Translational Animal Science, 2025, **9**, txaf028
https://doi.org/10.1093/tas/txaf028
Advance access publication 1 March 2025
Environmental Animal Science

The effect of Rumin8 Investigational Veterinary Product—a bromoform based feed additive—on enteric methane emissions, animal production parameters, and the rumen environment in feedlot cattle

Leanna Kelly,[†] Eleanor May Pressman,[†] John-Fredy Ramirez-Agudelo,[†] Hannah Chernavsky,[†] Pablo Alvarez-Hess,[‡] Silke Jacques,[‡] Matthias Hess,[†] and Ermias Kebreab[†,1]

[†]Department of Animal Science, University of California, Davis, CA 95616, USA
[‡]Rumin8 Pty, Ltd, West Perth, WA 6005, Australia

[1]Corresponding author: ekebreab@ucdavis.edu

ABSTRACT

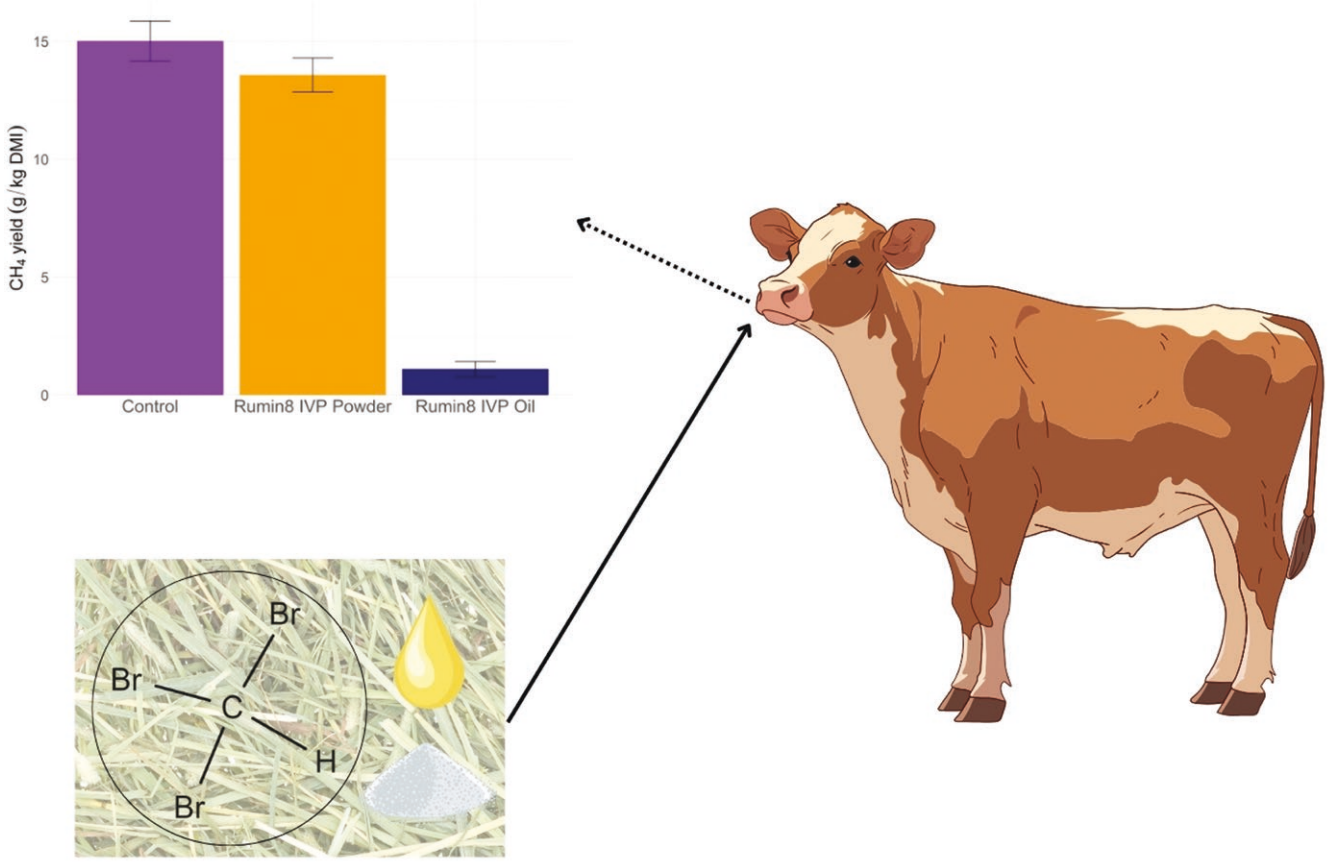
The livestock sector plays a crucial role in mitigating global climate change by reducing greenhouse gas emissions, with enteric fermentation as the largest source. Although various approaches have been proposed to decrease enteric methane (CH_4) emissions, feed additives containing bromoform ($CHBr_3$) have shown promise with minimal impact on animal production parameters. This study aimed to evaluate the effects of two Rumin8 Investigational Veterinary Products (IVP) containing synthetic $CHBr_3$ on enteric gas emissions, animal production parameters, and the rumen environment. Twenty-four Angus beef steers were randomly assigned to one of three treatment groups: Control, Oil (8 mL Rumin8 oil IVP/kg DMI), and Powder (1.2 g Rumin8 powder IVP/kg DMI). The Rumin8 oil IVP treatment resulted in a $CHBr_3$ intake of 32.2 mg/kg DMI, while the Rumin8 powder IVP provided a $CHBr_3$ intake of 2.0 mg/kg DMI during weeks 1–8. In week 9, a new batch of Rumin8 powder IVP increased the $CHBr_3$ intake to 17.9 mg/kg DMI. The Oil group exhibited 95.0%, 95.0%, and 96.1% reductions in CH_4 production (g/day), yield (g/kg DMI), and intensity (g/kg average daily gain), respectively, accompanied by 925%, 934%, and 858% increases in H_2 production, yield, and intensity, respectively. Neither treatment significantly affected animal production parameters or rumen environment variables. These findings suggest that Rumin8 oil IVP containing synthetic $CHBr_3$ has the potential to reduce enteric CH_4 emissions. This warrants further investigation, as this is the first published in vivo study to assess compound efficacy.

Lay Summary

This study investigates the potential of using synthetic bromoform as a feed additive to reduce methane emissions in cattle, a key contributor to greenhouse gas emissions from the livestock sector. The research evaluated two Rumin8 products—an oil-based and a powder-based formulation—on their ability to reduce methane emissions, as well as their effects on animal production and the rumen environment. Twenty-four Angus beef steers were divided into three groups: a control group, a group receiving Rumin8 oil, and a group receiving Rumin8 powder. The Rumin8 oil treatment led to a significant reduction in methane emissions, with a ≈95.0% decrease in methane production, yield, and intensity, without any negative effects on the animals' growth or their digestive health. The powdered version of Rumin8 did not alter methane emissions, possibly due to a low concentration of the active ingredient and warrants further research. These findings demonstrate the potential of Rumin8 oil as a promising solution to significantly reduce methane emissions in cattle, offering a tool to mitigate the environmental impact of livestock production.

Received October 19, 2024 Accepted February 27, 2025.

Graphical Abstract



Key words: bromoform, cattle, enteric methane, Rumin8

INTRODUCTION

Livestock systems are responsible for approximately 12% of all global anthropogenic greenhouse gas (GHG) emissions, contributing about 6.2 gigatonnes of carbon dioxide (CO_2) equivalents annually (FAO, 2023). The largest share of these emissions arises from enteric fermentation from ruminants, which produces methane (CH_4), a GHG with a global warming potential 27 times greater than that of CO_2 over a 100-yr period (IPCC, 2023). Therefore, reducing enteric CH_4 emissions is crucial to mitigate the environmental impact of livestock systems and achieve national and international climate goals.

While a shift towards plant-based diets is often suggested as a strategy to reduce emissions from ruminant production, ruminants contribute to global food systems by supporting nutrition, sustainability, food security, and gender equity (Adesogan et al., 2020). Animal source foods contribute high-quality proteins and essential nutrients with high bioavailability, complementing plant-based sources to help meet nutritional needs, particularly in populations at risk of undernutrition (Leroy and Barnard, 2020). This is especially relevant in low- and middle-income countries, where increasing the consumption of animal food is a key strategy for addressing undernutrition (Beal et al., 2023). Additionally, the global demand for meat and milk is projected to rise by 73% and 58%, respectively, by 2,050, compared to the 2,010 levels (FAO, 2011). Ruminants also support ecosystem services including nutrient cycling, biodiversity, and, in some cases, carbon sequestration, when managed appropriately (Teague et al., 2016). Additionally, reducing enteric CH_4 is desirable because CH_4 production represents a loss of dietary energy that could otherwise be used by animals (Morgavi et al., 2023).

Enteric CH_4 is produced in the rumen because of the breakdown of dietary carbohydrates. Carbohydrates are broken down into glucose and other monomers, which are further metabolized into volatile fatty acids (VFAs) such as propionate, acetate, and butyrate. These VFAs serve as the principal energy source for ruminants, supporting various physiological functions including maintenance, growth, and production. VFA production also generates CO_2 and H_2, which are used as substrates for CH_4 by methanogenic archaea (Hungate, 1966).

Several strategies have been proposed to decrease enteric CH_4 emissions, including increasing feeding levels, decreasing grass maturity, lowering dietary forage-to-concentrate ratios, using CH_4 inhibitors, incorporating tanniferous forages, providing electron sinks, and adding oils and fats (Arndt et al., 2022). Among these strategies, CH_4 inhibitors have shown marked promise. For example, seaweed (*Asparagopsis taxiformis, AT*) reduces enteric CH_4 emissions by up to 80% in beef steers (Roque et al., 2021). The CH_4 inhibiting activity of seaweed is primarily attributed to the presence of the naturally occurring bromoform ($CHBr_3$) (Machado et al., 2016a). Bromoform, a halogenated CH_4 analog (HMA), inhibits CH_4 production by interfering with coenzyme M methyltransferase and methyl coenzyme M reductase, which catalyze methyl transfer and methyl group reduction, respectively, in the final steps of methanogenesis (Glasson et al., 2022).

