



# Effect of bromoform-based feed additive on methane emissions and performance of Nellore cattle in feedlot backgrounding and finishing phases

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## Abstract

This study evaluated the effects of a bromoform based feed additive (BBFA) on growth performance and enteric methane emissions in Nellore cattle during feedlot backgrounding and finishing phases. Ninety-six Nellore bulls (10 ± 2 mo old, body weight (BW) 315 ± 23 kg (block 1), and BW = 360 + 7 kg [block 2]) were allocated to 1 of 2 treatments: CONTROL (basal diet) and BBFA (basal diet plus BBFA [to target 23 mg bromoform/kg dry matter]). Bulls were housed in pens ( $n = 6$  animals/pen) and monitored over 218 d (108 d backgrounding, 110 d finishing). Performance parameters were measured in all bulls, while enteric methane emissions were assessed in a subset of 36 animals using the sulfur hexafluoride (SF<sub>6</sub>) tracer technique. Supplementation of the BBFA did not influence dry matter intake (DMI), weight gain or feed efficiency ( $P > 0.05$ ). Compared with control, BBFA decreased methane production and yield by 26% and 25%, respectively, during backgrounding ( $P < 0.001$ ). While, in the finishing phase, BBFA supplementation resulted in reductions of 58% in methane production, 59% in yield, and 60% in intensity ( $P < 0.001$ ). These findings demonstrate the potential to supplement the BBFA throughout the lifecycle of cattle in Brazil, reducing methane emissions without any detrimental impacts on productivity.

## Lay Summary

This study investigated whether adding a bromoform-based feed additive (BBFA) to cattle diets could reduce methane emissions without harming animal performance. Researchers worked with 96 young Nellore bulls, during backgrounding and finishing phases, for about 7 mo. The animals were divided into 2 groups: 1 received a control diet (basal diet), and the other received the basal diet plus the BBFA. Animal performance and feed intake were measured, and methane emissions were monitored using the sulfur hexafluoride (SF<sub>6</sub>) tracer technique. The BBFA did not affect overall performance. Bulls receiving the BBFA produced about 25% to 26% less methane in the backgrounding phase, and about 58% less methane in the finishing phase. Overall, the study showed that this bromoform-based feed additive can significantly reduce methane emissions in feedlot cattle without compromising productivity.

**Keywords** bromoform, greenhouse gas, ruminant nutrition

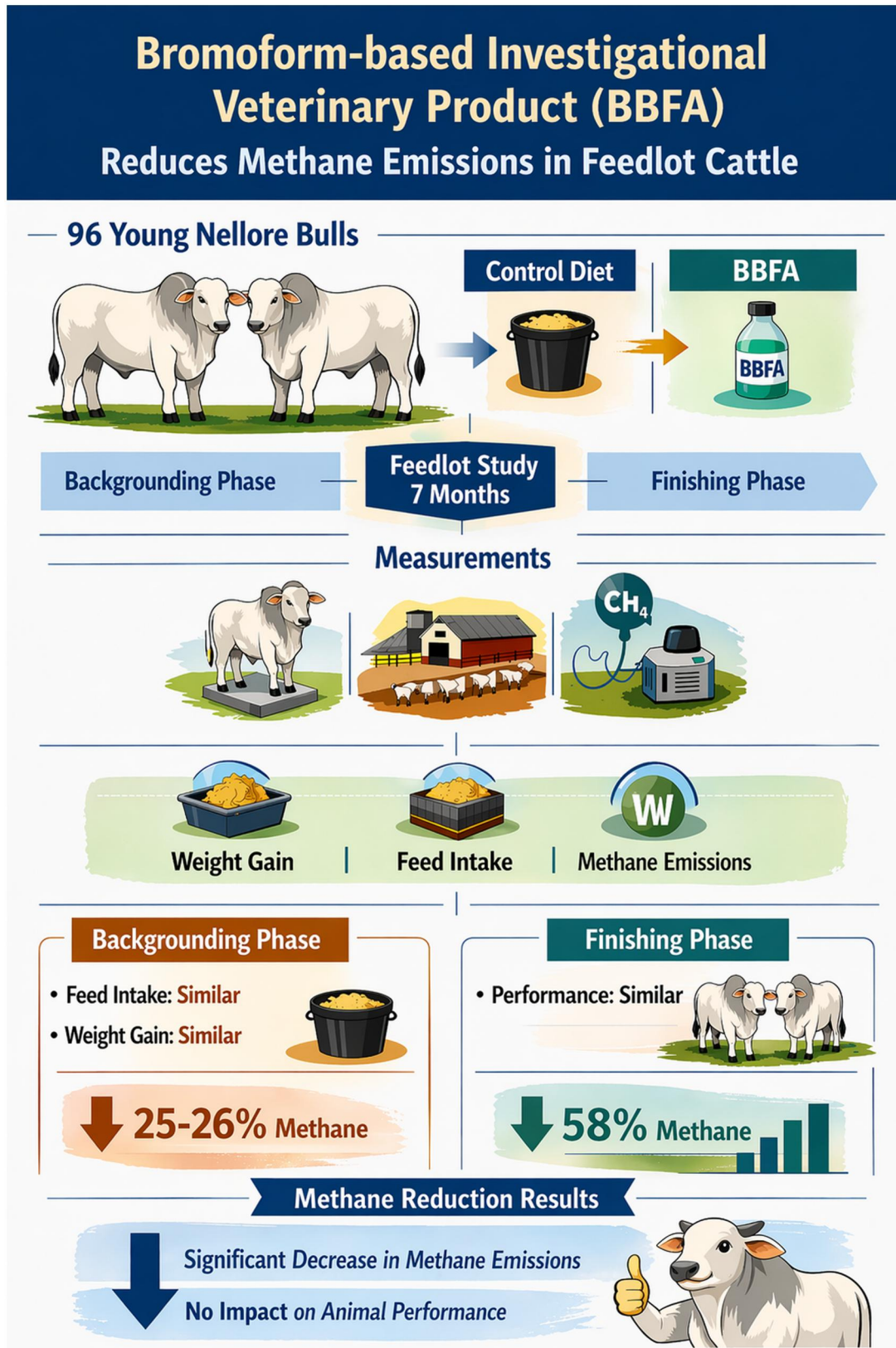
**Abbreviations:** ADG, average daily gain; BW, body weight; DDG, dried distiller's grain; DM, dry matter; FE, feed efficiency; FCE, feed conversion efficiency; GC, gas chromatography; HCY, hot carcass yield; TMR, total mixed rations

