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Ruminant Nutrition

Effect of SeaFeed, a canola oil infused with *Asparagopsis armata*, on methane emissions, animal health, performance, and carcass characteristics of Angus feedlot cattle

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ABSTRACT

The objectives of this project were to measure the effect of SeaFeed, a canola oil infused with *Asparagopsis armata*, on methane emissions, animal health, performance, and carcass characteristics of feedlot cattle. Angus steers (n = 160) with an initial body weight of 474.4 kg were fed a steam-flaked wheat and barley ration for 200 d in a large, commercial feedlot. A single-blinded randomized complete block design compared two treatments including control and SeaFeed included at a rate of 25 mg bromoform per kg dry matter intake (DMI). Monthly testing of bromoform levels in the canola demonstrated that SeaFeed maintained a stable bromoform concentration for 6 mo. The inclusion of SeaFeed had no effect on daily DMI. However, steers fed SeaFeed were more efficient with a 6.6% higher ($P < 0.01$) gain-to-feed ratio as compared to control steers over the 200-d feeding period. This improved efficiency resulted in 0.094 kg higher ($P < 0.01$) average daily gain and 19.7 kg higher ($P < 0.01$) live exit weight in steers fed SeaFeed as compared to control steers. Steers fed SeaFeed produced 51.7% less ($P < 0.01$) methane and yielded 50.5% less ($P < 0.01$) methane over the 200-d feeding period as compared to cattle fed the control ration. Peak methane inhibition occurred on day 29 on feed with 90.6% less methane production in cattle fed SeaFeed as compared to control animals. SeaFeed reduced ($P < 0.01$) methane intensity by 55.4% over the 200-d feeding period. Hot carcass weight tended ($P = 0.097$) to be 6.9 kg heavier in cattle fed SeaFeed as compared to controls. Cattle fed SeaFeed had similar ($P > 0.20$) marbling, meat color, eye muscle, area, and ultimate pH to control cattle. Interestingly, cattle fed SeaFeed tended ($P = 0.054$) to have slightly higher fat color scores. Rumen papillae from cattle fed SeaFeed were more ($P < 0.01$) gray in color and more oval ($P < 0.01$) in shape as compared to control animals; however, rumen damage was not different between treatment groups. In regards to food safety and residues, all muscle, fat, and kidney samples were free from bromoform residues. Bromine residues in kidney and meat samples were higher ($P < 0.01$) in the SeaFeed group as compared to controls. Cattle fed SeaFeed produced strip loin steaks similar ($P > 0.05$) in eating quality to control cattle. These results demonstrate that SeaFeed reduced methane emissions, improved performance, and produced safe beef with similar eating quality to conventional beef.

LAY SUMMARY

Practical strategies to reduce enteric methane emissions from cattle are urgently required to achieve global methane reduction goals. While several candidate feed additives are safe and effectively mitigate methane emissions, commercial trials that measure the effects on performance and carcass characteristics are lacking in the literature. This project measured the effect of a commercially available low-emission feed technology, SeaFeed, a canola oil infused with the red seaweed *Asparagopsis armata*, on productivity, carcass characteristics, beef palatability, and greenhouse gas emissions of Angus cattle fed in a large, commercial feedlot. While there was no difference in dry matter intake, Angus steers fed SeaFeed for 200 d converted feed more efficiently into carcass weight with higher average daily gain and exit weight as compared to cattle fed a control ration. Cattle fed SeaFeed demonstrated a significant reduction in methane production, yield, and intensity. Strip loin steaks from control and SeaFeed-fed cattle were similar in regards to consumer sensory attributes including juiciness and flavor. Residue testing confirmed the muscle, fat, and kidney from cattle fed SeaFeed were free from bromoform residues. These results demonstrate that SeaFeed reduced methane emissions, improved performance, and produced safe beef with similar eating quality to conventional beef.

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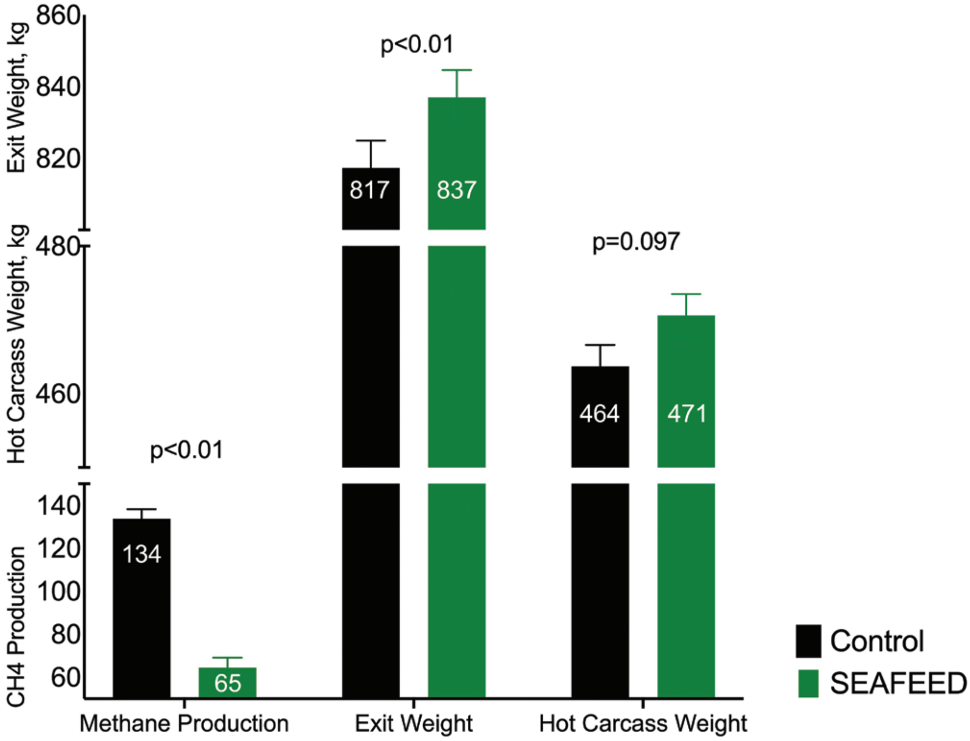
GRAPHICAL ABSTRACT