Although seaweed-based CH_4 mitigation shows great promise, there are certain challenges that need to be addressed for its broader application. Currently, the seaweed used in experimental studies is often manually collected from the wild, which can be costly, and presents considerations for sustainable production and commercial scalability (Black et al., 2021). Moreover, naturally harvested seaweeds can show variability in growth rates and bromoform ($CHBr_3$) concentrations, even within the same species (Mata et al., 2017). Aquaculture presents a promising solution for sustainably producing seaweed on a larger scale, although it also comes with its own set of considerations, such as managing potential environmental impacts and ensuring efficient production methods (Vijn et al., 2020). However, as interest and innovation in this area continue to grow, so does the potential to scale up seaweed production to make it a viable and environmentally friendly feed additive (Nilsson and Martin, 2022).

It is hypothesized that synthetically manufactured $CHBr_3$ could offer the same CH_4 mitigation benefits as naturally occurring $CHBr_3$ extracted from seaweeds. While no in vivo studies have directly tested this hypothesis, research on other synthetic HMAs, such as bromochloromethane (BCM), has shown promising results (Denman et al., 2007; Abecia et al., 2012). Bromochloromethane has been demonstrated to reduce CH_4 production (g/day) across various ruminant species without affecting animal production parameters (Mitsumori et al., 2012; Abecia et al., 2013). In steers fed a grain-based diet, BCM reduced CH_4 production by 50% to 60% without impacting performance (Tomkins et al., 2009).

The objective of this study was to evaluate the effectiveness of two Investigational Veterinary Products (IVP) containing synthetic $CHBr_3$ for reducing enteric CH_4 emissions in vivo. As this study is the first in vivo evaluation of Rumin8 IVP, both the powder and oil formulations were tested to compare their efficacy in delivering synthetic $CHBr_3$ and mitigating enteric CH_4 emissions, given their distinct applications in cattle feeding systems. Specifically, this study aimed to (1) quantify enteric emissions of CH_4, H_2, and CO_2 in terms of daily production (g/day), yield (g/kg DMI), and intensity (g/kg average daily gain, ADG); (2) assess key animal performance metrics, including DMI, ADG, and feed conversion efficiency (FCE); and (3) examine changes in the rumen environment, focusing on pH and VFA concentrations, resulting from the inclusion of synthetic $CHBr_3$ in the total mixed ration (TMR) delivered via two different carriers (powder and oil).

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis (Protocol No. 23094).

Study Design, Animals, and Diets

Twenty-four Angus beef steers, approximately 8 mo old and weighing 408 ± 18.0 kg at the start of the study, were obtained from the same ranch via the Western Video Market (Cottonwood, CA, USA). The steers were blocked by weight and randomly allocated to one of the three treatment groups: Control (n = 8), Powder (n = 6), and Oil (n = 8). The unequal distribution of steers across treatment groups was due to the removal of two steers from the Powder group: one due to subchronic ruminal acidosis and another due to severe chronic pneumonia. Data from these two steers were excluded from the statistical analysis.

The oil and powder IVPs were provided by Rumin8 Pty Ltd. (West Perth, WA, Australia), with each of the proprietary formulations containing different concentrations of $CHBr_3$. Both the Oil and Powder groups were intended to receive the same dose of $CHBr_3$ throughout the study; however, the concentration of $CHBr_3$ was lower that the oil. The Powder group received 2.0 mg $CHBr_3$/kg dry matter intake (DMI) from weeks 1–8, when the first batch of powder was depleted. A new batch of powder was then introduced, which had a higher concentration of $CHBr_3$, resulting in an increased dose of 17.9 mg CHBr3/kg DMI from weeks 9–12. To account for this change, weeks 1–8 were designated as period 1 (P1), and weeks 9–12 as period 2 (P2). Rumin8 powder IVP was incorporated into the TMR at 1.2 g/kg as fed in both periods. The Oil group received a consistent 32.2 mg $CHBr_3$/kg DMI throughout the 12-wk experiment (P1 and P2), with the Rumin8 oil IVP included in the TMR at 8 mL/kg. Both Rumin8 IVPs were thoroughly mixed into the TMR by hand for each treatment animal to ensure a uniform distribution. Given that the oil made up less than 0.80% of the overall diet, it is unlikely to have influenced methane reduction. Grainger and Beauchemin (2011) found that at practical feeding levels below 8% fat, a 10 g/kg increase in dietary fat reduces methane yield by only 1 g/kg DM intake. Additionally, initial in vitro tests of the Rumin8 oil IVP, conducted without $CHBr_3$, showed no effect on methane production.

Each steer was randomly assigned to an individual Roughage Intake Control (RIC, Hokofarm Group, Netherlands) feeding trough and was fed twice per day at 0,700 and 1,900 hours at 110% of the previous day's intake. Feed residuals were recorded daily before morning feeding to determine the previous day's intake. The steers were provided with a low-forage TMR typical of feedlot diets (Table 1) with water available ad libitum. The cattle were housed in three adjacent pens, each containing 8 animals. The pens had concrete flooring bedded with rice hulls, which were refreshed weekly. On days when enteric gas sampling occurred, alfalfa pellets offered through the GreenFeed device (C-Lock, Inc., Rapid City, SD, USA) were included as part of the daily feed intake. Diet and alfalfa pellet samples were collected at the end of each 2-wk increment and analyzed for various nutritional components (Table 1, Cumberland Valley Analytical Services, Waynesboro, PA).

The experiment followed a Randomized Complete Block Design. The first 2 wk served as a covariate phase to establish baseline measurements, followed by 12 wk of data collection. The 12-wk period was divided into six 2-wk increments, each with a 3-d enteric gas sampling session.

Gas Emission Measurements

Gas emissions (CH_4, CO_2, and H_2) were measured over three consecutive days at the end of the covariate phase and weeks 2, 4, 6, 8, and 12. Gas emissions from the covariate phase were used as the baseline. No measurements were taken in week 10 due to animal health concerns unrelated to treatment. Gas emissions were collected during 180-min sessions as follows: starting at 0,800, 1,700, and 2,300 (sampling day 1), 0,500, 1,400, and 2,000 (sampling day 2), and 0,200 and 1,100 (sampling day 3). Additional sessions were conducted to replace those that were canceled due to unforeseen circumstances, such as animal behavior, weather, or equipment malfunction, to ensure the accuracy of the data collection.

Table 1. Ingredients and nutrient composition of the experimental diet and alfalfa pellets

	TMR	Alfalfa pellets
Ingredients (% of DM)		
Forage		
Alfalfa hay	0.000	100.
Dried distiller's grain	28.8	0.000
Wheat hay	10.9	0.000
Concentrate		
Molasses	2.26	0.000
Rolled corn	56.2	0.000
Beef trace salt[1]	0.04	0.000
Calcium carbonate	1.53	0.000
Magnesium oxide	0.03	0.000
Salt	0.32	0.000
Nutrient composition[2] (% DM, unless noted)		
Organic matter	83.3	90.9
Crude protein	16.5	15.9
ADF (% NDF)	43.8	87.6
NDF	21.6	44.2
Lignin (% NDF)	12.4	18.7
Crude fat	4.63	2.01
TDN	78.6	51.8
Ash	4.91	13.9
Calcium	0.530	1.81
Phosphorus	0.430	0.220
Magnesium	0.250	0.330
Potassium	0.830	1.88
Sodium	0.140	0.210
Iron (PPM)	88.8	1,660
Manganese (PPM)	37.6	58.3
Zinc (PPM)	71.2	20.8
Copper (PPM)	8.40	8.75

ADF, acid detergent fiber; DM, dry matter; NDF, neutral detergent fiber; TDN, total digestible nutrients; TMR, total mixed ration.
[1]Beef trace salt sourced from A.L. Gilbert (Oakdale, California) contains: salt, manganous oxide, vegetable oil, zinc oxide, copper sulfate, ethylene, diamine dihydriodide, sodium selenite.
[2]Average over all samples taken throughout the course of experiment.

During sampling, steers were individually moved to a designated pen containing one GreenFeed system, where they voluntarily entered the unit to consume bait feed. Each steer had an individual sampling session lasting 3 to 5 min, followed by a 2-min background gas collection period. All steers were sampled within a total window of 180 min. The GreenFeed unit was calibrated the evening before each sampling day with a standard gas mixture containing (mol %) 5,000 ppm CO_2, 500 ppm CH_4, 10 ppm H_2, 21% O_2 and nitrogen as a balance (Air Liquide America Specialty Gases, Rancho Cucamonga, CA). The recovery rates of CO_2, CH_4, and H_2 were within ±3% of the known gas quantities. Alfalfa pellets were offered at each sampling interval as bait feed and kept below 10% of the total DMI during the measurement interval.

The gas emissions for each steer were calculated as the average of the emissions recorded during each session throughout the sampling interval. Data were filtered to include only steers who visited GreenFeed for at least four sessions during an interval. Nine datapoints from seven steers were excluded from certain intervals due to insufficient visits to GreenFeed.

Body Weight and ADaily Gain Measurements

Body weight was measured at the beginning and end of the covariate phase and at weeks 2, 4, 6, 8, and 12, before the emissions sampling interval, using a hydraulic squeeze chute with a scale (Silencer Ranch Model, Dubas Equipment Stapleton, NE, USA). Average daily gain (ADG) was calculated as the change in weight between consecutive weeks divided by the number of days. The final measurement from the covariate phase (end of covariate) served as the baseline for calculating week 2 ADG. One negative value in week 2 was removed from the Powder group to maintain consistency in calculating the emissions per ADG.

Rumen Fluid Sampling and Analysis

Rumen fluid samples were collected from four animals per treatment during the covariate phase and at weeks 4, 8, and 13 (Powder and Oil group steers remained on the feed additive through week 13 rumen fluid collection). Samples were obtained approximately 90 min after morning feeding using a stainless steel rumen probe inserted to a pre-measured depth. The rumen fluid samples were stored at –20 °C until analysis.

