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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

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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.

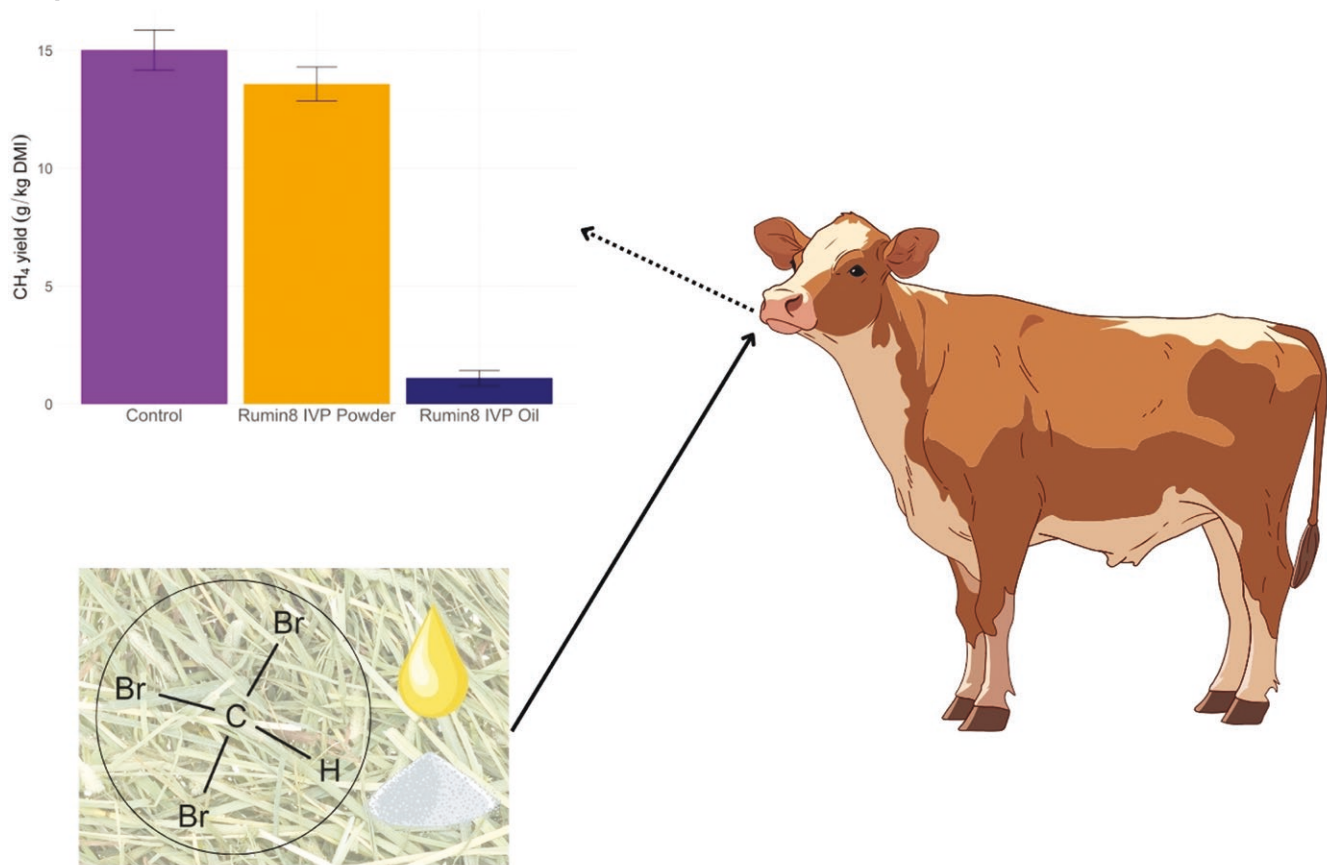
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Graphical Abstract



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 (CO₂) equivalents annually (FAO, 2023). The largest share of these emissions arises from enteric fermentation from ruminants, which produces methane (CH₄), a GHG with a global warming potential 27 times greater than that of CO₂ over a 100-yr period (IPCC, 2023). Therefore, reducing enteric CH₄ 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 CH₄ is desirable because CH₄ production represents a loss of dietary energy that could otherwise be used by animals (Morgavi et al., 2023).