Key words: *Asparagopsis*, cattle, feedlot, methane, seaweed

Abbreviations: CH_4, methane;DM, dry matter;kg, kilogram;Mcal, megacalorie;mg, milligram;MJ, megajoule;Ne_g, net energy of gain;Ne_m, net energy of maintenance;PPM, parts per million;SeaFeed, *Asparagopsis armata* infused canola oil

Introduction

Practical strategies to reduce enteric methane emissions from cattle are urgently required to achieve global methane reduction goals. Methane emissions associated with beef production are a priority with accelerated efforts focused on the development and commercialization of mitigation strategies (Stackhouse-Lawson et al., 2012). Enteric fermentation in cattle is the largest contributor of methane from beef cattle (Glasson et al., 2022). Several enteric methane mitigation strategies have been identified (Hegarty et al., 2021) and developed, including the commercially available SeaFeed (Sea Forest Ltd, Triabunna, Tasmania, Australia), an *Asparagopsis armata* infused canola oil containing bromoform. While several candidate feed additives are safe and effectively mitigate methane emissions, commercial trials that measure the effects on performance and carcass characteristics are lacking in the literature. Feeding cattle to achieve optimal performance requires excellent formulation skills by doctoral-level ruminant nutritionists, but equally important are the daily execution of consistent grain processing, ration mixing and delivery, and strict adherence to animal health, comfort, and welfare parameters. This project demonstrates a unique collaborative effort between the government, a commercial feedlot, veterinarians, nutritionists, meat scientists, and a science-based technology company to seriously address the challenge of climate change in a commercial environment.

Asparagopsis armata is a red macroalgae that has been shown to reduce methane emissions in cattle (Roque et al., 2019). Although *Asparagopsis* is known to contain a wide range of organobromine compounds, the bioactive metabolite, bromoform, has been identified as the primary metabolite responsible for the activity of *Asparagopsis* in the reduction of methane emissions (Glasson et al., 2022). A novel method to stabilize the secondary metabolites of *Asparagopsis*, particularly bromoform, in a vegetable oil carrier by steeping *Asparagopsis* in canola oil was described (Magnusson et al., 2020). This process was shown to maintain the stability of bromoform over 6 mo in the canola oil carrier (Tan et al., 2023). SeaFeed is a commercially available canola oil that has been infused with *A. armata*, resulting in the stabilization of the oil-soluble metabolites such as bromoform in the canola oil. Vegetable oils are commonly fed to feedlot cattle and hence inclusion of a methane mitigation feed technology in the form of a vegetable oil is a valid delivery mechanism and solution to reduce methane emissions of feedlot cattle (Ridoutt et al., 2022).

Inhibition of methanogenesis by bromoform is due to the blocking of key metalloenzymes of the Wolfe cycle (Glasson et al., 2022). Specifically, bromoform and other halogenated alkanes react competitively with substrates of coenzyme M transferase and methyl coenzyme M reductase to block steps six and seven of the Wolfe Cycle of methanogenesis (Glasson et al., 2022). The human food safety of consuming products from cattle fed bromoform has been evaluated as methanogens in the rumen metabolize bromoform through reductive dehalogenation, which in combination with minimally effective inclusion levels of bromoform limits the transfer of bromoform to animal tissues and food products (Glasson et al., 2022). While mechanistically it is understood that food products from cattle fed bromoform should be free of bromoform residues, this trial provided highly sensitive residue data on a large number of animals fed SeaFeed for an extended period of time to fully answer the questions of potential residues.

Originally, the scientific community postulated that redirection of energy lost as methane would result in improved feed utilization and improved animal productivity (Patra, 2012). Unfortunately, many of the published articles have not identified performance benefits associated with methane mitigation feed additives in feedlot cattle (Alemu et al., 2020, 2023; Almeida et al., 2023; Araújo et al., 2023; Cowley et al., 2024). This is possibly due to the experimental design of many of the trials and the observation that the feed intake was unlikely maximized in several of the trials due to experimental design constraints. For example, many of the previous trials were performed in respiration chambers which limit natural behaviors and feed intake. The objective of this trial was to utilize commercial feedlot nutritional formulations, commercial ration adaptation protocols, consistency of feed preparation and delivery, pen conditions, and experimental design to achieve maximal feed intake and performance parameters to evaluate if there is truly a performance benefit to feeding methane mitigation feed technologies such as SeaFeed. While the methanogenesis inhibitory activity of bromoform and *Asparagopsis* have been well-established, the effect of SeaFeed on methane production, intensity, and yield has not been established in high-performing Angus cattle over a 200-d period. The results of this study provide daily individual animal methane emissions, intake, and body weight to allow important insights in regards to the effect of methane mitigation on performance parameters over an extended feeding period.

Consumer eating experiences can be significantly impacted by differences in flavor, tenderness, and juiciness. Hence, the addition of feed additives such as those derived from *Asparagopsis* raises questions in regards to impacts on consumer eating experience. This study was designed to answer important questions through an extensively trained sensory panel evaluation. The results of this trained sensory panel are essential for branded beef program owners, restaurants, retailers, and cattle producers to ensure the quality of the eating experience consumers will experience if cattle have been fed the mitigant.

Thus, the objectives of this project were to measure the effect of SeaFeed on methane emissions, animal health, performance, and carcass characteristics of Angus cattle fed for 200 d in a large, commercial feedlot. The authors hypothesized that cattle fed SeaFeed would produce less methane and hence have increased feed efficiency resulting in increased performance with no difference in carcass characteristics.

MATERIALS AND METHODS

Animal Ethics

This project was completed under the approval of the Queensland Government Department of Agriculture and Fisheries Animal Ethics Committee (Animal Ethics Committee Reference Number: SA 2022/12/865) by the registered scientific user of animals in Queensland, Bovine Dynamics (Registration Number: SUR001552).