Samples in 50 mL conical tubes were thawed, and 2 mL of each sample was centrifuged at 13,000 RPM for 10 min at 40 °C. After centrifugation, 20 µL of 85% metaphosphoric acid was added to 1 mL of the supernatant, followed by overnight incubation at 40 °C. The samples were centrifuged again at 13,000 RPM for 10 min at 40 °C and filtered through a 0.45-µm filter. Subsequently, 1 µL of the filtered sample was injected into a 30 m × 0.25-mm FFAP capillary column with a 0.25-µm film thickness, fitted to a 7820A GC system (Agilent Technologies, Santa Clara, CA) equipped with flame ionization detection. Helium was used as a carrier gas at a constant pressure of 57 kPa. Sample injection was carried out in split mode (10:1) with an injection volume of 1 µL and an injector temperature of 250 °C. The initial oven temperature was set to 60 °C for 2 min and then increased to 180 °C in steps of 10 °C/min. Standard curves for VFAs were prepared using eight different standard solutions containing a mixture of six VFAs ranging from 2.5 mM to 150 mM. Each sample was measured in duplicate, and VFA concentrations were calculated using a calibration curve.

After the feeding trial was completed, all steers in the control group were slaughtered at a USDA-inspected commercial packing plant, and all Powder and Oil group steers did not enter the food chain.

Statistical Analysis

Twenty gas emissions, fermentation profile and animal performance variables were analyzed to assess the effects of the treatments. All data analyses were performed in R (R Core Team, 2021), with Linear Mixed-Effects models fitted using the nlme (Pinheiro et al., 2017) and lme4 (Bates et al., 2015) packages. Six distinct model configurations were tested to account for potential sources of variation in the data, including covariate effects, heteroscedasticity, and autocorrelation. These included: (1) a basic random-effects model, (2) a covariate-adjusted model, (3–4) heteroscedasticity-adjusted models (varPower, varIdent), and (5–6) autocorrelation-adjusted models (corCompSymm, corAR1). The primary

objective was to identify the best-fitting model for each variable based on normality of residuals and the lowest Akaike Information Criterion (AIC). The configurations of these models are detailed below.

The differences in $CHBr_3$ concentration between the two formulations utilized in the Powder group introduced a confounding factor, which was addressed by comparing the effects of treatments across two periods (P1 and P2). To mitigate multicollinearity while capturing the significant effects of the $CHBr_3$ concentration change, the period was prioritized as a fixed effect over the nested effect of 2-wk within each period. Consequently, all models included the interaction between treatment and period as a fixed effect, with a focus on this confounding factor. Each model also incorporates random effects, weights, correlation structures, and control parameters.

The "Basic" model considered steer as random effects without weights or correlation structures. The "Covariate" model extended this by including an additional covariate in the fixed effects. To address heteroscedasticity, the "varPower" and "varIdent" models applied variance functions varPower() and varIdent(), respectively. VarPower (form = fitted(.)) function modeled variance as a power of the fitted values, whereas varIdent(form = ~1 | Period) specified different variances for each period level (Gałecki and Burzykowski, 2013). Models incorporating autocorrelation, namely "corCompSymm" and "corAR1," used correlation structures corCompSymm(form = ~1 | Steer), which assumes that all pairs of observations within a steer have the same correlation, and corAR1(form = ~1 | Steer), which assumes that the correlation between measurements within a steer decreases exponentially with the time lag between them (Gałecki and Burzykowski, 2013). In all models, the random effects were defined as (~ 1 | STEER). All models using lme, such as varPower, varIndent, corCommSymm, and corAR1, utilized the control parameters specified by lmeControl(optimizer = "nlminb," optCtrl = list(iter.max = 200, eval.max = 400, trace = 1), tolerance = 1e-4) to improve the optimization process and ensure that the algorithm reached a solution within a reasonable number of iterations. The covariate used in the "Covariate" model was the same variable measured during the covariate phase. Data from four steers, three from the Powder group and one from the Oil group, were excluded from the "Covariate" model analysis with gas data because these animals did not have Greenfeed data from the covariate phase.

A basic model with random intercepts for steers was initially fitted to account for baseline differences and partially address within-steer correlation. In this framework, the steer is the experimental unit, with individual time points treated as subsamples nested within each steer (Wu, 2009). At this stage, residuals were assumed to be independent across time points after accounting for random steer effects. However, it is acknowledged that this assumption could theoretically bias results if unmodeled temporal correlation exists. To address this, a stepwise model-building approach was adopted, guided by AIC and residual diagnostics, to assess whether explicit correlation structures (e.g., corAR1) or heteroscedasticity adjustments would significantly improve model fit. This approach ensured that additional complexity was introduced only when empirically justified.

For each combination of the response variable and model, residuals were assessed for normality using the Shapiro-Wilk test. If the P-value was > 0.05, indicating insufficient evidence to reject the null hypothesis that the residuals were normally distributed, the model was accepted without further modifications. If the P-value was ≤0.05, a series of iterative steps were conducted. Data were transformed using methods such as logarithmic, square root, inverse, or Box-Cox transformation. If transformation alone was insufficient to achieve normality of the residuals, the data were cleaned by removing outliers based on the Q-Q plot at progressively stricter percentile thresholds (0.975, 0.95, 0.925, and 0.90) without exceeding 10% data removal to avoid bias. For each transformation and threshold, the model was re-fitted to the transformed-cleaned data, and the residuals were reevaluated using the Shapiro-Wilk test. This process continued until either the residuals were normally distributed, or all thresholds were exhausted. Heteroscedastic models (e.g., varPower, varIdent) explicitly account for unequal variances across groups or levels of a predictor. These models do not inherently require normally distributed residuals, though normality is still desirable for valid inference (Gałecki and Burzykowski, 2013).

From the candidate models, the best model for each variable was selected based on the lowest AIC value. To ensure valid comparisons across models with differing fixed-effect structures (i.e., covariate-adjusted vs. the other models), Maximum Likelihood (ML) estimation was used instead of the default Restricted Maximum Likelihood (REML) in the nlme and lme4 packages. While REML is preferred for unbiased variance estimation, it is statistically inappropriate for comparing models with different fixed effects because its likelihoods are conditioned on fixed-effect parameters, rendering them incomparable. ML estimation, by contrast, provides likelihoods on a consistent scale, enabling robust AIC-based model selection (Gałecki and Burzykowski, 2013). Model selection criteria prioritized both the normality of residuals (assessed via Shapiro-Wilk tests) and AIC minimization, ensuring adherence to statistical assumptions and penalizing overfitting. This approach guarantees that subsequent analyses—including ANOVA, and post-hoc pairwise comparisons (conducted via the emmeans package)—are grounded in the most parsimonious and well-fitting model.

RESULTS

Model Selection

Table 2 presents the best model (normality of residuals and the lowest AIC) for each variable, including details, such as transformations and outlier elimination (number of datapoints removed to achieve normality of residuals). The most frequently selected model was the "corAR1" model, reflecting its effectiveness in accounting for autocorrelation within the data. The "Covariate" model was the best only for CH_4 outcomes, highlighting the importance of including the pre-experiment emissions to properly account for their influence and improve the accuracy of the results. The inverse transformation was the most commonly applied. No transformation was necessary for the FCE variable and logarithmic transformation was common for the three CH_4 outcomes. Outlier elimination was required for thirteen variables, most notably for H_2 intensity (10 datapoints) and DMI (8 datapoints).

Gas Emissions

The emissions, expressed as production (g/day), yield (g/kg DMI), and intensity (g/kg ADG) of CH_4, H_2, and CO_2 from the steers in the three treatment groups (Control,

Table 2. Estimated marginal means of Rumin8 Powder and Oil on gas emissions, fermentation profile, and animal parameters

				P1 (Week 1–8)			P2 (week 9–12)				
Variable	Model1	Transf.2	Outl.3	Control	Powder	Oil	Control	Powder	Oil	SEM4	P5
Bromoform (mg/kg DM)				0.000	2.0	32.2	0.000	17.9	32.2		
Methane											
Production (g/day)	covariable	log	5	145[a] (31)	133[a] (12)	7.26[b] (25)	187[a] (7)	135[a] (2)	11.4[b] (4)	10.8	<0.001
Yield (g/kg DMI)	covariable	log	3	14.6[a] (31)	13.3[a] (12)	0.738[b] (26)	20.8[a] (7)	16.1[a] (2)	0.929[b] (5)	1.46	<0.001
Intensity (g/kg ADG)	covariable	log	5	108[a] (30)	82.5[a] (12)	4.19[b] (26)	173[a] (7)	215[a] (2)	10.6[b] (4)	18.1	<0.001
Carbon Dioxide											
Production (g/day)	corAR1	inverse	3	8,936 (31)	9,342 (20)	8,810 (28)	9,462 (7)	8,907 (3)	8,866 (7)	256	0.450
Yield (g/kg DMI)	corAR1	boxcox	0	891 (31)	900 (21)	906 (28)	1,021 (7)	1,189 (4)	965 (8)	30.4	0.900
Intensity (g/kg ADG)	varIdent	inverse	3	6,240 (30)	4,846 (21)	5,673 (26)	8,281 (7)	1,0651 (4)	8,357 (8)	732	0.280
Hydrogen											
Production (g/day)	basic	sqrt	3	0.694[a] (31)	1.049[a] (21)	7.11[b] (27)	0.692[a] (7)	3.03[b] (4)	6.41[c] (6)	0.241	<0.001
Yield (g/kg DMI)	varIdent	sqrt	3	0.0710[a] (31)	0.102[a] (21)	0.730[b] (26)	0.0790[a] (7)	0.456[b] (4)	0.626[b] (7)	0.0316	<0.001
Intensity (g/kg ADG)	varPower	sqrt	10	0.453[a] (28)	0.561[a] (21)	4.34[b] (23)	0.660[a] (7)	4.71[b] (4)	4.94[b] (6)	0.324	<0.001
Fermentation profile											
pH	varIdent	inverse	0	5.97 (8)	6.28 (6)	6.44 (8)	5.81 (4)	6.34 (3)	6.30 (4)	0.153	0.00551
Acetic acid (mM)	corAR1	inverse	0	69.5 (8)	47.2 (6)	52.2 (8)	391 (4)	239 (3)	180 (4)	197	0.0389
Propionic acid (mM)	corCompSymm	inverse	1	75.3 (8)	62.9 (5)	66.8 (8)	117 (4)	54.7 (3)	88.4 (4)	23.7	0.101
Butyric acid (mM)	varPower	inverse	0	23.1 (8)	19.8 (6)	21.3 (8)	26.4 (4)	22.7 (3)	21.0 (4)	3.89	0.508
Valeric acid (mM)	varIdent	inverse	1	14.3 (8)	13.4 (6)	15.9 (7)	16.5 (4)	13.9 (3)	16.8 (4)	2.04	0.290
Isobutyric acid (mM)	basic	inverse	2	9.60 (7)	8.14 (6)	10.7 (7)	8.13 (4)	9.15 (3)	12.5 (4)	1.97	0.209
Isovaleric acid (mM)	corAR1	inverse	2	13.6 (8)	15.3 (5)	12.4 (7)	15.4 (4)	15.3 (3)	15.3 (4)	1.98	0.629
Ac:Pr Ratio	corAR1	sqrt	0	1.00 (8)	0.967 (6)	0.790 (8)	1.00 (4)	1.13 (3)	0.970 (4)	0.114	0.480
Animal parameters											
DMI (kg/day)	varPower	inverse	8	9.89 (30)	10.2 (21)	9.95 (26)	9.43 (6)	6.44 (2)	9.73 (6)	0.377	0.834
ADG (kg/day)	corAR1	sqrt	0	1.42 (31)	1.88 (21)	1.60 (28)	1.16 (7)	0.753 (4)	1.03 (8)	0.143	0.163
FCE (ADG/DMI)	corAR1	none	0	0.156 (31)	0.185 (21)	0.177 (28)	0.133 (7)	0.120 (4)	0.121 (8)	0.0130	0.200

