by APIS-GENE.

Early Detection of Chromosomal Abnormalities in Cattle using SNP beadchip intensity data

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Chromosomal abnormalities frequently induce high mortality rate during gestation or after birth. Their early detection is a major challenge for the industry and breeders. The AnEmBoV project aims to identify chromosomal abnormalities, both in in vitro produced embryos (IVP) within selection programs and in calves for herd renewal, to prevent genetic defects, improve fertility, and minimize economic losses and unproductive periods. To provide an early diagnosis solution, we developed a methodology for detecting chromosomal abnormalities based on intensity data from genotyping arrays used in genomic selection. This approach enables the identification of chromosomal anomalies in both embryos and calves, distinguishing individuals with aneuploidies or structural rearrangements. A total of 192 IVP blastocysts were produced and few cells from each embryo were biopsied for genotyping. Five embryos were excluded because of low call rates. By analyzing signal intensity (Log R Ratio or LRR) and B allele frequency (BAF) data, we identified aneuploidy for 26% of the IVP embryos with 1 haploid, 11 triploid, 25 monosomic, 8 trisomic, and 3 tetraploid. We then tested various indicators to estimate the status of each chromosome for each embryo. While genotyping quality can affect detection, we have adapted our approach to different types of aneuploidy and have been able to make reliable predictions. Based on these results, we are currently developing an aneuploidy detection pipeline for genotyped embryos within genomic selection programs. Additionally, we applied this methodology to known cases of bulls carrying chromosomal rearrangements that induce partial aneuploidy in their offspring. Through intensity data visualization, we confirmed the chromosomal status of their progeny, which had previously been predicted using haplotype analysis, even in cases involving genomic regions as small as 2Mb. An automated pipeline is in development for detecting partial aneuploidies and deletions in breeding candidates. The AnEmBoV project is funded by APIS-GENE.

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Poster 29

Copy number variations among draught and milk type zebu cattle of South Asia by whole genome sequencing

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Copy number variations (CNV) arise from losses or gains of DNA fragments and they significantly explain genetic and phenotypic diversity. This study aims to uncover CNV among draught and milk-type Zebu cattle breeds of South Asia that potentially contribute to differences in production potential and adaptation to local conditions. Asian Zebu is recognized for their ability to thrive in harsh environments, tolerance to tropical heat, and higher resistance to diseases and tropical vectors. We generated 40 short-read paired-end whole genome sequences on Asian Zebu cattle and used an additional 20 from publicly available sources. The samples comprised 12 breeds from five countries (Pakistan, India, Sri Lanka, Myanmar, and Cambodia) located in South Asia. An average coverage of 20x per raw sequence data was generated in addition to 20 publicly available sequences. Quality-controlled reads were aligned using the recently generated *Bos indicus* reference genome (NCBI Bioproject Accession No. PRJNA897888) and the output sequence was used as input for CNV analysis. The read-depth approach of CNVpytor software was used. A large number of deletions and insertions of different lengths were identified. We identified overlaps with genes, many of them related to metabolic pathways and tropical adaptation. In conclusion, we present the distribution and diversity of CNV in South Asian Zebu cattle populations and our results indicate their significance to genetic differences between draught and milk-type breeds.

Long-read Pangenome of Holstein Cattle to Resolve Difficulties in Simultaneously Calling Structural Variations and SNPs

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With the advance of long-read sequencing, structural variations (SVs), in addition to SNPs, are increasingly detected and studied in populations of agriculturally important species such as cattle. The calling and genotyping of SVs and SNPs however may interfere with each other, causing artifacts such as spurious SNPs, especially in highly variable regions such as immune genes. We therefore sequenced samples obtained from Holstein cattle using Nanopore long-read technology, assembled their genomes into a pangenome graph and studied how SVs and copy number variations (CNVs) affect the calling and genotyping of SNPs. For a preliminary test, we constructed a pangenome graph for a part of Chromosome 18 from 12 individuals using the minigraph-cactus pipeline. Initial findings reveal that the density of SVs coincides with the density of SNPs that are difficult to call, mainly in the tail of the chromosome (58-65MB). The reasons for SNP calling errors and difficulties appear to be complex and multi-faceted. One of the most likely causes is that when there are two or more copies of a sequence segment, the read counts supporting two alleles in the same individual strongly deviate from 1:1 ratio, which would behave neither like a homozygote or a heterozygote for the SNP caller. The preliminary results suggest that pangenome graphs allow to better resolve by now understudied regions of the genome and contribute as a solution to the missing heritability problem.