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## Graphical abstract



## Introduction

Feed additives that interfere with methanogenesis and reduce enteric methane emissions to varying degrees have recently been developed (Honan et al. 2022), offering the advantage of a rapid effect and being practical to integrate in total mixed rations (TMR) of feedlots and other livestock feeding systems (Crawford 2021). Bromoform has been identified as the primary active compound in seaweed-based feed additives, it can be administered either in freeze-dried form or as a vegetable oil formulation and have demonstrated enteric methane mitigation efficiencies of up to 95% when included in TMR for beef cattle (Kinley et al. 2020; Cowley et al. 2024). However, challenges around sustainably scaling seaweed cultivation and achieving a consistent quality of the product have been raised (Vijn et al. 2020; Zhu et al. 2021; Camer-Pesci et al. 2023).

The development of a synthetically produced product under controlled laboratory conditions offers a strategy to ensure scalability and product consistency. The recent development of a bromoform-based feed additive (BBFA) containing stabilized synthetic bromoform provides improved bromoform stability, accurate dosing concentration, and a complete product characterization (Gyeltshen et al. 2025; Sun et al. 2025). The methane emission mitigation efficacy of this BBFA has been evaluated in beef cattle under TMR and pasture-based feeding systems (Gyeltshen et al. 2025; Sun et al. 2025). Methane yield was reduced by 94% when an oil-based BBFA was fed to Angus-cross steers, delivering 30 mg bromoform/kg dry matter (DM) (Gyeltshen et al. 2025). Comparable reductions (95%) were reported by Kelly et al. (2025) when the BBFA was fed to Angus steers, delivering 32 mg bromoform/kg DM. The BBFA has also been demonstrated as a viable option for pasture-fed systems where supplementation is a feasible strategy. In a recent study, designed to mimic a potential delivery strategy for grazing animals, Sun et al. (2025) reported methane yield reductions of 24% when beef heifers were fed fresh pasture, and pulse dosed with the BBFA twice daily. These findings demonstrate that the BBFA represents a promising and scalable feed additive for methane mitigation across diverse cattle production systems.

