

Enteric Methane Mitigation and Monitoring: Updates Regarding Sensor Technologies, Fatty Acids, and Bromoform

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Introduction

Enteric methane (CH_4) is a major greenhouse gas associated with ruminant livestock, representing both an environmental challenge and an energetic loss to the animal. Recent advances in dairy cattle nutrition have focused on strategies to mitigate CH_4 production while maintaining animal health, productivity, and efficiency. Dietary fatty acids (FA) and halogenated compounds such as bromoform have emerged as promising feed-based interventions. Fatty acids can influence ruminal fermentation by altering microbial populations and hydrogen flow, while bromoform, a bioactive compound found in red seaweeds such as *Asparagopsis* spp., directly inhibits rumen methanogens. Although mechanistically different, both approaches illustrate the potential of feed additives to shift fermentation toward reduced methanogenesis while sustaining or enhancing nutrient utilization and performance.

Equally critical is the accurate quantification of enteric emissions under production conditions. Respiration chambers remain the reference standard for CH_4 measurement, yet practical on-farm technologies are increasingly adopted to evaluate mitigation strategies. The integration of innovative feed additives with robust measurement technologies provides an opportunity to validate dietary interventions and support evidence-based recommendations for the dairy industry. This paper presents results from three complementary studies: (1) a comparison of CH_4 measurement systems in lactating cows, (2) an evaluation of dietary FA supplementation on milk production and gas emissions, and (3) an assessment of the stability and dose-response efficacy of bromoform-based additives.

Evaluation of Methane Measurement Systems in Lactating Dairy Cows

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Accurate and scalable measurement tools are critical for quantifying enteric CH_4 and implementing effective mitigation strategies in dairy production. We aimed to evaluate the accuracy of a head-chamber system and a modified sniffer system for measuring enteric CH_4 emissions in lactating dairy cows, using open-circuit respiration chambers as the reference.

Methodology

Twelve multiparous lactating Holstein cows (202 ± 12 days in milk, 46 ± 6.5 kg of milk/day, and 710 ± 30 kg of body weight) were enrolled in a replicated 3×3 Latin square to compare 3 measurement technologies: (1) respiration chambers (RC; No Pollution Ltd., Leicester, UK), (2) the GreenFeed system (GF; C-Lock Inc., Rapid City, SD), and (3) the Agscent Air GHG $\times$ Optiweigh system (AO; (Agscent Ltd., Carwoola, NSW, Australia). Cows were blocked by parity, days in milk, and baseline CH₄ emission (determined previously with GF), and randomly assigned within blocks to one of the systems during each 3-d period. Animals were transported from the Cornell Dairy Research Center (Harford, NY) to the Cornell Large Animal Research and Teaching Unit (Ithaca, NY), housed in tiestalls, and acclimated to facilities and measurement devices for 2 weeks before data collection. For GF and AO, cows remained in their stalls and were led to the respective units at 3-h intervals across 8 time points (08:00, 11:00, 14:00, 17:00, 20:00, 23:00, 02:00, and 05:00 h). For RC, cows were individually housed in 1 of 4 chambers for 72 h with continuous gas exchange monitoring. All cows received a basal total mixed ration (DM basis: 55% corn silage, 12% haylage, and 33% concentrate) formulated to meet or exceed requirements (AMTS.Cattle.Professional v. 4.14; Agricultural Modeling and Training System LLC, Groton, NY). Feed was offered once daily (09:30 h) with ad libitum access, and an equal amount of pelleted bait (Purina Animal Nutrition, Shoreview, MN) was provided across systems. Cows were milked at 06:00 and 17:30 h using portable milking units, either in tiestalls or inside the RC.