DMI, dry matter intake; ADG, average daily gain; FCE, feed conversion efficiency.
[1]Model: Indicates the Linear Mixed-Effects model configuration used. Basic: Includes the interaction of treatment and period as fixed effects and (1|Steer) as random effects, without weights or correlation structures. Covariate: Extends the Basic model by incorporating an additional covariate, measured at the end of the covariate phase, in the fixed effects. varPower: applies a variance function varPower(form = ~fitted(.)) to address heteroscedasticity. varIdent: Utilizes a variance function varIdent(form = ~1|Period). corCompSymm: Incorporates a correlation structure corCompSymm(form = ~1|Steer), assuming equal correlation among all pairs of observations within a steer. corAR1: Uses a correlation structure corAR1(form = ~1|Steer), assuming an exponential decrease in correlation with increasing time lag between measurements within a steer.
[2]Transf.: Refers to the type of data transformation applied to achieve normality of residuals and improve model fit. none: No transformation was applied. log: Logarithmic transformation. sqrt: Square root transformation. inverse: Inverse transformation.
[3]Outl.: Refers to the number of datapoints removed to achieve normality of residuals.
[4]Standard Error of the mean; standard error pooled across treatments.
[5]P: p-values for treatment effect from the Anova analysis.
[a,b,c]superscripts note significant difference ($p \leq 0.05$) between treatments within each period (P1 and P2).
(Parentheses) indicate the number of observations used in the statistical analysis.

Powder, and Oil), are presented in Table 2. These were divided into two periods based on the $CHBr_3$ concentration levels in the Powder group (P1 and P2). It should be noted that P1 lasted 56 d with four sampling intervals, whereas P2 lasted 28 d with only one sampling interval, resulting in a difference in statistical power between the two periods. The greater number of sampling intervals in P1 provided higher precision and reduced variability compared to P2, which may impact the comparability of results across periods and the reliability of findings from P2. In Table 2, superscripts indicate significant differences ($P \leq 0.05$) between treatments within each period (P1 and P2). Significant treatment effects on CH_4 and H_2 are illustrated in Figure 1 (P1 and P2) and over time in Figure 2 (CH_4 and H_2). The inclusion of Rumin8 oil IVP in the TMR significantly reduced enteric CH_4 production, yield, and intensity by 95.0%, 95.0%, and 96.1%, respectively, in P1, and by 93.9%, 95.5%, and 93.9%, respectively, in P2, compared to the control group. In contrast, Rumin8 powder IVP did not significantly reduce CH_4 production, yield, or intensity during either period.

Hydrogen production, yield, and intensity in the Oil group increased significantly by 925%, 934%, and 858%, respectively, in P1, and by 827%, 696%, and 648%, respectively, in P2 compared to the control group. In the Powder group, H_2 production, yield, and intensity were unaffected in P1 but significantly increased ($P \leq 0.05$) in P2 by 339%, 480%, and 613%, respectively, compared to the control group. Carbon dioxide emissions showed no statistically significant variations across the different treatments, as indicated by the overall ANOVA *P*-values ($P > 0.05$). Overall, gas production, yield, and intensity were more significantly influenced by the inclusion of Rumin8 oil IVP than powder IVP in the TMR; however, the increase in $CHBr_3$ concentration in Rumin8 powder IVP from P1 to P2 had an impact on H_2 production, yield, and intensity.

Animal Production Parameters

The introduction of $CHBr_3$ through either powder or oil IVP did not significantly affect key animal performance indicators, across control and treatment groups. This suggests that the efficacy of $CHBr_3$ in reducing CH_4 emissions does not compromise overall animal performance.

Rumen Environment

The treatments had an overall significant effect on rumen pH and acetic acid ($P \leq 0.05$). However, when comparing specific treatment-period combinations, no significant differences were observed between individual pairs indicating variability across treatments. For rumen pH, the control groups showed lower values (P1: 5.97; P2: 5.81) compared to the oil-treated groups (P1: 6.44; P2: 6.30), with a SEM of 0.153. For acetic acid, a notable increase in concentration was observed in the P2 groups compared to P1. Specifically, control group had a substantially higher acetic acid concentration (391 mM) in P2 compared to P1 (69.5 mM). Similarly, oil-treated groups in P2 (180 mM) showed a marked increase over P1 (52.2 mM), with an SEM of 197. This highlights a significant rise in acetic acid levels in the P2 treatments, particularly in the control group. Despite these fluctuations in pH and acetic acid, the concentrations of the other volatile fatty acids remained unchanged.

DISCUSSION

Enteric Gas Emissions

Given the diurnal pattern of CH_4 emissions, spot samples must be distributed over 24 hours to attain representative estimates of average daily CH_4 production (Tedeschi et al., 2022; Hristov et al., 2025). In tie-stall and feedlot settings, respectively, Hristov et al. (2015) and Roque et al. (2021) used the same spot sampling scheme employed in our study, which divides the 24 hours of a day into 8 three-hour periods, distributes these periods over 3 d, and obtains a sample from each animal in each of these periods. The scheme used here is comparable to the "6.0_2.0" scheme (sampling every six hours beginning two hours after feeding) in van Lingen et al. (2023), under which estimates of daily CH_4 emissions did not differ significantly from the reference model. Daily H_2 emissions measured using the "6.0_2.0" scheme in cattle fed ad libitum and supplemented with CH4-inhibitor 3NOP also did not differ significantly from the reference model. van Lingen et al. (2023) concluded that the "6.0_2.0" sampling scheme accurately estimates daily CH_4 emissions under a twice-daily ad libitum feeding regimen. Lee et al. (2022) concluded that at least 8 samples per day were needed for accurate estimates of daily CH_4 production; the sampling scheme employed here is equivalent to taking 8 samples per day, spread over 3 d. Based on these findings, we believe that we obtained accurate estimates of daily CH_4 emissions in our study.

This study is the first to evaluate Rumin8 IVPs, two feed additives containing synthetic $CHBr_3$, in vivo in cattle. Compared with other studies on synthetic HMAs, the CH_4 reductions observed with Rumin8 oil IVP in this study are among the most substantial reported. The greatest reduction in CH_4 yield observed in previous in vivo HMA studies was 91% reduction (Mitsumori et al., 2012) using 211 mg/kg DMI of a synthetic BCM additive in Japanese miniature goats. Tomkins et al. (2009) reported a 59.6% reduction seen in CH_4 yield in beef steers with a much lower dose of synthetic BCM (20.4 mg/kg DMI). Abecia et al. (2012) observed only a 33% reduction in the CH_4 yield at the same dosage in dairy goats. Higher doses of chloroform (55.3 and 92.6 mg/kg DMI) were used by Martinez-Fernandez et al. (2016), resulting in CH_4 yield reductions of 37% and 55% in beef steers, respectively. In this study, the concentration of $CHBr_3$ in the Oil group, which was the only group showing a significant ($P \leq 0.05$) decrease in CH_4 production, yield, and intensity, was 32.2 mg/kg DMI. The 95.0% reduction in CH_4 yield observed here exceeds those reported in other HMA in vivo studies, suggesting that $CHBr_3$ may be a more effective HMA for CH_4 reduction.

The results show that the effectiveness of the Rumin8 oil IVP remained consistent from P1 to P2. This contrasts with other studies on synthetic HMAs, such as that of Tomkins et al. (2009), who found that only 40% of the CH_4 reduction response could be maintained over a prolonged period (60 to 90 d) when using BCM. Knight et al. (2011) reported that CH_4 emissions in non-lactating dairy cows initially decreased but returned to 62% of pretreatment levels by day 42 of treatment when using chloroform. The persistence of the efficacy of Rumin8 oil IVP over the course of the experiment (Figure 2) aligns with findings from Roque et al. (2021) and Cowley et al. (2024), both of which tested *Asparagopsis taxiformis* and showed similar results. However, Cowley et al. (2024) reported that low-dose groups (17 mg $CHBr_3$/kg DMI)

experienced a resurgence in CH_4 production and yield after day 12, with no significant difference from the control by day 56, suggesting rumen microbiome adaptation to low $CHBr_3$ levels. In this study, a similar dose of $CHBr_3$ (17.9 mg $CHBr_3$/kg DMI, Powder group P2) was used, but CH_4 was measured only once after 30 d, making it difficult to determine whether the effects of CH_4 changed over time.