To date, research assessing the methane-mitigating effects of bromoform containing seaweed, bromoform sourced from seaweed or synthetic BBFA's beef cattle has primarily consisted of short- to medium-term trials, typically lasting between 14 and 90 d (Kinley et al. 2020; Altman et al. 2024; Cowley et al. 2024; Kinley et al. 2024; Meo-Filho et al. 2024; Gyeltshen et al. 2025; Kelly et al. 2025; Sun et al. 2025). Only 3 longer-term studies have investigated sustained efficacy and productivity responses of bromoform containing additives, with trial durations of 147 d (Roque et al. 2021), 200 d (George et al. 2024), and 275 d (Cowley et al. 2023). Therefore, the objective of this research was to evaluate the response of feeding a BBFA oil to Nellore cattle during feedlot backgrounding and finishing phases (218 d) on enteric methane emissions and growth performance.

## Materials and methods

The experimental procedures used in this experiment followed the animal care and handling by the Brazilian College of Animal

Experimentation (COBEA—College of Animal Experimentation Guidelines) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee of the São Paulo State University (Unesp Jaboticabal, SP, Brazil, Protocol #9539/23).

The study was conducted in the Forage Farming sector of the Faculty of Agricultural and Veterinary Sciences, Sao Paulo State University (Unesp Jaboticabal), Brazil (latitude of 21°14'05"S, a longitude of 48°17'09"W, and an altitude of 615 m).

## Animals, treatments, and experiment design

The experiment evaluated the effect of a BBFA in oil form, on performance and methane emissions on beef cattle during 2 feedlot phases: backgrounding (from May 2024 to August 2024) and finishing (from September 2024 to December 2024).

Ninety-six Nellore bulls (10 ± 2 mo old, body weight (BW) 315 ± 23 kg (block 1), and BW = 360 + 7 kg (block 2) at the start of the backgrounding phase; 14 ± 2 mo old, 517 ± 29 kg BW at the start of the finishing phase) were used to evaluate performance parameters, of which 36 were also assessed for individual intake and enteric methane emissions.

Animals were allocated to 1 of 2 treatments in a randomized block design (initial BW (2 BW block levels) with 8 pens per treatment). The 2 treatments were: CONTROL, fed a basal diet only and BBFA, fed a basal diet plus BBFA. The BBFA was delivered at 5.3 mL/kg DMI, which equates to 23 mg broform/kg DMI. The BBFA was provided by Rumin8 (Rumin8 Pty Ltd, Perth, Western Australia, Australia) and mixed with the TMR and fed immediately each day.

The experiment had a total duration of 218 d, where the backgrounding phase lasted 108 d and the finishing phase lasted 110 d. The animals were housed in group pens; a total of 16 pens (10 × 8 m) were used with 6 bulls per pen. Water was delivered ad libitum. Each pen had a feed bunk, providing 1.2 m of bunk feeder space per animal, where the TMR was fed once daily. The backgrounding ration was composed of 60% corn silage and 40% of a concentrate composed of soybean hulls, cracked corn, dried distiller grains (DDG) and mineral salt, while the finishing ration was composed of 30% sugar cane silage and 70% of the concentrate (Table 1).

Prior to the experiment, bulls were adapted to the feedlot backgrounding diet for 14 d using a step-up protocol of 80% corn silage and 20% concentrate for 7 d followed by 60% corn silage and 40% concentrate for another 7 d. Total mixed rations were offered daily with adjustments based on feed refusals of the previous day (maintained around 5%), allowing for an ad libitum intake of TMR. During the adaptation phase (d 1 to 14) the animals gradually received increasing doses of the BBFA in four 3-d steps, with each step delivering 25, 50, 75, and 100% of the target dose. This was followed by three 28-d periods, with body weight recorded every 28 d, and the final 10 d dedicated to individual intake and enteric methane measurements, to ensure that for each sampled animal, there were at least 6 viable days of consecutive 24 h sampling. During the finishing phase, bulls were transitioned to the finishing diet over 14 d, followed by three 28-d periods, with BW recorded every 28 d, and individual intake and enteric methane emissions measured during the final 12 d of the experiment, to ensure that for each sampled animal, there were at least 6 viable days of consecutive 24 h sampling.

**Table 1** Ingredient proportion and chemical composition of backgrounding and finishing diets.

Item	Backgrounding diets		Finishing diets	
	Control	BBFA	Control	BBFA
<i>Ingredients proportion, %</i>				
Corn silage	60.00	60.00	0.00	0.00
Sugar cane silage	0.00	0.00	30.00	30.00
Soybean hulls	16.00	16.00	22.00	22.00
Cracked corn	12.00	12.00	30.00	30.00
DDG <sup>a</sup>	10.00	10.00	16.00	16.00
Mineral salt	2.00	1.91	2.00	1.95
BBFA <sup>b</sup>	0.00	0.09	0.00	0.05
<i>Chemical composition, %</i>				
Dry matter	52.80	52.80	69.56	69.56
Organic matter	94.87	94.87	94.73	94.73
Crude protein	11.98	11.98	12.12	12.12
Eter extract	3.32	3.32	3.39	3.39
Starch	18.50	18.50	23.00	23.00
Neutral detergent fiber	50.75	50.75	43.62	43.62
Nutrients digestible total	68.88	68.88	69.45	69.45

<sup>a</sup>DDG, dried distillers grain.