Specifications and operation of the RC units followed Machado et al. (2016) and Keller et al. (2022). Chambers maintained controlled conditions (18°C, 55% relative humidity). Calibration was conducted monthly using certified CH₄ and carbon dioxide (CO₂) standards (Airgas USA, Radnor, PA), with zero calibration via nitrogen. Gas recovery tests using CO₂ were performed regularly to verify accuracy. Total gas production (g/d) was calculated from inlet-outlet concentration differences, corrected for airflow rate, pressure, and temperature. The mean recovery was 99.6% for CO₂ and 99.4% for CH₄. The AO system collected exhaled breath through a vacuum-driven, open-circuit setup and analyzed samples in real time using a tunable diode laser spectroscopy sensor for CH₄ (0 - 40,000 ppm, 0.01 ppm resolution) and a nondispersive infrared sensor for CO₂ (0 - 20,000 ppm, 5 ppm resolution), with integrated temperature, pressure, and humidity probes. Measurements were recorded every second. The GF system used an open-flow design with nondispersive infrared sensors for CH₄ and CO₂, with automatic calibration using certified span and zero gases (Airgas USA). A CO₂ recovery test at the start and end of the trial yielded 99.6% recovery (SD = 2.5).

Ambient temperature and humidity were monitored with HOBO data loggers (model LMX2300; Onset Computer Corp., Bourne, MA). Samples of the total mixed ration were collected 3 times per week for DM determination. Feed ingredients were sampled twice weekly, composited by week, dried at 55 °C for 48 h, ground through a 1-mm screen (Wiley mill; Thomas Scientific, Philadelphia, PA), and stored in sealed bags until analysis. Milk yield was recorded daily. Milk samples were collected every 3 days over 2 consecutive milkings (n = 9) into vials containing 2-bromo-2-nitropropane-1,3-diol (Broad

Spectrum Microtabs II; Advanced Instruments Inc.) and stored at 4 °C. Analyses for fat, true protein, lactose, and milk urea nitrogen were conducted by Dairy One DHIA Laboratory (Ithaca, NY) using Fourier-transform infrared spectroscopy (Milkoscan FT+; Foss Inc.). Data were analyzed using the MIXED procedure of SAS (v9.4; SAS Institute Inc., Cary, NC). The model included measurement method, block, repetition, and their interaction as fixed effects, with cow nested within square as a random effect. Least squares means were separated with Tukey's adjustment. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Preliminary Results

During the experimental period, ambient temperature and relative humidity in the facilities were comparable to those in the RC, averaging $18 \pm 0.5^\circ\text{C}$ and $57 \pm 7.4\%$, respectively. Dry matter intake, milk yield, energy-corrected milk, and 3.5% fat-corrected milk averaged 24.6, 33.3, 42.0, and 35.6 kg/d, respectively, with no differences among measurement systems. Milk fat content was also unaffected (mean = 5.27%). True protein concentration was slightly higher for GF (3.43%) than AO (3.36%) or RC (3.33%; $P < 0.01$), and total solids tended to be greater for GF (14.7 vs. 14.4%; $P = 0.07$), although these differences were not biologically relevant. Yields of milk components, milk urea nitrogen, and feed efficiency were similar across systems. Despite reports that chamber housing can reduce intake, no differences in DM intake were observed, suggesting that the acclimation protocol effectively minimized housing effects.

Daily CH_4 production differed by method ($P < 0.01$), averaging 394 g/d (AO), 403 g/d (GF), and 546 g/d (RC). Respiration chambers measured ~38% higher emissions than spot-sampling approaches. Carbon dioxide showed a similar pattern (8.2, 13.3, and 15.6 kg/d for AO, GF, and RC; $P < 0.01$). Methane yield (g/kg DMI) and intensity (g/kg milk or ECM) were also greatest for RC, reflecting its continuous capture of emissions.

Methane estimates from AO were moderately correlated with RC ($r = 0.57$), whereas GF showed weaker agreement with RC ($r = 0.36$). The two spot-sampling systems were moderately correlated ($r = 0.41$). Concordance with RC was poor for both spot methods ($\text{CCC} \leq 0.09$). Methane production was positively associated with DMI, strongest for RC ($r = 0.77$), intermediate for AO ($r = 0.62$), and weak for GF. Relationships with ECM were moderate for RC and AO but not significant for GF. Continuous chambers provided the most robust intake-emission associations, whereas spot-sampling methods tended to underestimate absolute emissions and attenuate correlations.