The concentration of $CHBr_3$ plays a crucial role in the magnitude of CH_4 reduction, as supported by in vitro work of Machado et al. (2016b), where CH_4 decreased with increasing concentrations of *Asparagopsis taxiformis*. In this study, three $CHBr_3$ concentrations were tested: powder in P1 (2.0 mg $CHBr_3$/kg DMI), powder in P2 (17.9 mg $CHBr_3$/kg DMI), and oil in both P1 and P2 (32.2 mg $CHBr_3$/kg DMI). As shown in Table 3, the CH_4 production, yield, and intensity generally decreased with increasing $CHBr_3$ concentration. Although the differences in CH_4 production, yield, and intensity were not significant ($P > 0.05$) between the Powder and Control IVP groups during either period, the downward trend suggests that the $CHBr_3$ concentration is an important factor for CH_4 abatement. This is further supported by the 339%, 480%, and 613% increases in H_2 production, yield, and intensity, respectively, in the Powder group in P2 compared to the control group. An increase in H_2 emissions is often associated with a reduction in CH_4 emissions, suggesting a potential CH_4-lowering effect; however, no reduction was observed in this study, which may be due to the limited number of observations in P2. The findings of Alvarez-Hess et al. (2024) and Cowley et al. (2024) further support this, demonstrating that $CHBr_3$ concentration plays a key role in CH_4 mitigation. Experimental formulations of the powder were assessed for this experiment, but further research is required to elucidate the optimal $CHBr_3$ dosing when administered via a solid carrier.

The highest concentration of $CHBr_3$ used in this study (32.2 mg $CHBr_3$/kg DMI) resulted in a CH_4 yield (0.738 g/kg DMI) greater than that observed by Cowley et al. (2024) (0.20 g/kg DMI at 34.0 mg $CHBr_3$/kg DMI) (Table 3). Kinley et al. (2020) reported a similar CH_4 yield (0.20 g/kg DMI) at a lower $CHBr_3$ concentration (26.6 mg $CHBr_3$/kg DMI). Roque et al. (2021) reported a higher CH_4 yield (5.67 g/kg DMI) at a higher $CHBr_3$ concentration (71.5 mg/kg DMI). Storage conditions can significantly affect the concentration of halogenated compounds in *Asparagopsis taxiformis*, as reported by Stefenoni et al. (2021), who found a 75% decrease in $CHBr_3$ concentrations over time depending on storage conditions. Given that $CHBr_3$ concentrations were not analyzed over time in this study, it is not possible to report the stability of synthetic $CHBr_3$ compared to that of naturally occurring $CHBr_3$. However, the efficacy of the Rumin8 oil IVP over the course of this study suggests the stability of the synthetic $CHBr_3$.

Another important consideration is the form of the additive. Initial studies using *A. taxiformis* provided freeze-dried whole algal biomass (Roque et al., 2019a, 2021; Kinley et al., 2020). However, steeping seaweed in vegetable oil has become a potentially viable alternative because of logistical challenges associated with flash-freezing. Vegetable oils, commonly used in TMR for both beef and dairy cattle, have the added benefit of inhibiting CH_4 production (Rasmussen and Harrison, 2011). In in vitro work by (Kinley et al., 2022) showed that *A. taxiformis* steeped in oil performed better than those freeze-dried, showing greater CH_4 reductions at lower $CHBr_3$ concentrations. Additionally, studies have confirmed that the

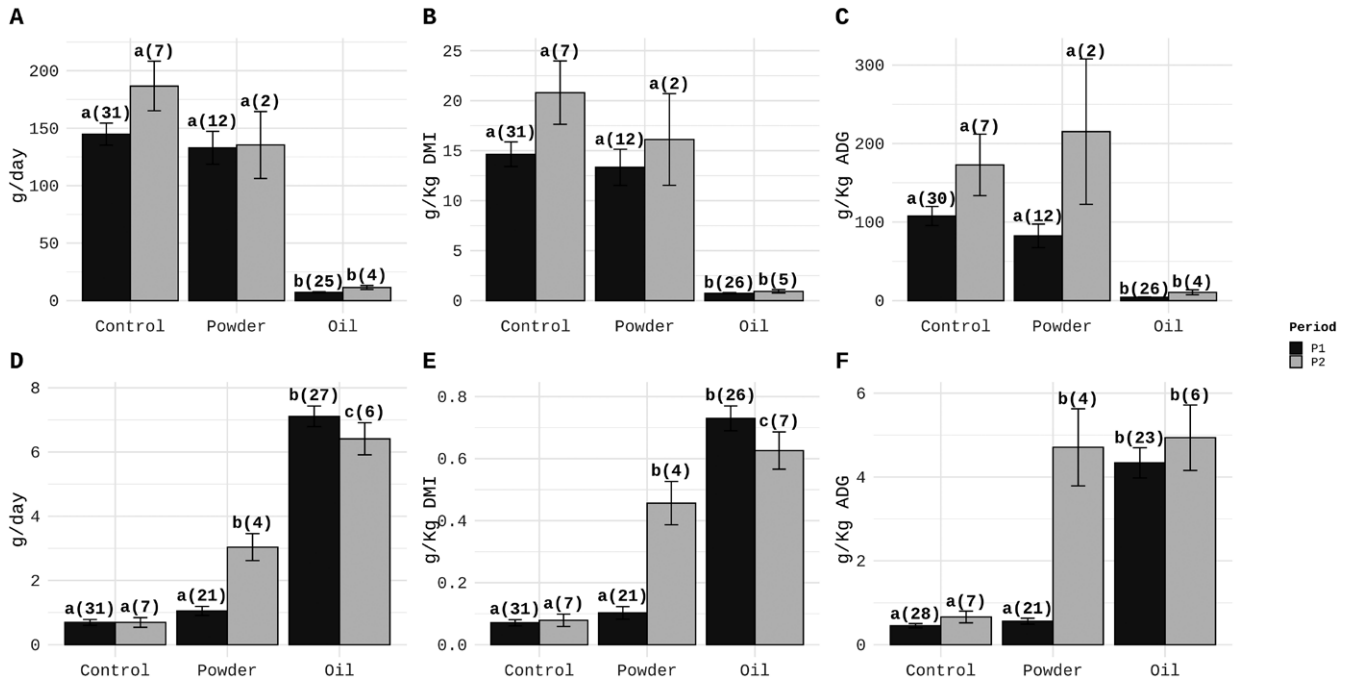


Figure 1. Methane (CH_4) production (A), yield (B) and intensity (C), and hydrogen (H_2) production (D), yield (E) and intensity (F) for control and treatment groups supplemented with Rumin8 IVP powder and oil for period 1 (P1) and period 2 (P2). Different letters indicate significant differences between treatment groups ($P \leq 0.05$) within each period (P1 and P2). The number in parentheses next to each letter represent the number of observations used in the statistical analysis. Four steers (three from the Powder group and one from the Oil group) were excluded from the analysis of CH_4 outcomes with the "Covariate" model due to missing Greenfeed data from the covariate phase.

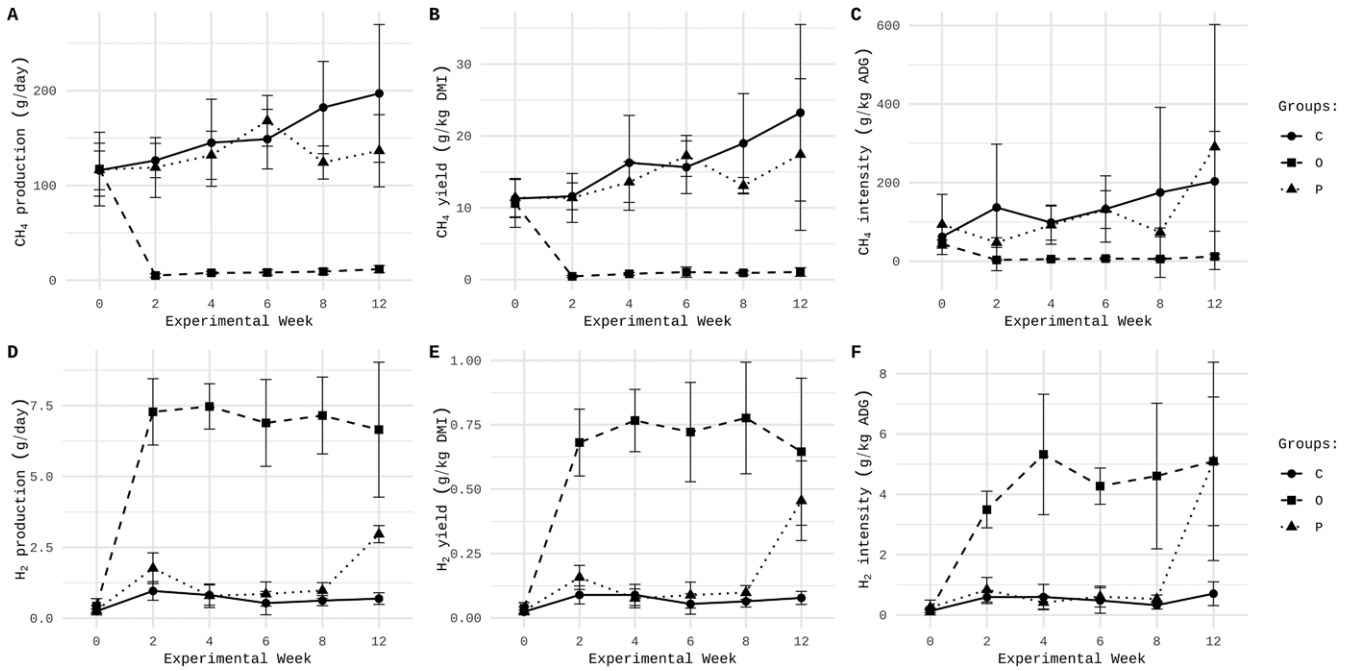


Figure 2. Methane (CH_4) production (A), yield (B) and intensity (C), and hydrogen (H_2) production (D), yield (E) and intensity (F) for control (C) and treatment groups supplemented with Rumin8 IVP powder (P) and oil (O) over the 12-wk experimental period.