<sup>b</sup>BBFA, Bromoform based feed additive.

Bulls were fed once daily at 0700 h. The concentrates were first mixed using a mixer and then combined manually with the silage in the feed bunks. Feed refusals were collected and weighed before the morning feeding, and feed delivery was adjusted daily to maintain refusals at approximately 5% of the feed offered. In the BBFA treatment, 5.3 mL of BBFA per kg of TMR DM was included. Containers of BBFA were shaken daily before use, and the required amount was poured over the TMR and manually mixed for 20 s before feeding once daily and offered immediately.

## Proximate analyses

The sample of supplement ingredients diet and feces were dried at 55 °C for 72 h and ground in a Wiley mill (Thomas Scientific) to pass through a 1-mm screen, and analyzed for DM (method 934.01), OM (method 942.05), and EE (method 920.85) according to the Association of Official Analytical Chemists (AOAC1995). Nitrogen was determined using a LECO FP-528 nitrogen analyser (LECO Corp.). The NDF was determined using a-amylase and without the addition of sodium sulfite, according to the method reported by (Van Soest et al. 1991) and adapted for the Ankom200 Fibre Analyser (Ankom Technology, Fairport, NY, USA).

## Feed intake

Thirty-six animals, 18 per treatment were randomly selected from the 8 pens and were used for intake estimation, which were performed by marker methods. From the 8 pens in each treatment, 2 animals were selected from 6 pens, and 3 animals were selected from 2 pens. Fecal production was assessed using chromium oxide (Cr<sub>2</sub>O<sub>3</sub>) as an external marker. For that, a Cr<sub>2</sub>O<sub>3</sub> capsule (10 g/animal/d) was directly introduced into the esophagus of the animals at 0900 h for the last 10 consecutive days in

each experimental phase (d 98 to 108 and 207 to 218). Fecal samples were collected in the last 3 d (106 to 108 and 216 to 218 d) of the dosage period at 1100 and 1600 h, 0900 and 1500 h, and 0700 and 1400 h, on the first, second, and third days of sample collection, respectively. Fecal samples were dried at 55 °C for 72 h in forced air and ground (Wiley mill; Thomas Scientific) through a 1-mm sieve. The Cr<sub>2</sub>O<sub>3</sub> concentration in fecal samples was determined by atomic absorption spectrophotometry (Williams et al. 1962). Fecal production was estimated using the following equation: FP = Cr<sub>2</sub>O<sub>3</sub> supplied / (Cr<sub>2</sub>O<sub>3</sub> in feces / DM 105 °C) where FP = fecal production obtained by Cr<sub>2</sub>O<sub>3</sub> (g DM/d), Cr<sub>2</sub>O<sub>3</sub> supplied = amount of Cr<sub>2</sub>O<sub>3</sub> supplied to the animals per day (10 g), Cr<sub>2</sub>O<sub>3</sub> in feces = Cr<sub>2</sub>O<sub>3</sub> concentration in feces (%), and DM 105 °C = feces DM at 105 °C. Dry matter intake (DMI) was then estimated using indigestible neutral detergent fiber (iNDF) as an internal marker. For that, feces and ingredients of diet samples were dried at 55 °C for 72 h under forced air and ground to pass through a 2-mm screen sieve in a Wiley mill (Thomas Scientific). Then, samples were weighed, placed into ANKOM bags (Filter bag F57; ANKOM Technology Corporation), and incubated in the rumen of cannulated Nellore animals for 288 h (Valente et al. 2011). The NDF concentration of the bags was determined by an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY, USA), and DMI was calculated according to the equation: DMI = ((FP × [iMF]) / [iMH]) where FP = fecal production; iMF = internal marker concentration in feces, and iMH = internal marker concentration in the feed (diet/ingredients). Fecal samples were collected in the last 3 d of the dosage period as previously described for Cr<sub>2</sub>O<sub>3</sub>. Additionally, fecal samples were processed as previously described for Cr<sub>2</sub>O<sub>3</sub>.