Experimental Design

A single-blinded randomized complete block design was conducted to evaluate the effect of SeaFeed (Sea Forest Ltd), a bromoform-containing canola oil on methane emissions, performance, animal health, and carcass characteristics of Angus feedlot cattle. Individual animal was the experimental unit as the pens contained individual feed intake, weight, and methane emissions monitoring systems. Individual steers (n = 160) were sequentially allocated to one of four groups of cattle with 40 individuals per group as they completed induction processing to the feedlot. Four pens were blocked by row with two adjacent pens in the same row allocated to each block. Within a block, a random number generator was used to allocate pen to treatment. A random number generator was used to assign four groups of 40 individuals to one of the four pens. The study was powered to detect a 2.0-kg hot standard carcass weight response at 80% power between control and SeaFeed treatments.

Cattle Processing

Angus steers (n = 160) were sourced from a single vendor in Northern New South Wales, Australia. Steers arrived to the trial site on April 6, 2023, were scanned using individual RFID ear tags, individually weighed in a calibrated hydraulic chute (Sumo, Thompson Longhorn, Goombura, Queensland, Australia), vaccinated with Rhinogard Bovine Herpesvirus 1 Live Intranasal Vaccine for Cattle (Zoetis, Parsippany, NJ, USA, 2 mL, intranasal) and Bovishield MH One Mannheimia Haemolytica Vaccine for Cattle (Zoetis, 2 mL, subcutaneous), treated with Dectomax V Dual Combination Injection for Cattle (Zoetis, 5 mg/mL doramectin, 150 mg/mL levamisole, 20 mL, subcutaneous), Flukare C (Virbac Australia, Milperra, New South Wales, Australia, 120 g/L triclabendazole, 50 mL), and backgrounded for 28 d days prior to the commencement of the trial. Steers gained 1.28 kg/d over the backgrounding phase.

Steers were individually weighed on a calibrated scale that was tested with 200 kg of certified weights prior to processing on May 2, 2023. Steers were held overnight on an arrival ration. Steers were reweighed on May 3, 2023 to produce an average induction weight over 2 d. Steers were inducted to a 20,000-head commercial feedlot in south-eastern, Queensland, Australia on May 3, 2023.

At induction processing, steers were tagged in the ear with individual visual identification and lot number, Rhinogard Bovine Herpesvirus 1 Live Intranasal Vaccine for Cattle (Zoetis, 2mL, intranasal) and Bovilis MH Mannheimia Haemolytica Vaccine for Cattle (Coopers Animal Health, East Bendigo, Victoria, Australia, 2 mL, subcutaneous), treated with Dectomax V Dectomax V Dual Combination Injection for Cattle (Zoetis, 5 mg/mL doramectin, 150 mg/mL levamisole, 21 mL, subcutaneous) and Nitrofluke (Virbac Australia340 g/L nitroxynil, 67 g/L clorsulon, 17 mL, subcutaneous), and orally drenched with Lactipro Advance (Axiota Animal Health, Fort Collins, CO, USA, Megasphaera eldensii, 50 mL, oral). Steers (n = 160) selected for study enrollment were within a body weight range of 476 to 522 kg with a mean of 502.2 and 11.87 kg SD. Steers were excluded from the group if outside the targeted body weight range or had an average daily gain of less than 0.6 kg/d during the backgrounding phase.

Pen Designs and Monitoring Systems

Feedlot pens were cleaned at the commencement of the trial and water troughs were cleaned twice a week. There were a total of four feedlot pens with 40 heads per pen. Each pen

Table 1. Composition and analyzed nutrient content (DM basis) of diets

Item	Dietary treatment	SeaFeed
	Control	
Finisher ration ingredient, %		
Steam-flaked wheat	52.87	52.87
Steam-flaked barley	25.17	25.17
Corn silage	6.88	6.88
Finisher supplement[1]	5.15	5.15
Vegetable oil	3.82	3.82
Cereal hay	2.50	2.50
Spent grains	2.25	2.25
Protein meal	0.88	0.88
Control canola oil	0.49	0.00
SeaFeed canola oil	0.00	0.49
Active ingredient concentration		
Bromoform, mg/kg	0.00	25.00
Finisher ration formulated composition, %		
Ne_m, Mcal/kg	2.18	2.18
Ne_g, Mcal/kg	1.49	1.49
Metabolizable energy, MJ/kg	13.36	13.36
Crude protein, %	13.50	13.50
Calcium, %	0.73	0.73
Phosphorus, %	0.37	0.37
Finisher ration analyzed composition, %		
Dry matter, % (as-fed basis)	72.13	71.93
Neutral detergent fiber, %	20.70	20.51
Crude protein, %	14.35	14.53
Fat, %	6.75	6.74
Ash, %	5.71	5.75
Crude fiber, %	6.01	5.84
Metabolizable energy, MJ/kg	13.58	13.57

[1]Finisher supplement contained vitamins, minerals, and monensin at 23.99 PPM.

was equipped with eight Feed Intake Nodes (Vytelle, Lenexa, KS, USA) to measure the dry matter intake (DMI) of individual animals. Pens also contained in-pen weighing position scales (Vytelle) associated with the water troughs. Two pens in a single row contained GreenFeed Pasture Systems (C-Lock, Rapid City, SD, USA) to allow emissions monitoring for control and SeaFeed-treated cattle. The 200-d feeding period commenced at the time when cattle were placed into the pens with the feed intake nodes.

Ration Formulations and Feeding

A mixing test was performed with a 2-tonne batch of feed (as-fed) which demonstrated coefficients of variation for fat, zinc, and moisture of 4.3%, 7.8%, and 3.5%, respectively, demonstrating the ration was adequately mixed to provide consistent levels of the active ingredient, bromoform, throughout the ration delivery.

Following processing, steers were transitioned onto finisher ration using a three-step program over 18 d with each of the three rations fed for 6 d (Supplementary Table S1). For the SeaFeed cattle, the inclusion level of the SeaFeed canola oil containing bromoform was increased in each ration resulting in a bromoform level of 0 mg bromoform/kg dry matter for days 1 to 6, 8.15 mg/kg for days 7 to 12, 16.56 mg/kg for days 13 to 18, and 25.06 mg/kg from days 19 to 200.