Enteric CH₄ 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 CO₂ and H₂, which are used as substrates for CH₄ by methanogenic archaea (Hungate, 1966).

Several strategies have been proposed to decrease enteric CH₄ emissions, including increasing feeding levels, decreasing grass maturity, lowering dietary forage-to-concentrate ratios, using CH₄ inhibitors, incorporating tanniferous forages, providing electron sinks, and adding oils and fats (Arndt et al., 2022). Among these strategies, CH₄ inhibitors have shown marked promise. For example, seaweed (*Asparagopsis taxiformis*, AT) reduces enteric CH₄ emissions by up to 80% in beef steers (Roque et al., 2021). The CH₄ inhibiting activity of seaweed is primarily attributed to the presence of the naturally occurring bromoform (CHBr₃) (Machado et al., 2016a). Bromoform, a halogenated CH₄ analog (HMA), inhibits CH₄ 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 than 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 CHBr_3 /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 composition² (% 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.

¹Beef trace salt sourced from A.L. Gilbert (Oakdale, California) contains: salt, manganous oxide, vegetable oil, zinc oxide, copper sulfate, ethylene, diamine dihydride, sodium selenite.

²Average 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 CO₂, 500 ppm CH₄, 10 ppm H₂, 21% O₂ and nitrogen as a balance (Air Liquide America Specialty Gases, Rancho Cucamonga, CA). The recovery rates of CO₂, CH₄, and H₂ were within $\pm 3\%$ 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 $\times$ 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 $10^{\circ}\text{C}/\text{min}$. 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 (Galecki 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 (Galecki and Burzykowski, 2013). In all models, the random effects were defined as (~ 1 | STEER). All models using lme, such as `varPower`, `varIdent`, `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 (Galecki 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 (Galecki 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

Variable	Model1	Transf.2	Outl.3	P1 (Week 1–8)			P2 (week 9–12)		
				Control	Powder	Oil	Control	Powder	Oil
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)
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)
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)
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)
Yield (g/kg DMI)	corAR1	boxcox	0	891 (31)	900 (21)	906 (28)	1,021 (7)	1,189 (4)	965 (8)
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)
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)
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)
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)
Fermentation profile									
pH	varIdent	inverse	0	5.97 (8)	6.28 (6)	6.44 (8)	5.81 (4)	6.34 (3)	6.30 (4)
Acetic acid (mM)	corAR1	inverse	0	69.5 (8)	47.2 (6)	52.2 (8)	391 (4)	239 (3)	180 (4)
Propionic acid (mM)corCompSymm		inverse	1	75.3 (8)	62.9 (5)	66.8 (8)	117 (4)	54.7 (3)	88.4 (4)
Butyric acid (mM)	varPower	inverse	0	23.1 (8)	19.8 (6)	21.3 (8)	26.4 (4)	22.7 (3)	21.0 (4)
Valeric acid (mM)	varIdent	inverse	1	14.3 (8)	13.4 (6)	15.9 (7)	16.5 (4)	13.9 (3)	16.8 (4)
Isobutyric acid (mM)basic		inverse	2	9.60 (7)	8.14 (6)	10.7 (7)	8.13 (4)	9.15 (3)	12.5 (4)
Isovaleric acid (mM)corAR1		inverse	2	13.6 (8)	15.3 (5)	12.4 (7)	15.4 (4)	15.3 (3)	15.3 (4)
Ac:Pr Ratio	corAR1	sqrt	0	1.00 (8)	0.967 (6)	0.790 (8)	1.00 (4)	1.13 (3)	0.970 (4)
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)
ADG (kg/day)	corAR1	sqrt	0	1.42 (31)	1.88 (21)	1.60 (28)	1.16 (7)	0.753 (4)	1.03 (8)
FCE (ADG/DMI)	corAR1	none	0	0.156 (31)	0.185 (21)	0.177 (28)	0.133 (7)	0.120 (4)	0.121 (8)

DMI, dry matter intake; ADG, average daily gain; FCE, feed conversion efficiency.