## Animal performance

The assessment of animal performance was determined by weighing the animals at the beginning and end of each experimental phase, after a 16-h food and water fast. All animals were weighed non-fasting every 28 d to adjust the feed offered. Average daily gain (ADG) was calculated by the difference between the final and initial BW, divided by the number of experimental days. Average daily DM intake (DMI), during this period (1 to 98 and 123 to 206 d), was calculated by difference between feed offered and feed refusal of each pen and divided by the number animal per pen. Feed conversion efficiency (FCE) was determined as the ADG/DMI.

At the end of the experimental period, cattle were transported to a commercial beef processing facility and slaughtered on the same day. Pre-slaughter handling complied with recommended animal welfare practices, and slaughter procedures followed the Regulation of the Sanitary and Industrial Inspection of Products of Animal Origin. Immediately after slaughter, carcasses were weighed to obtain the hot carcass weight (HCW). Hot carcass yield (HCY) was calculated as the ratio of HCW to final body weight (BW).

## Methane emissions measurements

Methane emissions were measured using the sulfur hexafluoride (SF<sub>6</sub>) tracer technique (Johnson et al. 1994) over 5 consecutive 24-h measurement periods. Thirty-six animals, 18 animals per treatment, the same animals used for individual intake measurement were equipped with gas collection halters 14 d before

gas sampling to allow for adaptation to the equipment. To allow tracer gas to equilibrate in the rumen, 3 d before the first collection period started, a pair of permeation tubes with a constant and known SF<sub>6</sub> release rate was introduced directly into the rumen of each animal via the esophagus. In this experiment, 1345.83 ± 58.05 mg of SF<sub>6</sub> was introduced in the permeation tube, and the permeation rate of SF<sub>6</sub> from the permeation tubes averaged 1.623 ± 147.56 ng/min. Lifetime of the permeation tubes was 17.37 ± 1.6 mo, ie enough to maintain a constant diffusion rate of the SF<sub>6</sub> throughout the 218-d experiment.

Expired gases were continuously collected with a sampling apparatus consisting of a halter-mounted stainless-steel capillary tube (0.127 mm) connected to an in-line filter (15 µm). This system was attached to a pre-evacuated stainless-steel collection cylinder positioned on the animal's back. The negative pressure within the cylinder enabled continuous sampling as it gradually dissipated, drawing expired air from around the animal's mouth and nostrils. To account for background concentrations of methane and SF<sub>6</sub>, 2 additional pre-evacuated cylinders were placed daily in different sites at a consistent location throughout the feedlot (2 opposite ends of the feed stall corridor), and at ~1.0 m above ground level (height of the steers' nostrils), during each sampling period. Having 36 animals and 6 d of collection (1 cylinders/animal/d) in each study phase, 216 cylinders with breath samples were handled. However, data were analyzed only from 180 of those cylinders. As 2 background canisters were collected every sampling day, in total, 12 cylinders were handled, and data were analyzed only from 10 of those cylinders. Data were not analyzed from those cylinders with gas leakage (detected when pressure was measured after circadian circle). On average, 1 cylinders per animal was lost every period due to handling (gas leakage).

During the gas sampling period, animals were moved to a cattle chute, and collection cylinders were replaced daily at 0900 h with newly evacuated cylinders. At the end of each collection period, the remaining internal negative pressure of each cylinder (typically 40% to 60% of the initial pressure) was recorded. Initial pressure in the device averaged -13.14 PSI, and final pressure before changing averaged -6.85 PSI.

Methane concentrations in the collected samples were determined by gas chromatography (GC) equipped with a flame ionization detector. Analyses were performed in duplicate or triplicate to ensure reproducibility, with measurement error maintained below 3%. The GC system was calibrated against certified standard reference materials (SRM; NIST) containing known methane concentrations. SF<sub>6</sub> concentrations were determined using a GC equipped with an electron capture detector, calibrated with certified standards spanning 30 to 1000 µg/L. A 100 µg/L SF<sub>6</sub> standard was included as a routine control.

## Statistical analysis

Statistical analyses were performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). Normality and homogeneity of the data were tested using the UNIVARIATE procedure of SAS. Means were reported as least square means. Treatments were considered different when  $P < 0.05$  by Tukey's test.

The statistical model used was:  $Y_{ij} = \mu + T_i + B_j + e_{ij}$ , in which  $Y_{ij}$  represents the observation on experimental unit treatments  $i$

in block  $j$ ;  $\mu$  = the overall mean;  $T_i$  = Treatment  $i$ ;  $B_j$  = the block effect  $j$ ; and  $e_{ij}$  = the residues corresponding to each observation. The performance, intake, methane emissions and carcass characteristics data were compared between treatments by ANOVA as a randomized block considering the pen as the experimental unit. The fixed effects considered were treatments, block, treatments error, and the random effects of residues corresponding to the model. The statistical analysis of each phase, background or finishing were the same.