Overall, RC yielded higher CH_4 and CO_2 values and stronger relationships with intake than either spot-sampling system, reflecting their complete diurnal coverage and controlled environment. Spot methods (AO and GF) produced comparable but lower emission estimates and weaker associations with productivity, emphasizing the need to account for methodological differences when interpreting or comparing enteric gas measurements.

Effects of Dietary FA on Milk Production and Ruminal Greenhouse Gas Emissions in Lactating Dairy Cows

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Supplementing dairy cow diets with lipids has been explored as a strategy to mitigate enteric CH₄ emissions. Meta-analyses report reduction in CH₄ production (g/day) and intensity (g/kg of milk) when fats are incorporated, although responses vary with lipid type, inclusion level, and diet composition (Beauchemin et al., 2008; Patra, 2013; Arndt et al., 2022). Our objective was to investigate the effects of alternative dietary FA supplementation strategies, substituting either fiber or starch (non-isoenergetic and isoenergetic, respectively) on milk production and composition, nutrient digestibility, and enteric gas emissions in lactating Holstein cows.

Methodology

Forty-eight multiparous Holstein cows (94.4 ± 20.7 days in milk; 46.3 ± 6.5 kg milk/day) were housed at the Cornell Dairy Research Center (Harford, NY). Following a 3-week acclimation period and GreenFeed™ training, cows were allocated to 1 of 2 main plots (24 cows per plot): non-isoenergetic (NONISO) and isoenergetic (ISO). Within each plot, cows received 1 of 4 treatments in a replicated 4×4 Latin square with 21-day periods: (1) no FA supplementation (CON), (2) palmitic acid (PA; 85% C16:0; PeliPalm85; Pelican Solutions, Inc. Novi, MI), (3) calcium-salts of FA (CSFA; 59% C16:0, 25% oleic acid [C18:1], and 3% eicosapentaenoic acid plus docosahexaenoic acid; Virtus Nutrition, Corcoran, CA), or (4) whole cottonseed plus canola oil (OIL). Diets supplied ~2% supplemental FA (DM basis). The feeding level for canola oil was targeted at 0.5% of ration DM with the remainder of FA derived from whole cottonseed. Diets were primarily composed of corn silage, almond hulls, cornmeal, alfalfa hay, and a vitamin-mineral premix. Diets were formulated using AMTS.Farm.Cattle (Pro). Total mixed rations were offered once daily at 0700 h and cows were milked 3 times daily at 0600, 1400, and 2200 h. Feed refusals were maintained between 5 and 10% of total feed offered.

Individual feed, total mixed ration, and orts samples were collected for DM analysis and composited every period for chemical analyses. Oven-dried samples were analyzed according to AOAC (2000) methods for DM (934.01), crude protein (990.03), ether extract (2003.05), and ash (942.05). Starch content was determined using the method described by Hall (2009). Fiber fractions were analyzed for acid detergent fiber (973.18) and amylase-treated neutral detergent fiber (aNDFom; Van Soest et al., 1991). Undigestible neutral detergent fiber was used as an internal marker to determine total apparent tract digestibility and was determined after 240 h of in-vitro fermentation following Goering and Van Soest (1970). All analyses were conducted by Cumberland Valley Analytical Services Inc. (Waynesboro, PA). Milk samples were collected during the final 3 days of each experimental period (9 milkings per period). Samples with preservative were analyzed for concentrations of fat, true protein, and lactose using Fourier transform infrared spectroscopy. Fecal samples were obtained either via rectal collection or voluntary

defecation every 9 h over the final 3 days of each experimental period. Methane, CO₂, and hydrogen (H₂) emissions were measured over 3 consecutive days at the end of each experimental period using 3 GreenFeed units. A total of 3 spot measurements per animal per period were obtained at 0230, 1030, and 1830 h. A custom pelleted bait feed was offered within the GreenFeed units. A CO₂ recovery test was conducted at the beginning of each experimental period (101% CO₂ recovery, SD = 3.85, n = 4). Statistical analyses were carried out using the MIXED procedure of SAS. The mixed-effects model included treatment, plot, period, square, and treatment × plot interaction as fixed effects, and cow nested within square as a random effect. Least squares means were separated using Tukey's adjustment.