¹Model: 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.

²Transf.: 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.

³Outl.: Refers to the number of datapoints removed to achieve normality of residuals.

⁴Standard Error of the mean; standard error pooled across treatments.

⁵P: 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 CH_4 -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 CH₄ production and yield after day 12, with no significant difference from the control by day 56, suggesting rumen microbiome adaptation to low CHBr₃ levels. In this study, a similar dose of CHBr₃ (17.9 mg CHBr₃/kg DMI, Powder group P2) was used, but CH₄ was measured only once after 30 d, making it difficult to determine whether the effects of CH₄ changed over time.

The concentration of CHBr₃ plays a crucial role in the magnitude of CH₄ reduction, as supported by in vitro work of Machado et al. (2016b), where CH₄ decreased with increasing concentrations of *Asparagopsis taxiformis*. In this study, three CHBr₃ concentrations were tested: powder in P1 (2.0 mg CHBr₃/kg DMI), powder in P2 (17.9 mg CHBr₃/kg DMI), and oil in both P1 and P2 (32.2 mg CHBr₃/kg DMI). As shown in Table 3, the CH₄ production, yield, and intensity generally decreased with increasing CHBr₃ concentration. Although the differences in CH₄ 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 CHBr₃ concentration is an important factor for CH₄ abatement. This is further supported by the 339%, 480%, and 613% increases in H₂ production, yield, and intensity, respectively, in the Powder group in P2 compared to the control group. An increase in H₂ emissions is often associated with a reduction in CH₄ emissions, suggesting a potential CH₄-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 CHBr₃ concentration plays a key role in CH₄ mitigation. Experimental formulations of the powder were assessed for this experiment, but further research is required to elucidate

the optimal CHBr₃ dosing when administered via a solid carrier.

The highest concentration of CHBr₃ used in this study (32.2 mg CHBr₃/kg DMI) resulted in a CH₄ yield (0.738 g/kg DMI) greater than that observed by Cowley et al. (2024) (0.20 g/kg DMI at 34.0 mg CHBr₃/kg DMI) (Table 3). Kinley et al. (2020) reported a similar CH₄ yield (0.20 g/kg DMI) at a lower CHBr₃ concentration (26.6 mg CHBr₃/kg DMI). Roque et al. (2021) reported a higher CH₄ yield (5.67 g/kg DMI) at a higher CHBr₃ 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 CHBr₃ concentrations over time depending on storage conditions. Given that CHBr₃ concentrations were not analyzed over time in this study, it is not possible to report the stability of synthetic CHBr₃ compared to that of naturally occurring CHBr₃. However, the efficacy of the Rumin8 oil IVP over the course of this study suggests the stability of the synthetic CHBr₃.

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 CH₄ 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 CH₄ reductions at lower CHBr₃ concentrations. Additionally, studies have confirmed that the