Cattle were fed steam-flaked wheat and barley diets including a vitamin and trace mineral supplement that exceeded recommended requirements (NASEM, 2016). The ingredient and nutrient composition of the finishing rations are provided in Table 1. Feed bunks were evaluated visually at approximately 0600 hours and a residual feed estimate was made and cattle were adjusted using the standard bunk procedure to maintain ad libitum feed intake. Orts were removed and weighed to ensure the feed bunk was clean every other day to maintain fresh feed free of spoilage. Feed was delivered one time daily at 0630 hours using a truck mounted with a Roto-Mix 920 delivery box.

Samples of steam-flaked wheat, steam-flaked barley, and finisher ration were collected daily from the feed bunk, and subsamples of the ration were dried at 100 °C to determine the daily dry matter of the ration. Monthly control and SeaFeed ration samples were collected and submitted for proximate analysis. Daily dry matters were averaged weekly and used to calculate dry matter.

Pellets were used as an attractant to the GreenFeed Pasture Systems in two pens (one control pen and one SeaFeed pen). The ingredient and nutrient composition of the pellets are provided in Supplementary Table S2. Daily feed intake of pellets was monitored and accounted for in daily feed intakes.

Testing for Bromoform Concentration of Water, Canola Oil, and Rations

Samples were tested by gas chromatography-mass spectrometry. Duplicate samples of the control and SeaFeed canola oils and control and SeaFeed finisher rations were submitted monthly to two laboratories (Analytical Services Tasmania, New Town, Tasmania, Australia and National Measurement Institute, Port Melbourne, Victoria, Australia) for testing of bromoform concentration. Water was tested in duplicate and bromoform levels were less than 1.0 mg/kg. Monthly bromoform levels for canola oil and rations are displayed in Table 2.

Testing for Iodine Concentration of Water, Canola Oil, and Rations

Iodine level testing was performed by the NATA-accredited method (VL345) at the National Measurement Institute. Samples were homogenized and extracted with a solution of 25% tetramethylammonium hydroxide at 90 °C for 3 h. Following dilution and filtration, the iodine level in the solution was determined by Inductively Coupled Plasma Mass Spectrometry. The limit of reporting was 0.01 mg/kg. Water was tested in duplicate and the iodine level was 0.075 mg/L. Monthly iodine levels for canola oil and rations are displayed in Table 2.

Animal Health

Animal health was monitored daily by trained pen riders. Animals expressing clinical signs of morbidity were taken to the hospital for further evaluation, and treatment was performed according to the feedlot health protocol designed by a veterinarian. Tulathromycin plus ketoprofen (Draxxin KP Plus Ketoprofen Injectable Solution for Cattle, Zoetis, 1 mL/40 kg, subcutaneous) was the first line of treatment for Bovine Respiratory Disease. Oxytetracycline dihydrate

Table 2. Bromoform and iodine levels in control and SeaFeed canola oil and rations over 6 mo[1]

Item	Canola Oil		Rations	
	Control[2]	SeaFeed	Control	SeaFeed
Bromoform, mg/kg				
Month 1	43.3	5,700.0		
Month 2	<10.00	6,290.0	<1.00	7.00
Month 3	11.0	6,170.0	<1.00	10.00
Month 4	26.0	6,070.0	<1.00	7.00
Month 5	29.0	5,660.0	<1.00	10.00
Month 6	13.0	6,290.0	2.00	10.00
Iodine, mg/L				
Month 1	<0.01	1.70	0.71	0.70
Month 2	<0.01	1.40	0.51	0.48
Month 3	<0.01	1.40	0.66	0.65
Month 4	0.01	1.60	0.70	0.68
Month 5	<0.01	1.60	0.54	0.56
Month 6	<0.01	1.40	0.64	0.54

[1]Iodine levels were tested by inductively coupled plasma mass spectrometry at the National Measurements Institute. Bromoform levels were tested by gas chromatography-mass spectrometry at Analytical Services Tasmania.
[2]Bromoform was detected in control canola oil and was determined to be a likely result of the canola oil extraction procedure. The result was not a result of contamination.

(Bivatop 200 Long Acting Injectable, Boehringer Ingelheim Animal Health, Ingelheim, Germany, 1mL/10 kg, subcutaneous) was the second line of treatment for Bovine Respiratory Disease. There were two mortalities in the trial. The causes of death as confirmed by a field necropsy were acidosis ($n = 1$) and broken leg ($n = 1$). Two animals were diagnosed with severe polioencephalomalacia. These animals were treated, improved, and completed the feeding period, but never fully recovered in terms of growth and productivity and hence were removed from the study as the disease was unrelated to treatment. Importantly, during this period there was an increased number of polioencephalomalacia cases in the Australian feedlot industry associated with very high feed intakes and animal performance.

Emissions

Methane emissions were measured using the GreenFeed Pasture systems with one system placed in a control pen and one system placed in a SeaFeed pen within the same row for the 200-d duration of the trial. The GreenFeed Pasture systems were fitted with auto-calibration and hence there was no need for system rotation between pens. The GreenFeed Pasture systems were protected by metal paneling around the perimeter of the trailer and paneling and wood panels positioned at the alley to ensure that only a single animal could access the system at one time. Settings for the GreenFeeder Pasture systems were: 8 maximum drops per feeding period, 30-s drop interval, 18,000-s feeding period duration, 5 maximum feeding periods per day, 40 maximum drops per day per animal, and head proximity sensor at 800. Weekly maintenance included cleaning the primary air filter, refilling the bin with pellets, cleaning the head proximity sensor, cleaning the air inlet with a wire brush, and cleaning the solar panels. Carbon dioxide recoveries were performed monthly. Auto-calibrations were performed every 3 d.