Summary of Results

In brief, DMI, milk yield, and energy-corrected milk yield were modified by treatment ($P < 0.01$). In the NONISO plot, cows fed OIL had greater milk fat concentration (4.08%), relative to CON (3.60%; $P < 0.01$). In the ISO plot, cows fed PA had higher milk fat concentration (4.49%), relative to CON (3.85%; $P < 0.05$). Milk protein concentration in the ISO plot was lower across all FA treatments (~3.33%; $P < 0.01$), relative to CON (3.50%). Milk fat yield in the NONISO plot was highest in cows fed OIL (1.82 kg/day), compared to other treatments (~1.62 kg/day; $P = 0.02$). In the ISO plot, PA increased milk fat yield (1.87 kg/day), relative to CON (1.63 kg/day; $P < 0.02$). Milk protein yield was not affected by treatment in the NONISO plot but was reduced by PA and OIL in the ISO plot (1.37 and 1.39 kg/day, respectively; $P = 0.04$), relative to CON (1.46 kg/day).

Yields of milk C16:0 and mixed FA were greatest in cows fed PA, relative to CON ($P < 0.01$; both plots). Yields of milk C16:0 and mixed FA were greatest in cows fed PA, relative to CON ($P < 0.01$; both plots). Specifically, yield of C16:0 was greatest in ISO plot when cows were fed PA, (629 g/day; treatment × plot; $P = 0.01$), relative to CON (497 g/day). Mixed FA yield in the ISO plot was greatest when cows were fed PA (644 g/day; treatment × plot; $P = 0.02$), relative to CON (515 g/day). Yields of milk C18:0 and performed FA were greatest in cows fed OIL, relative to all other treatments ($P < 0.01$; both plots). Specifically, concentration of C18:0 was greatest in NONISO and ISO plot in cows fed OIL (9.34% and 9.22%, treatment × plot; $P = 0.04$), relative to CON (~5.62%). Similarly, Yields of C18:0 was greatest in NONISO and ISO plots were greatest in cows fed OIL (172 g/day and 168 g/day; treatment × plot; $P = 0.01$), relative to CON (~90.5 g/day). Performed FA concentration was greatest in cows fed CSFA and OIL in both NONISO and ISO plots, relative to CON. In the NONISO plot, cows fed OIL (614 g/day) had greater preformed FA yield, relative to CON (435 g/day, treatment × plot; $P = 0.04$). In the ISO plot, cows fed CSFA and OIL (523 and 584 g/day, treatment × plot; $P = 0.04$) had the greatest preformed FA yields, relative to CON (439 g/day). In NONISO and ISO plots, *de novo* FA concentrations were numerically reduced across all FA treatments; however, no significant differences were observed in *de novo* FA concentration and yield across all FA treatments. In the NONISO plot, cows fed CSFA had greater yields of C18:2 *trans*-10, *cis*-12 (0.48 g/day), relative to CON (0.37 g/day; treatment × plot; $P = 0.02$). In the ISO plot, cows fed CSFA had greater yields of C18:2 *trans*-10, *cis*-12 (0.61 g/day), relative to CON (0.38 g/day; treatment × plot; $P = 0.02$).

In the NONISO plot, the apparent total-tract digestibility of DM, organic matter, and fiber (aNDFom) followed a pattern. In the NONISO plot, cows fed PA had greater digestibility of DM (71.3 vs. 66.7%; treatment $\times$ plot, $P < 0.01$), organic matter (72.8 vs. 68.8%; treatment $\times$ plot, $P < 0.01$), and aNDFom (50.2 vs. 41.5%; treatment $\times$ plot, $P < 0.01$), relative to CON. In contrast, in the NONISO plot, cows fed OIL had lower digestibility of dry matter (63.0 vs. 66.7%; treatment $\times$ plot, $P < 0.01$), organic matter (64.9 vs. 68.8%; treatment $\times$ plot, $P < 0.01$), and aNDFom (33.4 vs. 41.5%; treatment $\times$ plot, $P < 0.01$), relative to CON.