$CHBr_3$ concentration in *A. taxiformis* steeped in oil is more stable over time than that in freeze-dried material (Tan et al., 2023). The effectiveness of $CHBr_3$-containing feed additives might also be influenced by the concentrate-to-roughage ratio of the diet. Preliminary research has suggested that as the concentrate-to-roughage ratio increases, the efficacy of *A. taxiformis* increases (Kinley et al., 2020; Roque et al., 2021). However, Kinley et al. (2021) reported the opposite in in vitro work, with an increase in CH_4 observed as the concentrate in the diet increased. Further research is needed to determine the effects of concentrate-to-roughage ratio on CH_4 emissions for feed additives containing synthetic $CHBr_3$.

It was previously believed that reducing methanogenesis would require alternative H_2 sinks to prevent H_2 accumulation in the rumen, which could otherwise hinder fermentation and lead to energy loss. However, Hristov et al. (2015) indicated that H_2 could be effectively removed from the rumen without the need for alternative sinks. Increases in enteric H_2 yields have been reported in studies using both synthetic HMA (Mitsumori et al., 2012; Martinez-Fernandez et al., 2016) and seaweed (Roque et al., 2019b, 2021; Kinley et al., 2020; Stefenoni et al., 2021). The 934% increase in H_2 yield observed in the Oil group in P1 is one of the largest reported in vivo studies testing HMAs or seaweed. A slightly higher concentration of $CHBr_3$ used by Roque et al. (2021) (71.5 mg/kg DMI) resulted in a lower increase in H_2 yield (590%), while a lower concentration (26.6 mg/kg DMI) used by Kinley et al. (2020) resulted in a higher increase in H_2 yield (1,700%). Although the increase in H_2 production seems high when calculated as a percentage, daily H_2 production increased from 0.694 g/head to 7.11 g/head. Given the low global warming potential of H_2 (GWP100 of 11, Hristov and Solomon, 2025) compared to CH_4 (GWP100 of 28), the negative effects of increasing H_2 production do not negate the positive effects of decreasing CH_4 emissions.

The CH_4-inhibited rumen adapts to high H_2 levels by both expelling H_2 gas and shifting fermentation (Mitsumori et al., 2012). The variability in H_2 yield can be explained by the degree to which each of these pathways is followed. Roque et al. (2021) suggested that while feeding seaweed can increase enteric H_2 yield, its effect is less pronounced than that of other CH_4-reducing feed additives because H_2 is likely redirected into alternative pathways that may be more beneficial to the animal. Given that the increase in H_2 yield observed in this study falls within the 1.25 to 17-fold reported by Roque et al. (2021), it is possible that synthetic $CHBr_3$-based feed additives also redirect H_2 from CH_4 production to other pathways that could benefit the animal. One potential pathway is the production of propionate, however, the VFA data in this study did not reflect increase in propionate concentration. It is important to note that rumen fluid samples were only collected from a subset of animals, which may have limited our ability to detect changes in fermentation end products. The redirection of H_2 could also explain why no increase in H_2 yield was observed by Martinez-Fernandez et al. (2018) in their study using chloroform in beef cattle. Additionally, Martinez-Fernandez et al. (2016) found that animals supplemented with concentrate expelled more H_2 than those on a hay-only diet, suggesting that a hay diet may more efficiently redirect H_2 into other microbial production pathways. This study was conducted using a high-concentrate diet, which is typical of a feedlot and could explain the elevated H_2 yield observed.

Animal Production Parameters

The addition of Rumin8 oil or powder IVPs to the TMR had no significant effects on DMI, ADG, or FCE compared with the control group in this study. These results are consistent with findings from HMA studies by Martinez-Fernandez et al. (2018), Mitsumori et al. (2012), Tomkins et al. (2009), and seaweed studies by Alvarez-Hess et al. (2023), Cowley

et al. (2024), and Williams et al. (2024), where reductions in CH_4 were observed without affecting the DMI or other animal production parameters. Although stable animal production parameters are desirable, an increase in productivity without changes in DMI would be more beneficial, as this would suggest enhanced efficiency in ruminants. This was observed in a study conducted by (Kinley et al., 2020), where steers receiving *A. taxiformis* at 0.10% and 0.20% diet organic matter demonstrated no changes in DMI and an increase in weight gain. George et al. (2024) reported a 6.6% increase in the gain-to-feed ratio and a 5% increase in ADG over a 200-d feeding period using canola oil infused with *A. armata*. Similarly, Abecia et al. (2012) observed an increase in milk yield and a decrease in CH_4 with no effects on DMI, when dairy goats were supplemented with BCM. Roque et al. (2021) observed a decrease in DMI but no difference in ADG in treatments, potentially indicating an increase in production efficiency. These studies support the theory of beneficial redistribution of energy, otherwise lost as CH_4.

Conversely, some studies have reported declines in production parameters with the use of HMAs or seaweeds. For example, Roque et al. (2019b) observed a decrease in DMI by 10.8% and 38% when *A. armata* was supplemented to dairy cattle at 0.5% and 1% inclusion on an organic matter basis, resulting in 11.6% reduction in milk yield at the higher level. Similarly, Stefenoni et al. (2021) observed a decrease in DMI with the highest *A. taxiformis* supplementation (0.50% DM), leading to a subsequent decrease in milk yield. Alvarez-Hess et al. (2024) also observed a linear decrease in milk yield with increasing levels of *A. armata* supplementation, although no changes in the DMI were observed in this study.

These studies can be categorized into three broad groups based on their effects on animal production parameters: no change in production or DMI (production±); increased production with no change in DMI (production+); and decreased production with or without a decrease in DMI (production–). The range of concentrations of $CHBr_3$ in the production±, production+, and production– groups are 11.9 to 51, 6.66 to 36.5, and 5.89 to 22.6 mg/kg DMI, respectively. As the ranges of $CHBr_3$ concentrations overlap between the groups, it is challenging to draw definitive conclusions about the relationship between $CHBr_3$ concentration and animal production parameters in studies where CH_4 is decreased. Similarly, the range of HMA concentrations in the production±, production+, and production– groups was 20.4 to 71.1, 12.6, and N/A mg/kg DMI, respectively. Given the limited number of HMA studies with relevant data, it is challenging to establish a clear association between HMA concentrations and animal production parameters. However, DMI was not affected in studies that used oil formulations (e.g., Cowley et al. 2024; George et al., 2024; Williams et al., 2024).

Alvarez-Hess et al. (2023) proposed that decreases in DMI occur when there is inadequate adaptation to the feed additive and recommended that future studies employ a longer adaptation period to avoid decreases. Although this study initially had a 14-d adaptation period, no adaptation period was provided when a different batch of Rumin8 IVP powder was introduced at the end of week 8, resulting in an 8.5-fold increase in $CHBr_3$. This led to a decrease in the DMI in the Powder group from P1 to P2. However, it should be noted that the other two groups also had a decrease in DMI in P2 due to adverse climatic events/non-experiment-related events. Overall, the inconsistency in findings regarding the diversion

Table 3. Enteric methane (CH_4) production and yield observed in studies using feed additives containing bromoform ($CHBr_3$) in cattle

Paper	Species	mg $CHBr_3$/kg DMI	CH_4 yield (g/kg DMI)
This study	Beef	2.00	13.3
		17.9	16.1
		32.2	0.738*
Roque et al. (2019b)	Dairy	12.1	12.0*
		24.3	8.00*
Kinley et al. (2020)	Beef	6.66	10.0
		13.32	6.80*
		26.6	0.200*
Roque et al. (2021)	Beef	35.8	9.75*
		71.5	5.67*
Alvarez-Hess et al. (2023)	Dairy	19.9	16.7*
		21.2	20.4*
Alvarez-Hess et al. (2024)	Dairy	5.89	23.8
		11.7	22.4
		19.6	20.2*
		22.6	16.3*
Cowley et al. (2024)	Beef	17.0	3.50*
		34.0	0.200*
		51.0	0.100*
Williams et al. (2024)	Dairy	11.9	15.7*
		12.4	17.2*

DMI, dry matter intake.
*Significant difference ($P \leq 0.05$) between treatment group and the corresponding study's control group.

of H_2 and energy from CH_4 production to beneficial sinks underscores the need for more targeted research into the potential benefits of feeding HMAs and seaweed to improve productive efficiency.

Rumen Environment

Analysis of VFA and pH levels in this study revealed no significant differences ($P > 0.05$), within periods (P1 and P2), between the groups treated with either Rumin8 powder or oil IVPs and the control group. These findings are consistent with those of Cowley et al. (2024) and Martinez-Fernandez et al. (2018), who found no changes in VFA production or relative concentrations when examining the effects of *A. taxiformis* and chloroform, respectively, on beef cattle. The stability of overall VFA production is desirable given the critical role these substrates play in animal performance. However, most studies analyzing the effects of seaweeds or HMAs on enteric CH_4 production, yield, and intensity have reported different results. Both in vitro and in vivo studies by Alvarez-Hess et al. (2023, 2024), Kinley et al. (2016, 2020), Machado et al. (2016b), Roque et al. (2019b), Stefenoni et al. (2021), and Williams et al. (2024) consistently observed decreases in acetate and increases in propionate, leading to lower acetate:propionate (A:P) ratios. Similar results were documented by Abecia et al. (2012) Denman et al. (2007), Goel et al. (2009), Knight et al. (2011), Martinez-Fernandez et al. (2016) and Mitsumori et al. (2012) in studies using BCM and chloroform. The reduction in the A:P molar ratio in the rumen is a common feature of antimethanogenic compounds, indicating a concurrent decrease in CH_4 formation and redirection of H_2 to propionic metabolic pathways once the rumen has had time to adapt (McAllister and Newbold, 2008; Roque et al., 2021). Therefore, the decrease in the A:P ratio observed in these studies suggests that H_2 was redistributed to propionate (Roque et al., 2019a). The increase in propionate can be attributed to the action of *Prevotella spp.* and *Selenomonas spp.* which capture excess H_2 to produce propionate (Denman et al., 2015). In some cases, such as in Kinley et al. (2020) and Abecia et al. (2012), this shift in fermentation has been linked to improvements in animal performance, potentially due to the increased availability of propionate as an energy source. Although no significant shifts in VFA profiles were observed in this study, definitive conclusions are limited by the small sample size. While no significant negative effects on productivity were detected, further research is needed to elucidate the effects of $CHBr_3$ on H_2 dynamics, VFA profiles, and overall animal performance.