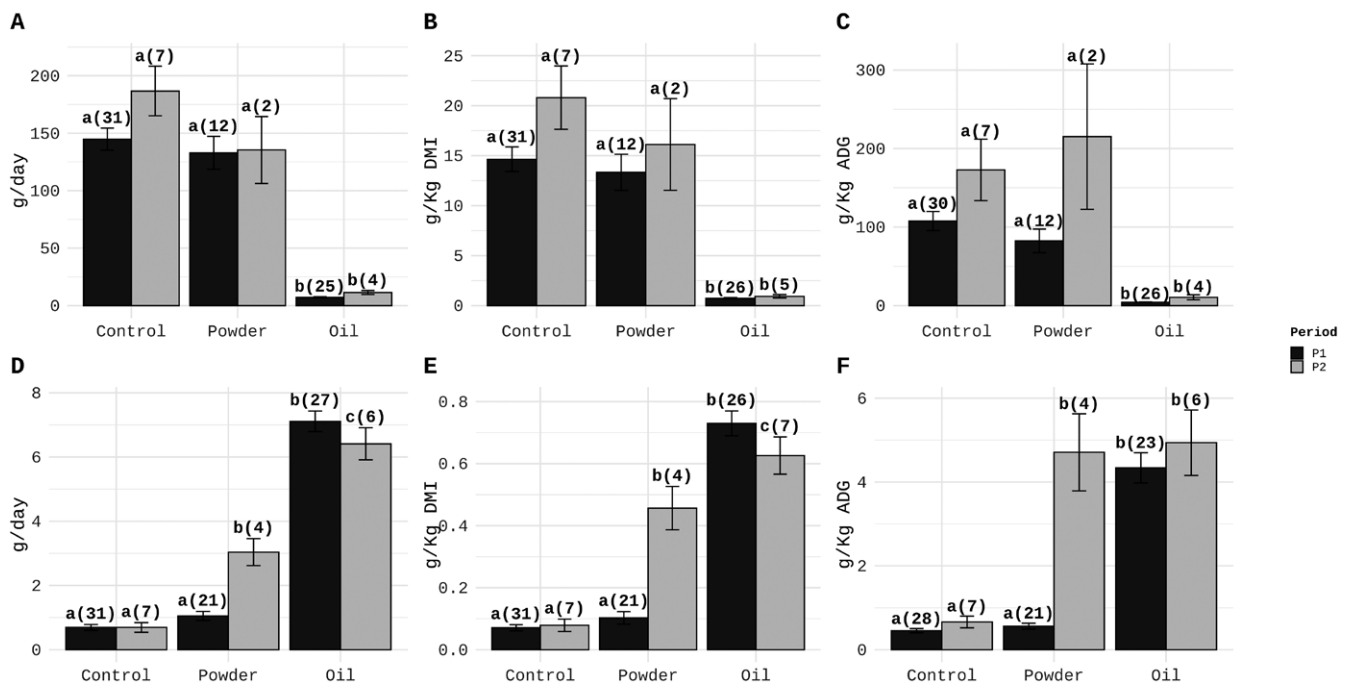


Figure 1. Methane (CH₄) production (A), yield (B) and intensity (C), and hydrogen (H₂) production (D), yield (E) and intensity (F) for control and treatment groups supplemented with Rumin8 IVP powder and oil for period 1 (P1) and period 2 (P2). Different letters indicate significant differences between 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 CH₄ outcomes with the “Covariate” model due to missing Greenfeed data from the covariate phase.