Methane emissions were notably low across treatments (328 to 385 g/d). In the NONISO diets, CH₄ was not affected by lipid source. When starch was replaced (ISO diets), all fat supplements increased CH₄ (~381 vs. 345 g/d for control; $P = 0.05$). Hydrogen emissions were greater with PA than with other treatments in the NONISO plot (1.43 vs. ~1.23 g/d; $P = 0.03$), and all fat sources increased H₂ production in the ISO plot (~1.75 vs. 1.41 g/d for CON; $P = 0.03$).

In summary, this study generated several key insights. Methane emissions were consistently low (328 to 385 g/d) in cows fed low-forage, high-concentrate diets, regardless of lipid supplementation, despite their high DM intake. For comparison, high-producing cows offered rations containing more than 50% forage typically emit 450 to 600 g of CH₄/day, highlighting the inherently low methane yield (g/kg of DM intake) observed across all animals in this trial. A moderate depression in milk fat was evident in some treatments but was alleviated when PA replaced starch, highlighting the role of dietary FA profile in maintaining milk fat under high-concentrate feeding. The results also indicate that low CH₄ emissions may occur at the expense of milk fat synthesis. Substituting starch with PA, calcium-salts of long-chain FA, or oil increased CH₄ production, whereas replacing fiber with lipids did not. Overall, these findings suggest that, in high-intake, low-forage systems, reducing dietary starch may be more effective than lipid supplementation for reducing CH₄ emissions. Furthermore, when baseline emissions are already low, the inclusion of FA alone is unlikely to deliver additional mitigation.

Stability and Dose-Response Evaluation of Novel Bromoform-Based Methane Mitigation Products in Lactating Dairy Cows

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Reducing enteric CH₄ from ruminants is a critical priority for improving the environmental sustainability of dairy production. Bromoform-containing feed additives, particularly those derived from *A. taxiformis*, have shown substantial potential to suppress ruminal methanogenesis. However, their adoption requires evidence that these products remain stable during storage and can be fed safely and effectively to lactating cows. To address these needs, we evaluated 3 bromoform-based CH₄-mitigation products, a synthetic bromoform preparation, an *A. taxiformis* pellet, and an *A. taxiformis* oil, in two

studies: a 16-week shelf-life trial assessing how storage temperature and light exposure influence bromoform retention, and a pilot dose-response trial assessing efficacy, safety, and effects on intake, milk yield and composition, and residue transfer in Holstein cows.

Methodology

For the shelf-life stability study, solid formulations (synthetic bromoform and *A. taxiformis* pellet) were packaged in stand-up barrier pouches made of either clear polyethylene/nylon laminate or metallized polyethylene (3.1 × 5.1 × 2 in.; Uline, Pleasant Prairie, WI, USA). The oil formulation (*A. taxiformis* in canola oil) was dispensed into 4-mL clear or amber screw-top glass vials (15 × 45 mm; Sigma-Aldrich, St. Louis, MO, USA). Three replicate samples of each product were prepared for every storage condition and sampling time. Packages were stored for 16 weeks under combinations of temperature (-20, 4, 25, or 37°C) and light (dark at all temperatures, with continuous fluorescent illumination at 25 and 37°C). Bromoform concentration (mg/kg DM) was analyzed at 0, 2, 4, 8, and 16 weeks by gas chromatography at Bigelow Laboratory for Ocean Sciences Analytical Services, and retention was expressed as a percentage of the initial concentration.