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Poster 31

Identification of genomic regions with transmission ratio distortion associated with potential lethal alleles in beef cattle

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Transmission Ratio Distortion (TRD) describes deviations from Mendelian inheritance in which alleles are transmitted at frequencies differing from the expected 50%. This study aimed to i) identify genomic regions exhibiting TRD in Nellore cattle (*Bos taurus indicus*); ii) detect TRD regions potentially carrying semi-lethal or lethal alleles; and iii) investigate candidate genes and conduct functional genomic enrichment analyses to uncover biological processes and metabolic pathways. A genomic dataset comprising 3,351 animals and 612,154 autosomal SNP markers (ARS-UCD1.2 *Bos taurus* genome assembly) was analyzed using TRDscan v.2. TRD effects were assessed through allelic (overall and parent-specific) and genotypic (additive and dominance) parametrizations, with regions showing effects $\geq [0.20]$ and $\geq 1,000$ underrepresented offspring classified as potentially harboring lethal alleles. A total of 37,783 SNPs and 174,190 haplotypes exhibited TRD across 1,249 genomic regions (802 Overall TRD, 191 Parent-Specific TRD, and 256 Genotypic TRD). Among these, 73 allelic and 30 genotypic TRD regions were identified as potentially carrying lethal alleles, with the most affected region showing up to 1,374 underrepresented offspring (BTA29, 35.28 – 35.48 Mb). Functional genomic analyses revealed 2,265 candidate genes, 200 significant Gene Ontology (GO) terms, and two pathways related to embryo development, morphogenesis, and growth regulation. These findings highlight the relevance of incorporating TRD information into genomic selection strategies to improve productivity while minimizing the propagation of deleterious alleles affecting reproductive success and embryo viability in Nellore cattle. This study was supported by FAPESP (grants 2017/10630-2, 2017/50339-5, 2021/11922-2, 2022/11852-7, 2023/11176-4, and 2024/01909-7).

Incorporating Breed-Specific Recombination Maps in Selection Signature Analysis of Cattle Breeds

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Recombination and selection are key evolutionary processes shaping genetic variation in livestock populations. Recombination promotes genetic diversity by breaking associations between alleles, thereby reducing linkage disequilibrium (LD) across the genome. In contrast, selection can lead to extended LD regions by driving the rapid increase in frequency of beneficial alleles. To improve the accuracy of selection signature detection, we constructed breed-specific recombination maps for eight cattle breeds using LINKPHASE3, separately estimating recombination rates for males and females. We analyzed genotype data from these breeds, with sample sizes ranging from 4,181 to 367,056 individuals. Our results show that males display higher recombination rates across all cattle breeds. The resulting maps spanned 26.64–29.63 Morgan in males and 23.10–25.62 Morgan in females. These maps were included in selection signature analysis using the rehh package. Our analysis identified genomic regions under selection, revealing breed- and sex-specific differences in selection pressure. Functional annotation of these regions uncovered candidate genes associated with economically important traits, highlighting their potential role in breed-specific adaptation. Additionally, we will investigate the advantages of using breed-specific recombination maps compared to a consensus map to assess their impact on selection signature detection. These findings emphasize the importance of incorporating breed-specific recombination maps into selection signature analysis to better understand the interplay between recombination and selection in shaping genetic diversity. Key-words: Cattle breeds, recombination map, recombination rate, selection signature

Further characterization of the structural variant upstream of the KIT gene underlying head depigmentation across a diverse panel of cattle breeds

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A previous study based on a taurine pangenome uncovered a structural variant upstream of the KIT gene associated with depigmentation in white-headed cattle. In this study, we aimed to deepen our understanding of this region by analysing a collection of 80 genome assemblies from 20 different cattle breeds, including several not previously investigated. Using a combination of complementary approaches, we constructed a 2 Mb pangenome graph encompassing the KIT gene and the depigmentation-associated region to investigate the spectrum of structural variations within this region. Our analysis revealed a more fragmented structure than previously reported, mainly due to the inclusion of a larger number of assemblies (80 vs. 24) with varying sequencing technologies' quality (CLR and HiFi). Despite this increased complexity, the identified structural variant's alleles closely mirrored those reported in earlier study and depicting clear segregation of the population into two distinct groups. The first group includes breeds characterized by a white-headed phenotype and the second group contains all non-white headed breeds. We further validated this structural variant by aligning short-reads to the pangenome graph and genotyping several hundred animals for this structural variant. Our results provide new insights into the genetic mechanisms underlying head depigmentation in cattle and demonstrate the power of pangenome-based analyses for detecting trait-associated structural variations. This research was supported by funding from the Agreenium programme, with contributions from the Biosphera Graduate School, the ABIES doctoral school, the INRAE Animal Genetics division, and the INRAE GBOS team from the GABI department.