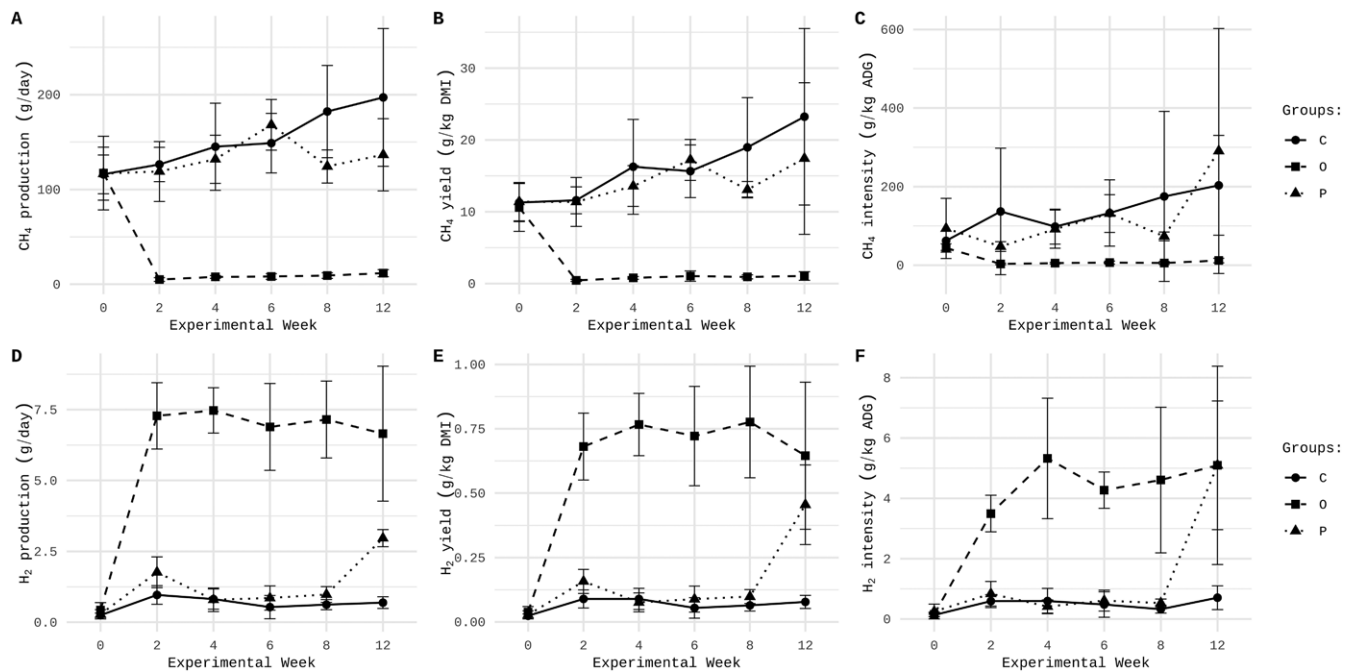


Figure 2. Methane (CH₄) production (A), yield (B) and intensity (C), and hydrogen (H₂) production (D), yield (E) and intensity (F) for control (C) and treatment groups supplemented with Rumin8 IVP powder (P) and oil (O) over the 12-wk experimental period.

CHBr₃ 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 CHBr₃-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 CH₄ observed as the concentrate in the diet increased. Further research is needed to determine the effects of concentrate-to-roughage ratio on CH₄ emissions for feed additives containing synthetic CHBr₃.

It was previously believed that reducing methanogenesis would require alternative H₂ sinks to prevent H₂ accumulation in the rumen, which could otherwise hinder fermentation and lead to energy loss. However, Hristov et al. (2015) indicated that H₂ could be effectively removed from the rumen without the need for alternative sinks. Increases in enteric H₂ 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; Stefanoni et al., 2021). The 934% increase in H₂ 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 CHBr₃ used by Roque et al. (2021) (71.5 mg/kg DMI) resulted in a lower increase in H₂ yield (590%), while a lower concentration (26.6 mg/kg DMI) used by Kinley et al. (2020) resulted in a higher increase in H₂ yield (1,700%). Although the increase in H₂ production seems high when calculated as a percentage, daily H₂ production increased from 0.694 g/head to 7.11 g/head. Given the low global warming potential of H₂ (GWP100 of 11, Hristov and Solomon, 2025) compared to CH₄ (GWP100 of 28), the negative effects of increasing H₂ production do not negate the positive effects of decreasing CH₄ emissions.

The CH₄-inhibited rumen adapts to high H₂ levels by both expelling H₂ gas and shifting fermentation (Mitsumori et al., 2012). The variability in H₂ 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 H₂ yield, its effect is less pronounced than that of other CH₄-reducing feed additives because H₂ is likely redirected into alternative pathways that may be more beneficial to the animal. Given that the increase in H₂ yield observed in this study falls within the 1.25 to 17-fold reported by Roque et al. (2021), it is possible that synthetic CHBr₃-based feed additives also redirect H₂ from CH₄ 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 H₂ could also explain why no increase in H₂ 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 H₂ than those on a hay-only diet, suggesting that a hay diet may more efficiently redirect H₂ 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 H₂ 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 $\pm$); 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 $\pm$, 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 $\pm$, 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 re-direction 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 $CHBr_3$ -containing seaweed studies without the challenges of seaweed production.

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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.

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