For the pilot dose-response study, 50 multiparous Holstein cows (121 ± 27 days in milk; 2.6 ± 0.3 parity) were housed in a free-stall barn equipped with Calan gates to allow individual feed intake measurements. Cows were fed once daily a total mixed ration (52:48 forage-to-concentrate ratio, DM basis) and milked 3 times per day (05:00, 13:00, and 21:00 h). Before the experiment, cows completed a 17-d training period, consisting of 10 d of adaptation to Calan gates and 7 d to GreenFeed units for gas-flux measurement. Animals underwent a 17-d pre-trial period consisting of 10 d of adaptation to Calan gates and 7 d of habituation to GreenFeed units for gas-flux measurement. The experimental period lasted 4 weeks and followed a randomized complete block design. Treatments consisted of 4 bromoform inclusion rates (0, 15, 22.5, and 30 mg/kg of DM intake) tested separately within each of 3 formulations: synthetic bromoform (SYN), *A. taxiformis* pellet (PAT), and *A. taxiformis* in canola oil (OIL). Supplements were top-dressed onto the total mixed ration once daily.

Dry matter intake and milk yield were recorded daily. Weekly composite milk samples were submitted to the Barbano Laboratory at Cornell University for analysis of fat, true protein, lactose, milk urea nitrogen, and FA composition using mid-infrared spectroscopic methods. Iodine content was analyzed by Michigan State University, and bromoform residues by Bigelow Laboratory for Ocean Sciences Analytical Services. Enteric emissions of CH₄, CO₂, and H₂ were measured daily with 3 GreenFeed units positioned across the pen to allow individual access except during milkings. A pelleted bait feed was dispensed automatically. Each cow was allowed a maximum of 2 visits per 24 h period to each GreenFeed unit, with no more than 6 drops (~30 g pellets/drop) per visit. On average, cows completed 3.65 ± 0.29 visits per day, with mean visit duration of 4.21 min. Body weight and body condition score were assessed at the beginning and end of the study.

Data were analyzed using the MIXED procedure of SAS. The model included treatment, week, and their interaction as fixed effects, with cow as a random effect. Repeated measurements across weeks were modeled using an appropriate covariance structure selected by the lowest AIC. Least squares means were estimated for each treatment and compared using Tukey's adjustment. Significance was declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

Preliminary Results

Bromoform stability was significantly affected by storage conditions. After 16 weeks, all formulations retained $\geq 95\%$ of their initial concentration at -20°C and $>90\%$ at 4°C . Degradation became apparent at 25°C , particularly with light exposure, where retention ranged from $\sim 45 - 55\%$ for the synthetic product to $75 - 80\%$ for the oil formulation. At 37°C , degradation was highest, and the combination of heat and light reduced bromoform to $<10\%$ of its original content in the synthetic product and to $\sim 30 - 40\%$ in the oil. Overall, cold, dark storage preserved $>90\%$ of bromoform across formulations, with the oil matrix consistently providing the greatest protection under suboptimal conditions.

Bromoform supplementation decreased feed intake at the highest inclusion level. At 30 mg/kg of DM intake, we observed a decrease of 18% for PAT and 13% for OIL compared with control, whereas moderate doses (15 - 22.5 mg/kg) caused only minor, nonsignificant reductions (2 - 9%). Across all synthetic bromoform treatments, DMI was unaffected. Milk yield showed no significant treatment effect ($P = 0.08$), although numerical differences of ± 4 kg/day were observed. Energy-corrected milk (ECM) and 4% fat-corrected milk (FCM) showed significant treatment effects ($P < 0.01$). The pellet *A. taxiformis* reduced ECM and FCM at both 15 and 30 mg/kg ($\sim 15\%$ below control), whereas SYN at 22.5 mg/kg maintained or slightly exceeded control levels, and OIL produced intermediate responses. Body weight (~ 725 kg) and body condition (~ 3.3) were unaffected ($P > 0.40$), indicating no short-term negative energy balance.