## Results

### Animal performance

During the backgrounding phase, no effect of feeding the BBFA were observed on DMI ( $P=0.186$ ), ADG ( $P=0.678$ ) or FCE ( $P=0.402$ ; Table 2). In the finishing phase, feeding the BBFA resulted in no observed effect on DMI ( $P=0.343$ ), ADG ( $P=0.399$ ) or FCE ( $P=0.580$ ). There was also no effect of treatment on hot carcass yield ( $P=0.327$ ; Table 2).

### Methane emissions

Cattle supplemented with the BBFA exhibited significant reductions in methane emissions. During the backgrounding phase, there was a 26.4% decrease in total methane production, a 24.9% decrease in methane yield and a 27% decrease in methane intensity compared to the control group ( $P < 0.05$ ; Table 3). During the finishing phase, cattle fed the BBFA exhibited even greater reductions compared to the control group, 58.4% in methane production, 58.7% in methane yield, and 60% in methane intensity ( $P < 0.001$ ; Table 3).

**Table 2** Dry matter intake and performance parameters of Nellore cattle fed a control diet or a bromoform based feed additive (BBFA) in a feedlot backgrounding and finishing diet (kg, unless otherwise specified).

Item	Diets		SEM <sup>a</sup>	P-value
	Control	BBFA		
<i>n</i>	8	8		
<b>Backgrounding phase</b>				
Dry matter intake (kg/d)	9.60	9.26	0.056	0.186
Initial BW <sup>b</sup>	356	358	3.034	0.877
Final BW	494	493	3.827	0.966
ADG <sup>c</sup> (kg/d)	1.39	1.36	0.023	0.598
FCE <sup>d</sup> (ADG/DMI <sup>5</sup> )	0.14	0.15	0.002	0.597
<b>Finishing phase</b>				
Dry matter intake (kg/d)	9.22	9.01	0.542	0.343
Initial BW <sup>b</sup>	520	516	4.117	0.814
Final BW	584	585	4.524	0.924
ADG <sup>c</sup> (kg/d)	0.91	0.96	0.025	0.399
HCY <sup>d</sup> (%)	59.52	59.17	0.181	0.327
FCE <sup>e</sup> (ADG/DMI <sup>6</sup> )	0.10	0.11	0.005	0.580

<sup>a</sup>SEM, standard error of the mean;

<sup>b</sup>BW, body weight;

<sup>c</sup>ADG, average daily gain;

<sup>d</sup>HCY, hot carcass yield;

<sup>e</sup>FCE, feed conversion efficiency.

**Table 3** Methane production, methane yield and methane intensity of Nellore cattle fed a control diet or a bromoform based feed additive (BBFA) in a feedlot backgrounding and finishing diet.

Item <sup>a</sup>	Diets		SEM <sup>b</sup>	P-value
	Control	BBFA		
<i>n</i>	8	8		
<b>Backgrounding phase</b>				
Dry matter intake (kg/d)	10.94	10.71	0.176	0.632
Average daily gain (kg/d)	1.09	1.10	0.062	0.922
Methane production (g/d)	179.35	132.04	6.964	0.001
Methane yield (g/kg DMI <sup>a</sup> )	16.57	12.44	0.662	0.004
Methane intensity (g/kg ADG)	176.05	137.28	11.556	0.186
<b>Finishing phase</b>				
Dry matter intake (kg/d)	11.03	11.08	0.179	0.897
Average daily gain (kg/d)	0.96	0.99	0.048	0.752
Methane production (g/d)	126.45	52.53	8.472	<0.001
Methane yield (g/kg DMI <sup>a</sup> )	11.53	4.76	0.780	<0.001
Methane intensity (g/kg ADG)	141.95	57.40	11.159	0.001

Abbreviations:

<sup>a</sup>DMI, dry matter intake (marker methods);

<sup>b</sup>SEM, standard error of the mean;

<sup>c</sup>ADG, average daily gain.