Given the significant reduction in CH_4 observed in this study, we expected a corresponding increase in propionate production or a decrease in the A:P ratio. While a significant shift in propionate was not observed ($P > 0.05$ in post-hoc pairwise comparisons within periods, P1 and P2), there was a trend towards reduced acetate production compared to the control group within periods, which may explain the observed reduction in CH_4. Camer-Pesci et al. (2023) highlighted the impact of diet on VFA production; however, because the steers in this study were fed a high-quality TMR diet, this is unlikely to be a contributing factor. The higher levels of enteric H_2 production observed in this study compared to other reports suggest that an increase in propionate production may not have been evident due to excess H_2 being eructated. Mitsumori et al. (2012) noted that while the CH_4-inhibited rumen adapts to high H_2 levels by shifting fermentation towards propionate production, most of the H_2 is eliminated, which may explain the findings of this study. It should also be noted that while propionate is a major H_2 sink, other H_2 sinks exist, including the production of butyrate and formate, microbial mass, and reductive acetogenesis (Ungerfeld, 2015). However, no significant changes in VFA concentration were observed in this study.

CONCLUSIONS

The addition of Rumin8 oil IVP at 8 mL/kg intake, containing 32.2 mg $CHBr_3$/kg DMI, resulted in a substantial reduction in CH_4 emissions: 95.0% in CH_4 production, 95.0% in CH_4 yield, and 96.1% in CH_4 intensity, without compromising animal production parameters. In contrast, the Rumin8 powder IVP used in P1 at 1.2 g/kg intake, containing 2.0 mg $CHBr_3$/kg DMI, had no significant effect on CH_4 production, yield, or intensity. A different IVP powder used in P2, containing 17.9 mg $CHBr_3$/kg DMI, also had no significant effect on CH_4 production, but led to increases in H_2 production, yield, and intensity of 339%, 480%, and 613%, respectively. Unlike many other studies utilizing CH_4-inhibiting feed additives, no significant changes in VFA production or relative concentrations (post-hoc pairwise comparisons within periods, P1 and P2) were observed in any of the treatment groups. The findings of this study imply that a synthetic $CHBr_3$-based feed additive could offer the same CH_4 mitigation potential observed in $CHBr_3$-contining seaweed studies without the challenges of seaweed production.

Acknowledgments

This research received financial support from Rumin8 Pty, Ltd. We would like to thank the undergraduate students from UC Davis who contributed to this research, especially our lead interns: Hannah Chernavsky, Daisy Cuevas, Jeremy (Jooyong) Kwon, Sebastian Renteria, and AJ Zaragoza-Smith, as well as our dedicated team of interns: Judith Becerra, Angie Hernandez, Jacob Keer, Megan (Yi-Ting) Lu, Selina Lu, Annaís Mendoza, Bahar Nainy-Nejad, Ariana Valdez Cordero, Elise Wacha, and Ryder Wu. Your hard work and support were invaluable to this project.

Author Contributions

Leanna Kelly (Data curation, Investigation, Project administration, Writing—original draft), Eleanor Pressman (Data curation, Methodology, Writing—review & editing), John-Fredy Ramirez-Agudelo (Formal analysis, Writing—review & editing), Hannah Chernavsky (Data curation, Investigation), Pablo Alvarez-Hessb (Conceptualization, Funding acquisition, Resources, Writing—review & editing), Silke Jaques (Conceptualization, Funding acquisition, Resources, Writing—review & editing), Matthias Hess (Conceptualization, Investigation, Writing—review & editing), and Ermias Kebreab (Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing)

Conflict of Interest

P.A-H and S.J. were employed by Rumin8 Pty, Ltd. The rest of the authors report no declarations of interest.

Literature Cited

Abecia, L., A. I. Martín-García, G. Martínez, C. J. Newbold, and D. R. Yáñez-Ruiz. 2013. Nutritional intervention in early life to manipulate rumen microbial colonization and methane output by kid goats postweaning. J. Anim. Sci. 91:4832–4840. doi:10.2527/jas.2012-6142

Abecia, L., P. G. Toral, A. I. Martín-García, G. Martínez, N. W. Tomkins, E. Molina-Alcaide, C. J. Newbold, and D. R. Yáñez-Ruiz. 2012. Effect of bromochloromethane on methane emission, rumen fermentation pattern, milk yield, and fatty acid profile in lactating dairy goats. J. Dairy Sci. 95:2027–2036. doi:10.3168/jds.2011-4831

Adesogan, A. T., A. H. Havelaar, S. L. McKune, M. Eilittä, and G. E. Dahl. 2020. Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. Glob. Food Secur. 25:100325. doi:10.1016/j.gfs.2019.100325

Alvarez-Hess, P. S., J. L. Jacobs, R. D. Kinley, B. M. Roque, A. S. O. Neachtain, S. Chandra, and S. R. O. Williams. 2023. Twice daily feeding of canola oil steeped with Asparagopsis armata reduced methane emissions of lactating dairy cows. Anim. Feed Sci. Technol. 297:115579. doi:10.1016/j.anifeedsci.2023.115579

Alvarez-Hess, P. S., J. L. Jacobs, R. D. Kinley, B. M. Roque, A. S. O. Neachtain, S. Chandra, V. M. Russo, and S. R. O. Williams. 2024. Effects of a range of effective inclusion levels of Asparagopsis armata steeped in oil on enteric methane emissions of dairy cows. Anim. Feed Sci. Technol. 310:115932. doi:10.1016/j.anifeedsci.2024.115932

Arndt, C., A. N. Hristov, W. J. Price, S. C. McClelland, A. M. Pelaez, S. F. Cueva, J. Oh, J. Dijkstra, A. Bannink, A. R. Bayat, et al. 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050. Proc. Natl. Acad. Sci. U.S.A. 119:e2111294119. doi:10.1073/pnas.2111294119

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, and M. B. Bolker. 2015. Package 'lme4'. J. Stat. Softw. 67:1–48. doi:10.18637/jss.v067.i01

Beal, T., C. D. Gardner, M. Herrero, L. L. Iannotti, L. Merbold, S. Nordhagen, and A. Mottet. 2023. Friend or Foe? The role of animal-source foods in healthy and environmentally sustainable diets. J. Nutr. 153:409–425. doi:10.1016/j.tjnut.2022.10.016

Black, J. L., T. M. Davison, and I. Box. 2021. Methane emissions from ruminants in Australia: mitigation potential and applicability of mitigation strategies. Animals 11:951. doi:10.3390/ani11040951

Camer-Pesci, B., D. W. Laird, M. van Keulen, A. Vadiveloo, M. Chalmers, and N. R. Moheimani. 2023. Opportunities of Asparagopsis sp. cultivation to reduce methanogenesis in ruminants: a critical review. Algal Res. 76:103308. doi:10.1016/j.algal.2023.103308

Cowley, F. C., R. D. Kinley, S. L. Mackenzie, M. R. S. Fortes, C. Palmieri, G. Simanungkalit, A. K. Almeida, and B. M. Roque. 2024. Bioactive metabolites of Asparagopsis stabilized in canola oil completely suppress methane emissions in beef cattle fed a feedlot diet. J. Anim. Sci. 102:skae109. doi:10.1093/jas/skae109

Denman, S. E., G. Martinez Fernandez, T. Shinkai, M. Mitsumori, and C. S. McSweeney. 2015. Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analog. Front. Microbiol. 6:1087. doi:10.3389/fmicb.2015.01087

Denman, S. E., N. W. Tomkins, and C. S. McSweeney. 2007. Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. FEMS Microbiol. Ecol. 62:313–322. doi:10.1111/j.1574-6941.2007.00394.x

FAO. 2011. World Livestock 2011 - Livestock in food security. Rome: FAO.

FAO. 2023. Pathways towards lower emissions. Rome: FAO. doi:10.4060/cc9029en

Gałecki, A., and T. Burzykowski. 2013. Fitting linear mixed-effects models. In: Linear mixed-effects models using R: A step-by-step approach. New York: Springer Science & Business Media; p. 303–326.

George, M. M., S. V. Platts, B. A. Berry, M. F. Miller, A. M. Carlock, T. M. Horton, and M. H. George. 2024. Effect of SeaFeed, a canola oil infused with *Asparagopsis armata*, on methane emissions, animal health, performance, and carcass characteristics of Angus feedlot cattle. Transl. Anim. Sci. 8:txae116. doi:10.1093/tas/txae116

Glasson, C. R. K., R. D. Kinley, R. de Nys, N. King, S. L. Adams, M. A. Packer, J. Svenson, C. T. Eason, and M. Magnusson. 2022. Benefits and risks of including the bromoform containing seaweed Asparagopsis in feed for the reduction of methane production from ruminants. Algal Res. 64:102673. doi:10.1016/j.algal.2022.102673

Goel, G., H. P. S. Makkar, and K. Becker. 2009. Inhibition of methanogens by bromochloromethane: effects on microbial communities and rumen fermentation using batch and continuous fermentations. Br. J. Nutr. 101:1484–1492. doi:10.1017/S0007114508076198

Grainger, C., and K. A. Beauchemin. 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol. 166-167:308–320. doi:10.1016/j.anifeedsci.2011.04.021

Hristov, A. N., A. Bannink, M. Battelli, A. Belanche, M. C. Cajarville Sanz, G. Fernandez-Turren, F. Garcia, A. Jonker, D. A. Kenny, V. Lind, et al. 2025. *Feed additives for methane mitigation:* recommendations for testing enteric methane-mitigating feed additives in ruminant studies. J. Dairy Sci. 108:322–355. doi:10.3168/jds.2024-25050

Hristov, A. N., J. Oh, F. Giallongo, T. W. Frederick, M. T. Harper, H. L. Weeks, A. F. Branco, P. J. Moate, M. H. Deighton, S. R. O. Williams, et al. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Natl. Acad. Sci. U.S.A. 112:10663–10668. doi:10.1073/pnas.1504124112

Hristov, A. N., and S. Solomon. 2025. Perspective: enteric methane mitigation and its impact on livestock hydrogen emissions. J. Dairy Sci. 108:1–5. doi:10.3168/jds.2024-25568

Hungate, R. E. 1966. The rumen and its microbes. New York: Academic Press.