Milk composition was largely unaffected by treatment. Fat percentage remained unchanged (4.39 - 4.82%; $P = 0.17$), while true protein content showed a modest treatment effect ($P = 0.02$), with PAT at 22.5 mg/kg of DM intake being slightly lower than the control. Fat and protein yields declined only in treatments where intake was reduced (PAT at 15 and 30 mg/kg of DM intake). Milk iodine concentrations increased by $>35\%$ in all PAT treatments and in OIL at 30 mg/kg ($P < 0.01$). Bromoform residues were dose- and time-dependent but transient. All supplemented cows had detectable residues, averaging 48 ng/mL, whereas controls remained near assay limits (~ 9 ng/mL). Residues peaked at week 3 (33 cows with detectable concentrations), declined to 16 detections by week 4, and were below assay limits by weeks 5 and 6 (post experimental period), indicating clearance within two weeks after dosing ceased.

Analysis of milk FA showed few treatment effects on concentration (g/100 g milk), but several differences emerged for yields (g/d). De novo FA concentration (g/100 g milk) was unaffected ($P = 0.17$) but yield differed ($P = 0.04$), cows receiving SYN at 22.5 mg/kg

of DM intake produced the highest de novo yield (610 g/d), whereas PAT at 15 mg/kg was lowest (493 g/d). Mixed FA concentration showed a tendency to decline with higher bromoform doses ($P = 0.08$), and yields were reduced in PAT_15 compared with control (668 vs. 818 g/d; $P = 0.02$). Preformed FA concentrations and yields remained unchanged ($P \geq 0.17$). Among individual FA, palmitic (16:0) yield was affected ($P = 0.02$), cows receiving PAT at 15 mg/kg had the lowest output (627 g/d), whereas SYN at 22.5 mg/kg and control cows produced the highest (742 and 770 g/d, respectively). Concentrations and yields of stearic (18:0) and oleic acid (18:1 cis-9) were not different among treatments ($P \geq 0.18$). Neither average chain length nor level of unsaturation differed by treatment ($P \geq 0.25$). Weekly effects were evident for most response variables ($P < 0.01$), but no treatment $\times$ week interactions were detected.

All 3 formulations reduced methane emissions relative to control. The greatest absolute reduction occurred with OIL at 30 mg/kg of DM intake (~50%), followed by PAT at 22.5 and 30 mg/kg (~27-40%) and OIL at 22.5 mg/kg (~27%). Synthetic bromoform at 30 mg/kg of DM intake reduced CH₄ by 24%. Across formulations, the 22.5 mg/kg dose achieved a consistent ~24% reduction in CH₄ yield and intensity without compromising milk production. Carbon dioxide production remained unchanged (~15 - 16 kg/day), while H₂ increased, indicating inhibition of methanogenesis without affecting basal respiration or milk performance. Although high between-cow variability limited statistical significance in this short-term trial, the data suggest that moderate doses of bromoform products can substantially suppress methane while maintaining intake and production.

Summary

Comparisons between respiration chambers with spot-sampling systems (GreenFeed and AgScent Air $\times$ Optiweigh) demonstrated that chambers provide the most complete and precise assessment of enteric CH₄, whereas spot-sampling methods yield lower but consistent estimates suitable for large-scale monitoring when their inherent limitations are considered. Research on dietary FA showed that altering lipid source or substituting starch and fiber influenced milk fat synthesis and nutrient digestibility but produced little additional reduction in CH₄ where baseline emissions were already low, suggesting that starch management may be a more effective strategy in high-intake, low-forage diets. Research on bromoform-based additives integrated storage stability and feeding trials to support practical application. Bromoform remained stable under cool, dark conditions, with oil formulations offering the greatest protection under less favorable environments. A short-term feeding trial indicated that moderate inclusion of synthetic bromoform, seaweed pellets, or seaweed oil reduced CH₄ emissions without compromising intake, milk yield, or composition, and residues in milk were transient, clearing rapidly after supplementation ended. Overall, these findings highlight the importance of accurate emission measurement, targeted dietary strategies, and feed additives as complementary tools to reduce gas emissions while sustaining performance and milk quality in modern dairy systems.

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