## Discussion

This study shows that after 218 d of feeding the BBFA, methane yield was abated by 59%. This result shows that methane abatement from bromoform is sustained after more than 200 d of feeding. Our findings align with those of Roque et al. (2021), who showed sustained methane mitigation efficacy in Angus-Hereford steers supplemented with seaweed, providing 35 and 70 mg of bromoform/kg DM for 147 d, achieving 45% and 68% reduction in methane yield, respectively. Also, Cowley et al. (2023) demonstrated persistent methane yield reduction of 22% when Wagyu steers received seaweed sourced bromoform at 25 mg/kg DM for 275 d. However, contrasting results have been reported by George et al. (2024), who found a progressive decline in methane abatement efficacy in steers fed 25 mg of seaweed sourced bromoform/kg DM over 200 d, with methane yield reductions decreasing from 62.3% in the first 50 d, to 55.8% in days 51 to 100, 45.8% in days 101 to 150, and 36.2% in the final 50 d of the study. A decline in methane mitigation efficiency has also been reported in a medium-term trial (59 d) where heifers were supplemented with a low dose of seaweed sourced bromoform (17 mg of bromoform/kg DM) (Cowley et al. 2024). However, in the study by Cowley et al. (2024), the medium (34 mg of seaweed sourced bromoform/kg DM) and the high (51 mg of seaweed sourced bromoform/kg DM) doses showed no decline in efficiency over time. The reasons behind the decline in methane mitigation efficiency in some trials is unknown, and several suggestions have been made. George et al. (2024) suggested that the decline in methane mitigation might be attributed to an increase in body weight, together with a decrease in DMI, resulting in a lower dose of bromoform per kilogram of body weight. On the other hand, Cowley et al. (2024) attributed the decline to suboptimal bromoform dosing, as this decline was not observed in the medium- and high-bromoform treatments. In a study by Stefenoni et al.

(2021), 20 lactating Holstein cows were fed seaweed-sourced bromoform in a Latin Square design for 4 periods of 28 d each. They observed a gradual decline of enteric methane inhibition by periods 3 and 4 compared to periods 1 and 2. However, further stability testing demonstrated pronounced degradation of bromoform over time in the seaweed, independent of storage conditions, which was further validated by a corresponding loss of in vitro efficacy after 90 d (Stefenoni et al. 2021). When rumen fluid samples from the study by Stefenoni et al. (2021) were used for microbiome analyses by Indugu et al. (2024), a significant reduction in *Methanosphaera* in period 1 samples was associated with an effective reduction in methane emissions, whereas by period 3 there were no significant differences in relative abundance of *Methanosphaera* between control and seaweed-fed groups (Indugu et al. 2024). It is possible that the results reported by Indugu et al. (2024) were driven by the loss of bromoform from the seaweed and not from microbial resistance. The observed variability across studies suggests that the decline in methane mitigation, observed in some cases, may be driven by factors such as bromoform source (synthetic or natural), bromoform formulation (in oil or freeze dried), bromoform stability, host physiology, dosing regime, and microbial communities. This is the first study to show that a BBFA can be fed to Nellore cattle under typical Brazilian feedlot conditions for over 200 d and still show strong methane abatement.

Most medium-term experiments (lasting 80 to 90 d) have also demonstrated the sustained efficacy of methane reduction over time. Kinley et al. (2020) and Kinley et al. (2024) fed bromoform for 90 and 81 d, respectively, with methane abatement of over 70%, and no loss of efficacy was reported. Furthermore, in the study by Kelly et al. (2025), Angus steers were fed a BBFA (same product as in the current experiment) for 84 d, resulting in a sustained methane reduction of over 90%. The methane abatement reported in the current experiment was lower than that reported by Kelly et al. (2025). However, the methane mitigation difference between the 2 studies is most likely driven by the different doses, as Kelly et al. (2025) fed 32 mg bromoform/kg DM, whereas the current study fed only 23 mg bromoform/kg DM. Interestingly, despite using modest inclusion levels, the methane reductions observed in this study exceed those reported at higher bromoform doses for long term trials. For example, methane yield reductions of 22%, 45%, and 36.2% were achieved when feeding bromoform sourced from seaweed at 25 mg/kg DM for 275 d to Wagyu steers (Cowley et al. 2023), at 35 mg/kg DM for 147 d to Angus-Hereford steers (Roque et al. 2021), and at 25 mg/kg DM in the last 50 d of a 200-d study to Angus steers (George et al. 2024), respectively, highlighting the long-term efficiency of the current formulation.