IPCC. 2023. Climate Change 2021 – The physical science basis: Working Group I contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. 1st ed. Cambridge, UK: Cambridge University Press. doi:10.1017/9781009157896

Kinley, R. D., G. Martinez-Fernandez, M. K. Matthews, R. de Nys, M. Magnusson, and N. W. Tomkins. 2020. Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. J. Clean Prod. 259:120836. doi:10.1016/j.jclepro.2020.120836

Kinley, R. D., R. de Nys, M. J. Vucko, L. Machado, and N. W. Tomkins. 2016. The red macroalgae Asparagopsis taxiformis is a potent natural antimethanogenic that reduces methane production during in vitro fermentation with rumen fluid. Anim. Prod. Sci. 56:282–289. doi:10.1071/AN15576

Kinley, R. D., S. Tan, J. Turnbull, S. Askew, and B. M. Roque. 2021. Changing the proportions of grass and grain in feed substrate impacts the efficacy of Asparagopsis taxiformis to inhibit methane production in vitro. Am. J. Plant Sci. 12:1835–1858. doi:10.4236/ajps.2021.1212128

Kinley, R. D., S. Tan, J. Turnbull, S. Askew, J. Harris, and B. M. Roque. 2022. Exploration of methane mitigation efficacy using asparagopsis-Derived bioactives stabilized in edible oil compared to freeze-dried Asparagopsis in vitro. Am. J. Plant Sci. 13:1023–1041. doi:10.4236/ajps.2022.137068

Knight, T., R. S. Ronimus, D. Dey, C. Tootill, G. Naylor, P. Evans, G. Molano, A. Smith, M. Tavendale, C. S. Pinares-Patiño, et al. 2011. Chloroform decreases rumen methanogenesis and methanogen populations without altering rumen function in cattle. Anim. Feed Sci. Technol. Special Issue: Greenhouse Gases in Animal Agriculture - Finding a Balance between Food and Emissions 166-167:101–112. doi:10.1016/j.anifeedsci.2011.04.059

Lee, C., K. A. Beauchemin, J. Dijkstra, D. L. Morris, K. Nichols, P. J. Kononoff, and D. Vyas. 2022. Estimates of daily oxygen consumption, carbon dioxide and methane emissions, and heat production for beef and dairy cattle using spot gas sampling. J. Dairy Sci. 105:9623–9638. doi:10.3168/jds.2022-22213

Leroy, F., and N. D. Barnard. 2020. Children and adults should avoid consuming animal products to reduce risk for chronic disease: NO. Am. J. Clin. Nutr. 112:931–936. doi:10.1093/ajcn/nqaa236

Machado, L., M. Magnusson, N. A. Paul, R. Kinley, R. de Nys, and N. Tomkins. 2016a. Identification of bioactives from the red seaweed Asparagopsis taxiformis that promote antimethanogenic activity in vitro. J. Appl. Phycol. 28:3117–3126. doi:10.1007/s10811-016-0830-7

Machado, L., M. Magnusson, N. A. Paul, R. Kinley, R. de Nys, and N. Tomkins. 2016b. Dose-response effects of Asparagopsis taxiformis and Oedogonium sp. on in vitro fermentation and methane production. J. Appl. Phycol. 28:1443–1452. doi:10.1007/s10811-015-0639-9

Martinez-Fernandez, G., S. E. Denman, C. Yang, J. Cheung, M. Mitsumori, and C. S. McSweeney. 2016. Methane inhibition alters the microbial community, hydrogen flow, and fermentation response in the rumen of cattle. Front. Microbiol. 7:1122. doi:10.3389/fmicb.2016.01122

Martinez-Fernandez, G., S. Duval, M. Kindermann, H. J. Schirra, S. E. Denman, and C. S. McSweeney. 2018. 3-NOP vs. halogenated compound: methane production, ruminal fermentation and microbial community response in forage fed cattle. Front. Microbiol. 9:1582. doi:10.3389/fmicb.2018.01582

Mata, L., R. J. Lawton, M. Magnusson, N. Andreakis, R. de Nys, and N. A. Paul. 2017. Within-species and temperature-related variation in the growth and natural products of the red alga Asparagopsis taxiformis. J. Appl. Phycol. 29:1437–1447. doi:10.1007/s10811-016-1017-y

McAllister, T. A., and C. J. Newbold. 2008. Redirecting rumen fermentation to reduce methanogenesis. Aust. J. Exp. Agric. 48:7–13. doi:10.1071/ea07218

Mitsumori, M., T. Shinkai, A. Takenaka, O. Enishi, K. Higuchi, Y. Kobayashi, I. Nonaka, N. Asanuma, S. E. Denman, and C. S. McSweeney. 2012. Responses in digestion, rumen fermentation and microbial populations to inhibition of methane formation by a halogenated methane analogue. Br. J. Nutr. 108:482–491. doi:10.1017/S0007114511005794

Morgavi, D. P., G. Cantalapiedra-Hijar, M. Eugène, C. Martin, P. Noziere, M. Popova, I. Ortigues-Marty, R. Muñoz-Tamayo, and E. M. Ungerfeld. 2023. Review: reducing enteric methane emissions improves energy metabolism in livestock: is the tenet right? Animal, Present and future challenges in energy and protein metabolism and their implication in animal nutrition. Animal 17:100830. doi:10.1016/j.animal.2023.100830

Nilsson, J., and M. Martin. 2022. Exploratory environmental assessment of large-scale cultivation of seaweed used to reduce enteric methane emissions. Sustain. Prod. Consum. 30:413–423. doi:10.1016/j.spc.2021.12.006

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, S. Heisterkamp, B. Van Willigen, and R. Maintainer. 2017. Package 'nlme'. *CRAN Repository*. https://CRAN.R-project.org/package=nlme

Rasmussen, J., and A. Harrison. 2011. The benefits of supplementary fat in feed rations for ruminants with particular focus on reducing levels of methane production. ISRN Vet. Sci. 2011:613172. doi:10.5402/2011/613172

R Core Team. 2021. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Roque, B. M., C. G. Brooke, J. Ladau, T. Polley, L. J. Marsh, N. Najafi, P. Pandey, L. Singh, R. Kinley, J. K. Salwen, et al. 2019a. Effect of the macroalgae Asparagopsis taxiformis on methane production and rumen microbiome assemblage. Anim. Microbiome 1:3. doi:10.1186/s42523-019-0004-4

Roque, B. M., J. K. Salwen, R. Kinley, and E. Kebreab. 2019b. Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. J. Clean Prod. 234:132–138. doi:10.1016/j.jclepro.2019.06.193

Roque, B. M., M. Venegas, R. D. Kinley, R. de Nys, T. L. Duarte, X. Yang, and E. Kebreab. 2021. Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. PLoS One 16:e0247820. doi:10.1371/journal.pone.0247820

Stefenoni, H. A., S. E. Räisänen, S. F. Cueva, D. E. Wasson, C. F. A. Lage, A. Melgar, M. E. Fetter, P. Smith, M. Hennessy, B. Vecchiarelli, et al. 2021. Effects of the macroalga Asparagopsis taxiformis and oregano leaves on methane emission, rumen fermentation, and lactational performance of dairy cows. J. Dairy Sci. 104:4157–4173. doi:10.3168/jds.2020-19686

Tan, S., J. Harris, B. M. Roque, S. Askew, and R. D. Kinley. 2023. Shelf-life stability of Asparagopsis bromoform in oil and freeze-dried powder. J. Appl. Phycol. 35:291–299. doi:10.1007/s10811-022-02876-y

Teague, W. R., S. Apfelbaum, R. Lal, U. P. Kreuter, J. Rowntree, C. A. Davies, R. Conser, M. Rasmussen, J. Hatfield, T. Wang, et al. 2016. The role of ruminants in reducing agriculture's carbon footprint in North America. J. Soil Water Conserv. 71:156–164. doi:10.2489/jswc.71.2.156

Tedeschi, L. O., A. L. Abdalla, C. Álvarez, S. W. Anuga, J. Arango, K. A. Beauchemin, P. Becquet, A. Berndt, R. Burns, C. De Camillis, et al. 2022. Quantification of methane emitted by ruminants: a review of methods. J. Anim. Sci. 100:skac197. doi:10.1093/jas/skac197

Tomkins, N. W., S. M. Colegate, R. A. Hunter, N. W. Tomkins, S. M. Colegate, and R. A. Hunter. 2009. A bromochloromethane formulation reduces enteric methanogenesis in cattle fed grain-based diets. Anim. Prod. Sci. 49:1053–1058. doi:10.1071/EA08223

Ungerfeld, E. M. 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. Front. Microbiol. 6:1272. doi:10.3389/fmicb.2015.01272

van Lingen, H. J., J. G. Fadel, E. Kebreab, A. Bannink, J. Dijkstra, and S. van Gastelen. 2023. Smoothing spline assessment of the accuracy of enteric hydrogen and methane production measurements from dairy cattle using various sampling schemes. J. Dairy Sci. 106:6834–6848. doi:10.3168/jds.2022-23207

Vijn, S., D. P. Compart, N. Dutta, A. Foukis, M. Hess, A. N. Hristov, K. F. Kalscheur, E. Kebreab, S. V. Nuzhdin, N. N. Price, et al. 2020. Key considerations for the use of seaweed to reduce enteric methane emissions from cattle. Front. Vet. Sci. 7:597430. doi:10.3389/fvets.2020.597430

Williams, S. R. O., A. S. O Neachtain, S. Chandra, R. B. S. Burgess, S. Labaf, G. Aylward, P. S. Alvarez-Hess, and J. L. Jacobs. 2024. The effects of feeding liquid or pelleted formulations of Asparagopsis armata to lactating dairy cows on methane production, dry matter intake, milk production and milk composition. Anim. Feed Sci. Technol. 309:115891. doi:10.1016/j.anifeedsci.2024.115891

Wu, L. 2009. Mixed effects models for complex data. New York: Chapman and Hall/CRC.