Daily DMI of pellets was recorded by the number of drops animals received according to the system and multiplied by the mass of the drop and corrected for dry matter. The intake of the pellets was included in the total DMI. The average daily methane emission rate per animal was calculated. Methane was expressed as yield (g methane/kg DMI) by dividing the average daily emission by the DMI for the animal.

Feed Intake

The DMI of each animal was calculated as the sum of the dietary dry matter consumed from the basal diets offered from the Feed Intake Nodes and the dry matter consumed of the pellets from the GreenFeed Pasture Systems. Daily feed delivered per pen according to truck weights was also recorded.

Live Weight Gain

Initial body weight was calculated as the average body weight across 2 d at the commencement of the trial (May 2, 2023 and May 3, 2023). Individual live weight was measured daily for 200 d by in-pen weighing position scales (Vytelle). Cattle were weighed over a calibrated group weigh bridge and a calibrated truck weighbridge at the exit.

Carcass Data

Cattle exited the feedlot at 0600 hours on November 19, 2023. Cattle remained on full feed until the time of exit. Cattle were transported 130 km (2 h) to the processing plant. Cattle remained on water in shaded lairage pens overnight. Cattle were processed from 0600 to 0900 hours on November 20, 2023. This resulted in a total time off feed prior to slaughter of approximately 24 h and a lairage duration of 22 h. Carcass data were collected by trained personnel from Bovine Dynamics Pty Ltd (Kenmore, Queensland, Australia). Individual animal visual identification tag was recorded and affixed to the harvest sequence number for each carcass. The time of stun was recorded manually. Electronic RFID tags were recorded at the time of stun.

Rumen gross pathology was scored using a published scoring rubric for papillae color, papillae shape, and damage in the ventral sac (Jonsson et al., 2020). Carcasses were dressed to AUS-MEAT carcass standards and hot standard carcass weight (kg) was measured after evisceration and trimming (AUS-MEAT, 2005).

Carcasses were chilled overnight for approximately 22 h, ribbed between the 12th and 13th ribs, and then graded by a single qualified Meat Standards Australia grader (Polkinghorne et al., 2008). Carcass characteristics measured include eye muscle area of the *longissimus thoracic et lumborum* at the carcass quartering site (cm^2), rib fat depth (mm) of subcutaneous fat of the *longissimus thoracic et lumborum* at the carcass quartering site, P8 fat depth (mm) of subcutaneous fat aligned with the crest of the third sacral vertebra, Meat Standards Australia marbling score which is the amount of fat between muscle fibers within the muscle of the *longissimus thoracic et lumborum* at the carcass quartering site (cm^2), Aus-Meat meat color of the *longissimus thoracic et lumborum* at the carcass quartering site, Aus-meat fat color of intermuscular fat lateral to the *longissimus thoracic et lumborum* at the carcass quartering site, Meat Standards Australia Index calculated using a predictive model of eating quality, and ultimate pH of the *longissimus thoracic et lumborum* (AUS-MEAT, 2005; Polkinghorne et al., 2008). Aus-Meat meat color was scored as 1A = 1.00, 1B = 1.33, 1C = 1.67, 2 = 2.00, 3 = 3.00, 4 = 4.00, 5 = 5.00, and 6 = 6.00.

Trained Sensory Evaluation

Striploin sections (International Meat Purchasing Specifications 180, 8-cm length) were shipped chilled to the Gordon W. Davis Meat Laboratory at Texas Tech University. Striploins were wet-aged for 32 d and then frozen at −20 °C until sensory testing. Each striploin section was cut into two face steaks, one 2.8-cm sensory steak, and one 2.8-cm extra steak. Steaks for trained sensory evaluation were thawed for 24 to 48 h at 2°C and reached an internal temperature of 0 to 4.3 °C at the time of cooking. Groups of steaks (n = 4) were singly layered and cooked on a grill grate (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) centrally located in a combi-oven (Model SCC WE 61 E; Rational) at 204 °C, 0% relative humidity, and default fan speed. Steak temperature was monitored in the cooking process using an oven core temperature probe (Model SCC WE 61 E; Rational) placed in the geometric center of a steak representative of the cooking group for size and shape. Steaks were removed from the oven at 69°C to target a peak internal temperature of 71°C. Peak internal temperature was measured in the geometric center of each steak using a calibrated type K thermocouple thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT, USA) and recorded. A raw weight (prior to cooking) and cooked weight (after cooking) were collected for each of the steaks.

Panelists were trained to identify and quantify attributes associated with beef according to the lexicon developed by for beef flavor in intact muscle (Adhikari et al., 2011). A continuous 100-point line scale (0 = tough, dry, not present; 100 = tender, juicy, extremely intense) was adapted and used to quantify attributes: tenderness, juiciness, beef flavor identity, browned, roasted, fat-like, buttery, grassy, liver-like, sour, overall beef flavor. Upon training, a sensory test was used to qualify a pool of 7 trained panelists for sensory evaluation. After peak internal steak temperature was measured, each steak was held on a cooling rack in an electric, insulated food transport box (Model UPCH400110, Cambro Manufacturing Company, Huntington Beach, CA) maintained at 55°C until serving. Cooked steaks were served to panelists within 15 min of their removal from the oven. Each of the 4 panel sessions consisted of 15 steaks (n = 60). No panelist served on more than 2-panel sessions per day, and a minimum of 1 h existed between the end of each session and the start of another. Panelists were seated in individual cubicles in a dark room under red incandescent lighting. Distilled water, apple juice, and unsalted saltine crackers were supplied as palate cleansers. Immediately before serving, cooked steaks were trimmed of any remaining external fat and connective tissue and cut into cubes (1 cm × 1 cm × steak thickness). Each panelist received 3 cubes for evaluation. Study samples were evaluated every 3.5 to 4 min by 6 to 7 panelists per session. Panelist responses were recorded on an electronic ballot generated by an online survey software (Qualtrics Surveys, Provo, UT) using a tablet (iPad, Apple, Inc., Cupertino, CA). Intensity ratings for each attribute were averaged among panelists for each sample.