Although the BBFA mitigated methane emissions in both backgrounding and finishing phases, the methane reduction observed in the finishing period was numerically greater than that observed during the backgrounding period. However, this difference was not analyzed statistically and should therefore be viewed with caution. This variation may be attributed to the difference in dietary composition, as the backgrounding diets had high fiber content, while the finishing diets were high in concentrates. Greater proportional reductions when concentrates are fed were reported by Kebreab et al. (2025), who reviewed 14 trials with 39 treatments that fed bromoform and found that, overall, dietary composition affects methane mitigation efficacy of bromoform based feed

additives, with greater reductions observed in diets higher in starch. In contrast, higher fiber levels attenuate this effect. This is consistent with the findings of Kelly et al. (2025) and Gyeltshen et al. (2025), where the BBFA reduced methane emissions by up to 95% in cattle fed a high grain diet, but when Sun et al. (2025) fed a BBFA to cattle fed fresh pasture, methane abatement was 25%. However, in the study by Sun et al. (2025), the BBFA was not mixed with the feed but fed as a pulse dose in a mineral mix before the fresh pasture was offered, which may also have contributed to the observed methane abatement in that study. We suggest that further research is required to understand the interaction between bromoform dose, feeding regime, and the base diet on methane abatement. It should be noted that homogeneity of mixing of the BBFA into the TMR was not tested in this trial, so the exact bromoform intake per bull is uncertain. Moreover, the stability of bromoform in the 2 feeds was also not analyzed in this study. In this context, Sena et al. showed that when seaweed sourced bromoform in vegetable oil was mixed with a lamb feed, 92% of the bromoform was lost within 24 h of exposure to environmental conditions. It is unclear if this is a characteristic of that specific formulation or if there is an interaction with diet type on stability.

The second objective of this research was to evaluate the effect of the BBFA feeding on animal performance. There have been reports of increased productivity in cattle as an outcome of reducing methane emissions through feed additives (Kinley et al. 2020; Pupo et al. 2025). It has been reported that the increase in productivity following methane abatement is driven by a shift in the rumen fermentation pathways, whereby the excess hydrogen favors the production of some volatile fatty acids (such as propionate) over others (such as acetate) (Janssen 2010; Ungerfeld 2015a). This occurs because the partial pressure of hydrogen in the rumen determines the thermodynamics of fermentation pathways (Janssen 2010; Morgavi et al. 2023). When hydrogen concentration is high after methanogenesis has been interrupted, the production of propionate becomes thermodynamically favorable (Martinez-Fernandez et al. 2016; Romero et al. 2023). Furthermore, there are other hydrogen sinks which have been identified when methanogenesis is interrupted, such as acetogenesis, nitrate reduction to ammonia, sulfate reduction to hydrogen sulfite, formate formation and increases of microbial biomass production, which are all nutritionally beneficial and are associated with improved animal performance (Joblin 1999; van Zijderveld et al. 2010; Leng 2014, Ungerfeld 2015a). Decreased methane production along with increased productivity has been reported in trials evaluating bromoform based feed additives in beef cattle. For example, in a study by Roque et al. (2021), 21 Angus-Hereford steers were fed seaweed delivering either a low (35 mg/kg DM) or a high (70 mg/kg DM) bromoform dose, and FCE tended to increase by 7% in the low treatment and by 14% in the high treatment. Moreover, Kinley et al. (2024), showed that steers that received bromoform sourced from seaweed in a finisher diet had FCE improved by 7.4%, while 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 bromoform sourced from seaweed. However, there are also reports showing no effect or even negative effects of bromoform on animal performance. For example, Angellotti et al. (2025) and Eikanger et al. (2024) have reported decreased milk yield in dairy cattle fed freeze dried seaweed containing bromoform. According to the review by Kebreab et al. (2025), high

bromoform doses lead to reduced feed intake and dairy cattle appear more sensitive to this effect than beef cattle. These findings highlight the complexity of the relationship between methane abatement and animal performance. Although the numerical difference observed here during the finisher stage may be promising, those results should be taken with caution, as they were not statistically significant. That no adverse effects on productivity were observed is also promising, as it shows that the BBFA can be fed for over 200 d with sustained methane abatement, with no negative effects on productivity.

## Conclusion

This research demonstrated the effect of feeding a bromoform based feed additive for 218 d to Nellore cattle in a Brazilian feedlot. Feeding the BBFA, which delivered 23 mg bromoform/kg DM, reduced methane yield by 25% during the backgrounding phase and by 59% during the finishing phase, with no negative effects on animal performance.

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## Conflicts of interest

The authors confirm that this article is original, is not being considered for publication elsewhere, and has been approved by all authors. P.S. Alvarez-Hess, S. Jacques and R. Tognelli were employed by Rumin8 Pty Ltd during this experiment and provided technical information on the storage and use of the BBFA.

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