Metafounders and accuracy of genomic prediction in tropical dairy herds for milk yield

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In smallholder dairy production systems, the limited use of artificial insemination results in a data structure with limited genetic connectedness between herds and thus separating the genetic and environmental effects is challenging. The lack of efficient performance recording systems results in sparsely recorded data and incomplete pedigree information, leading to less accurate or biased genetic evaluations. For genomic evaluations, it is crucial to assess the impact of missing pedigree links and methods to account for unknown parents, especially on data from smallholder dairy production systems. The aim of this study was to assess the impact of modelling metafounders (MF) instead of unknown parent groups on the accuracy of single-step genomic prediction. Data were from the Ethiopian national dairy cattle recording and extracted from the ILRI database. The data contained 246,291 test-day milk yield records from 33,653 dairy cows in 13,266 herds and the pedigree had 143,000 animals. In total, 6,876 animals were genotyped, and 4,200 cows had genotypes and phenotypes. After cleaning and quality control, 38,344 SNPs were used in the analysis. The MF approach with four MFs was used. The relationships within and between the MFs were modeled by a gamma matrix (Γ). Repeatability test-day animal models were used. Pedigree BLUP (pBLUP), single-step genomic model (ssGBLUP), and ssGBLUP with MFs (ssGBLUP_MF) were used to analyze the data. Models were compared using the Legarra-Reverter (LR) validation approach, with two validation animal cohorts born between 2019-2020 and 2020-2021. The LR regression coefficients varied between 0.86 and 0.89 for pBLUP, 0.98 and 0.99 for ssGBLUP, and 1.00 and 1.01 for ssGBLUP_MF. Prediction accuracy ranged from 0.31 to 0.33 for pBLUP, 0.61 to 0.62 for ssGBLUP and from 0.77 to 0.79 for ssGBLUP_MF for the two groups of validation animals. Compared to other models, ssGBLUP_MF showed the least bias in genetic trends and the least inflation in predictions, suggesting the suitability of the MF approach in handling unknown parents in genomic prediction in tropical dairy herds.

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Poster 15

The youth and animals: psychological benefits from interacting with pets

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The positive aspects of pet ownership are widely known, and their popularity contributed to a lot of research. Previous research shows multiple benefits from the presence of pets in people's lives, and in this paper we examined how much interaction with pets contributes to the psychological well-being of the youth. The intent was to establish in which aspects does interaction with pets contribute the most, as well as whether the type of pets they own influences their emotional well-being in any way. In this research, there were 100 participants, all students at the University of Belgrade. Participants filled out the Pets Questionnaire, which was purposefully designed for this research. The questionnaire has 32 items, which are given in the form of a 5-point Likert scale, and it measures five dimensions: Activity, Emotionality, Empathy towards people, Empathy towards animals, and General attitude towards animals. The results indicate that interaction with pets has multiple benefits for the psychological well-being of the youth. Pets contribute to young people changing their relationship with themselves, others, and especially towards animals. They also contribute to developing an overall positive attitude and empathy towards animals. Results also show that interaction with dogs contributes to activity and empathy towards other animals and people much more than interaction with cats. Dog owners are more active, organized, creative, successful, and responsible than cat owners. They pay much more attention to their attitude towards animals and to other people's attitudes as well; they are much more likely to help an animal in need, and to fight for a more positive attitude about animals in their surroundings. On the other hand, cat owners are more sensitive to animals and their problems in general, that is cat owners have a better attitude towards animals. Obtained results confirm that the presence of pets in the lives of the youth has multiple positive psychological benefits. In a time where people are less social and digital communication is much more prevalent, the importance of pets in strengthening social relationships and spreading positive influence becomes more important than ever before.

Survey on the management of newborn calves in Germany during extreme temperatures

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Impact factors for lifelong health and performance are the milk supply and the ambient conditions that calves are reared in. Extreme weather conditions are increasing due to climate change. Especially in extreme conditions, newborn calves are challenged to adapt their thermoregulation quickly to their surroundings. Various products exist on the market, that are supposed to help the young calves with maintaining their body temperature. A survey (n = 164) was conducted to determine which measures German farmers take to support their calves with adapting to extreme temperatures. Multiple answers per respondent and per question were possible. A preliminary statistical analysis revealed that the first action after birth was often to dry the calf off the amniotic fluid. Farmers reported to let the dam lick the calf (69%) and help drying by rubbing the calf with straw (68%) or using a towel (10%). The majority of farmers (41%) stated to dry the calf until the dryness is comparable to a wrung-out towel. To warm the newborn calf, 32% used a calf blanket on the dry calf, while 7% put the blanket on the calf without previous drying and 26% used an infrared lamp. Only 6% of farms used a heated calf hutch. The majority of farmers were not sure (39%) whether the calves ingest more first colostrum during extreme temperatures, or they observed no difference to normal temperatures (46%). The colostrum intake was claimed by 9% to be higher during cold temperatures, while 7% stated it to be higher during warm temperatures. A different picture emerged for slightly older calves: 44% of farmers suspected a higher milk intake during colder climates and only 7% reported the intake to increase during hot temperatures. As the most frequently used additional measures for warming calves during extreme cold, 78% of farms raised the nesting score, 58% used calf blankets, 36% offered more milk and 35% used group housing. In extreme heat, 65% of farms provided more water, 46% ensured shade, 38% offered cold water and 13% used ventilators to cool their calves. In conclusion, farmers predominantly seem to use traditional measures to support their calves, while the adoption of newer, more technical or costly solutions remain limited.

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