Bromoform, Bromine, and Iodine Residue Testing in Kidney, Fat, and Muscle

Kidney samples (n = 30 control, n = 30 SeaFeed) of approximately 70 g of tissue were obtained at the time of removal from the carcass. Subcutaneous fat samples (n = 30 control, n = 30 SeaFeed) of approximately 70 g of tissue were obtained from the ventral brisket region of the carcass. Lean skeletal muscle samples (n = 30 control, n = 30 SeaFeed) of approximately 70 g of tissue were obtained from the foreshank including the flexor carpi ulnaris and superficial digital flexor muscles.

All tissue samples were stored in sterile containers, chilled with ice bricks, and transported chilled overnight to the laboratory. Bromoform testing was completed by gas chromatography-mass spectrometry (Analytical Services Tasmania). Bromine and Iodine testing was completed by the NATA-accredited method (VL345) at the National Measurement Institute.

Statistical Analyses

The experimental unit was defined as the individual animal for all outcome variables. The fixed effect of treatment and random effect of block were included in the model. Statistical significance of interactions and main effects were defined at $P < 0.05$ and a trend at $P < 0.10$ levels. Variables were analyzed using a linear mixed model, PROC MIXED SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

The experiment was analyzed with dead cattle removed as the cause of death was not associated with treatment. One individual was euthanized due to a broken leg which was not associated with treatment and hence was removed from the study. The other mortality was diagnosed as acidosis on post-mortem examination and was removed from the study. Two additional animals with severe polioencephalomalacia were removed from the study and details of these animals are included above.

RESULTS AND DISCUSSION

Stability of Bromoform in SeaFeed Canola Oil and Ration Over Six Months

The level of bromoform in the SeaFeed canola oil was stable over the 6-mo experimental phase of the trial (Table 2). A low level, less than 50 mg/kg, of bromoform was detected in the control canola oil. It was confirmed that this low level of detection was not due to contamination, but a result of the extraction process used in canola oil manufacturing.

The level of bromoform in the SeaFeed and control rations are summarized in Table 2, demonstrating detection of bromoform in total-mixed rations using gas chromatography-mass spectrometry is possible. Bromoform levels in rations with SeaFeed were consistent over the 6 mo.

Iodine Levels in SeaFeed Canola Oil and Ration Over Six Months

The level of iodine in control and SeaFeed canola oil and rations was reported over 6 mo (Table 2). Iodine was not detected in the control canola oil. However, iodine levels ranged from 1.4 to 1.7 mg/L in the SeaFeed canola oil. Levels of iodine in control and SeaFeed rations were similar.

Animal Performance

Animal performance data including body weights and DMI are provided in Table 3. By experimental design, initial body weight did not differ between treatments ($P = 0.64$). DMI throughout the feeding period was not different between treatments ($P = 0.67$). The final body weight was 19.7 kg heavier ($P < 0.01$) for SeaFeed cattle as compared to control cattle. Average daily gain was 0.09 kg/d higher ($P < 0.01$) in cattle fed SeaFeed as compared to control cattle over the 200-d feeding period. Gain:feed was 0.01 higher ($P < 0.01$) for steers fed SeaFeed as compared to controls.

Animal Health

There were two mortalities in the trial, both in the control group. Causes of death as confirmed by a field necropsy were acidosis ($n = 1$) and broken leg ($n = 1$). There was no difference ($P = 0.52$) in morbidity between the treatment groups. Bovine respiratory disease was the most common ailment with both treatment groups treating 44.9% of the cattle (35/78 individuals). Two animals were diagnosed with severe polioencephalomalacia. These animals were treated, improved, and completed the feeding period, but never fully recovered in terms of growth and productivity and hence were removed from the study as the disease was unrelated to treatment. Importantly, during this period there was an increased

Table 3. The effect of SeaFeed canola oil on performance of Angus steers

Item	Treatment		SEM	*P*-value
	Control	SeaFeed		
Steers, n	78	78		
Body weight, kg				
Initial	473.9	474.9	1.40	0.64
Day 50	609.4	623.7	8.34	<0.01
Day 100	701.7	718.0	8.21	<0.01
Day 150	765.8	783.9	7.51	<0.01
Final	817.3	837.0	7.58	<0.01
Average daily gain, kg				
Days 1 to 50	2.71	2.98	0.152	<0.01
Days 51 to 100	1.82	1.84	0.040	0.67
Days 101 to 150	1.28	1.32	0.033	0.44
Days 151 to 200	1.00	1.03	0.038	0.58
Days 1 to 100	2.28	2.43	0.075	<0.01
Days 1 to 150	1.95	2.06	0.046	<0.01
Days 1 to 200	1.72	1.81	0.035	<0.01
Daily DMI, kg per individual				
Days 1 to 50	10.90	10.98	0.396	0.69
Days 51 to 100	12.37	12.32	0.270	0.76
Days 101 to 150	11.23	11.08	0.354	0.40
Days 151 to 200	10.47	10.36	0.321	0.46
Days 1 to 100	11.64	11.65	0.329	0.95
Days 1 to 150	11.50	11.46	0.336	0.78
Days 1 to 200	11.24	11.19	0.331	0.67
Gain:feed				
Days 1 to 50	0.249	0.271	0.0050	<0.01
Days 51 to 100	0.147	0.150	0.0034	0.52
Days 101 to 150	0.114	0.121	0.0047	0.07
Days 151 to 200	0.093	0.098	0.0040	0.27
Days 1 to 100	0.196	0.209	0.0023	<0.01
Days 1 to 150	0.169	0.181	0.0019	<0.01
Days 1 to 200	0.153	0.163	0.0020	<0.01

Table 4. The effect of SeaFeed canola oil on emissions of Angus steers

Item	Treatment		Reduction (%)	SEM	*P*-value
	Control	SeaFeed			
Steers with valid observations, n	37	34			
Methane production, g CH_4/d					
Days 1 to 20 (adaptation)	136.72	72.06	47.3	9.604 to 9.787	<0.01
Days 1 to 50	152.56	49.31	67.7	5.977 to 6.376	<0.01
Days 51 to 100	133.49	58.03	56.5	5.758 to 5.586	<0.01
Days 101 to 150	123.58	68.19	44.8	4.516 to 4.584	<0.01
Days 151 to 200	122.5	78.54	35.9	4.841	<0.01
Days1 to 100	141.99	56.23	60.4	5.388 to 5.469	<0.01
Days1 to 150	137.39	59.67	56.6	4.690 to 4.759	<0.01
Days 1 to 200	133.74	64.54	51.7	4.392 to 4.582	<0.01
Methane yield, g CH_4/kg total DM intake					
Days 1 to 20 (adaptation)	13.49	7.55	44.0	0.953 to 0.971	<0.01
Days 1 to 50	13.10	4.94	62.3	0.620 to 0.661	<0.01
Days 51 to 100	11.32	5.01	55.8	0.477 to 0.484	<0.01
Days 101 to 150	11.10	6.02	45.8	0.421 to 0.428	<0.01
Days 151 to 200	11.84	7.55	36.2	0.497	<0.01
Days 1 to 100	12.14	5.23	56.9	0.479 to 0.487	<0.01
Days 1 to 150	11.84	5.36	54.7	0.420 to 0.426	<0.01
Days 1 to 200	11.86	5.87	50.5	0.389 to 0.406	<0.01
Methane intensity, g CH_4/kg liveweight gain					
Days 1 to 20 (Adaptation)	70.97	21.54	69.6	8.500 to 8.662	<0.01
Days 1 to 50	61.79	18.50	70.1	2.687 to 2.733	<0.01
Days 51 to 100	71.84	34.13	52.5	3.618	<0.01
Days 101 to 150	99.42	57.87	41.8	5.857 to 6.223	<0.01
Days 151 to 200	162.49	88.19	45.7	17.455	<0.01
Days1 to 100	66.79	24.72	63.0	2.424 to 2.499	<0.01
Days1 to 150	73.35	29.70	59.5	2.472 to 2.508	<0.01
Days 1 to 200	82.73	36.86	55.4	2.794 to 2.914	<0.01

number of polioencephalomalacia cases in the Australian feedlot industry associated with very high feed intakes and animal performance.

Methane Emissions

GreenFeed Pasture System visitation data are provided in Supplementary Table S3. A total of 17,687 valid observations with a mean duration of 3 min 51 sec were included in the data set. Of the 76 individuals with access to the GreenFeed Pasture systems, 71 provided acceptable data, demonstrating a visitation rate of 93.4% of animals. The average number of observations per day was 88, 53.5 in the control pen, and 34.9 in the SeaFeed pen.

Methane emissions data are provided in Table 4. Steers fed SeaFeed produced 51.7% less ($P < 0.01$) methane and yielded 50.5% less ($P < 0.01$) methane over the 200-d feeding period as compared to cattle fed the control ration. Peak methane inhibition occurred on day 29 on feed with 90.6% less methane production in cattle fed SeaFeed as compared to control animals. Over the entire feeding period, methane production for control Angus steers was 133.74 g methane per individual per day and steers fed SeaFeed produced 65.54 g methane per individual per day. SeaFeed reduced ($P < 0.01$) methane intensity by 55.4% over the 200-d feeding period.

Carcass Characteristics

Carcass grading data and rumen gross pathology scores are provided in Table 5. Steers fed SeaFeed tended ($P = 0.097$) to produce carcasses with 6.9 kg additional hot carcass weight as compared to cattle fed control rations. There was no difference ($P > 0.10$) in marbling, meat color, eye muscle area, rib fat, ultimate pH, or MSA index. Cattle fed SeaFeed tended ($P = 0.054$) to have higher fat color scores than control carcasses; however, the magnitude of this difference was only 0.18 units on a 9-point scale. Rumen papillae of steers fed SeaFeed were more gray in color ($P < 0.01$) and more oval in shape ($P < 0.01$) as compared to steers fed control rations.

Residue Testing for Bromoform, Iodine, and Bromine

In regards to food safety and residues, all muscle, fat, and kidney samples were free from bromoform residues to a limit of detection of 0.05 mg/kg bromoform in 30 control and 30 SeaFeed animals (Table 6). Bromine residues in kidney samples were higher ($P < 0.01$) in the SeaFeed group as compared to controls. While bromine residues in meat samples were higher ($P < 0.01$) in the SeaFeed group as compared to controls, residues were much lower than those found in kidney samples. There was no difference ($P = 0.66$)

Table 5. The effect of SeaFeed canola oil on carcass weight and characteristics of Angus steers

Item	Treatment		SEM	*P*-value
	Control	SeaFeed		
Carcasses, n	78	78		
Hot carcass weight, kg	463.7	470.6	2.903	0.097
MSA marbling	568.3	549.3	25.668	0.26
Aus-Meat marbling	3.4	3.2	0.232	0.38
Meat color[1]	1.7	1.6	0.038	0.30
Fat color	0.5	0.6	0.065	0.054
Eye muscle area, cm^2	74.4	75.3	1.021	0.51
Rib fat, mm	8.3	8.2	0.144	0.57
P8 fat, mm	28.3	28.7	1.255	0.74
Chiller assessment pH	5.48	5.48	0.005	0.47
MSA index	64.89	64.84	0.280	0.83
Ossification, cold	158.1	154.7	1.605	0.14
Dentition	2.8	2.7	0.171	0.46
Rumen papillae color	0.43	0.91	0.139	<0.01
Rumen papillae shape	0.75	1.22	0.133	<0.01
Rumen papillae damage	0.01	0.01	0.016	1.00

[1]Meat color was scored as 1A = 1.00, 1B = 1.33, 1C = 1.67, 2 = 2.00, 3 = 3.00, 4 = 4.00, 5 = 5.00, 6 = 6.00.

Table 6. The effect of SeaFeed canola oil on bromoform, bromine, and iodine residues of Angus steers[1]

Item	Treatment		SEM	*P*-value
	Control	SeaFeed		
Carcasses, n	30	30		
Bromoform, mg/kg				
Fat	<0.05	<0.05	0.000	1.00
Kidney	<0.05	<0.05	0.000	1.00
Muscle	<0.05	<0.05	0.000	1.00
Bromine, mg/kg				
Fat	2.88	2.98	0.164 to 0.167	0.66
Kidney	14.73	21.66	0.295 to 0.296	<0.01
Muscle	5.2	7.4	0.171 to 0.174	<0.01
Iodine, mg/kg				
Fat	0.0036	0.0004	0.00101 to 0.00103	0.03
Kidney	0.0774	0.0782	0.00155 to 0.00157	0.72
Muscle	0.0180	0.0183	0.000591 to 0.000601	0.68

[1]Results presented on wet matter basis.

in bromine levels in fat. Iodine levels were higher ($P = 0.03$) in fat samples from control animals as compared to samples from SeaFeed animals. There was no difference ($P > 0.50$) in iodine levels of kidney and muscle.

Trained Panel Sensory Characteristics

Results of the trained sensory panel analyses are provided in Table 7. Cattle fed SeaFeed produced strip loin steaks that were similar ($P > 0.05$) in eating quality to control cattle.

DISCUSSION

These results demonstrate that SeaFeed is a safe and effective methane mitigant with performance benefits when fed to Angus cattle at a rate of 25 mg bromoform/kg dry matter for 200 d. The stability of the active metabolite, bromoform, in canola oil was demonstrated over the 6-mo duration of the project which is consistent with recent studies (Magnusson et al., 2020; Tan et al., 2023; Cowley et al., 2024).

While the stability of bromoform levels in the oil is established and consistent with the results of the present study, laboratory analyses of bromoform levels in total-mixed rations containing *Asparagopsis*-infused vegetable oil have not been previously published. Importantly, this study demonstrated consistent detection of bromoform in the ration throughout the study. While levels presented are lower than expected, this may be due to the volatility of bromoform during mixing, feeding, sampling, transport to the laboratory, and final testing. Additionally, it

Table 7. Sensory characteristics of striploin steaks (*n* = 60) from Angus steers fed SeaFeed canola oil

Attribute	Treatment		SEM	*P*-value
	Control	SeaFeed		
Tenderness	55.70	53.40	0.849	0.07
Juiciness	50.40	48.80	0.908	0.22
Flavor attributes				
Beef	54.10	53.60	0.340	0.27
Browned	50.90	51.00	0.425	0.90
Roasted	52.30	52.10	0.352	0.58
Fat-like	11.90	11.30	0.377	0.30
Buttery	1.19	1.22	0.250	0.93
Grassy	3.61	2.95	0.300	0.13
Liver-like	1.42	1.08	0.219	0.28
Sour	1.73	1.63	0.253	0.77
Overall beef flavor	53.00	52.70	0.681	0.79

may be possible that the levels are extremely low and hence difficult to accurately detect the true level with current analysis methods. Nevertheless, the efficacy of the bromoform was clearly evident in the emissions reduction results.

The inhibitory effect of SeaFeed on methane emissions was evident in the results throughout the duration of the feeding period with 51.7% reduction in methane emissions. As the cattle were adapted to the finisher diet, the level of inclusion of bromoform and total daily DMI increased and hence methane inhibition increased. The peak reduction in methane production occurred on day 29 of the feeding period with 90.6% less methane production in cattle fed SeaFeed as compared to control animals. SeaFeed also reduced methane intensity and yield. Over the 200-d duration of the feeding trial, it is evident that the inhibitory effect on methane emissions was reduced over time. This observation was identified by Cowley et al. (2024) in the low *Asparagopsis* inclusion group where bromoform was included at 17 mg bromoform/kg DMI. Cowley et al. (2024) explained that adaptation leading to loss of antimethanogenic efficacy has not occurred in published studies where *Asparagopsis* products have been fed at the higher end of the range of effective inclusion levels. There are several hypotheses for this observation which have been noted in other work. It is quite possible that methanogens develop tolerance to the bromoform over continuous exposure and hence their activity levels and efficacy decrease over time. An alternative hypothesis that the authors particularly support is that the dose rate of bromoform (mg bromoform/kg body weight) is associated with the efficacy of the mitigant and hence as cattle become heavier and feed intake is reduced, the dose rate of bromoform is reduced and hence the inhibitory effect is reduced. More research is required to determine the true mechanism behind the moderation in methane inhibition over the feeding period and to develop strategies to extend the mitigation effects of the additive. Further research should be completed on the effect of the dose rate of bromoform, not simply the dose delivered.

Originally, the scientific community postulated that redirection of energy lost as methane would result in improved feed utilization and improved animal productivity (Patra, 2012). Specifically, a 22% increase in average daily gain was identified in Brahman-Angus cross steers fed 0.2% organic matter *Asparagopsis taxiformis* over 90 d (Kinley et al., 2020). The present study identified a significant performance response with an additional 19.7 kg in live weight gain, 0.09 kg average daily gain, and 0.01 kg gain:feed when cattle were fed SeaFeed. However, recent publications have not identified a performance response to feeding *Asparagopsis* or vegetable oil products containing active metabolites of *Asparagopsis* (Li et al., 2018; Stefenoni et al., 2021; Cowley et al., 2024). It is likely that many of the recent trials had experimental design constraints such as the use of chambers or small sample sizes that limited feed intake and natural behaviors of cattle and hence performance was unlikely maximized. The authors believe that this trial allowed the cattle to reach their true performance potential due to the commercial realities of the trial being performed in a large, commercial feedlot. Specifically, the trial was completed with professional ration formulation, consistent feed preparation and delivery, excellent pen conditions, and reliable daily execution to achieve maximal feed intake and performance parameters.

This study identified mild changes in rumen color and shape associated with feeding SeaFeed; however, all rumens in the study were healthy and did not show signs of damage. The minor changes in the papillae shape and color are not alarming.

Meat, kidney, and fat from cattle fed SeaFeed for 200 d were free from bromoform residues, demonstrating the safety of feeding SeaFeed for extended periods of time. These results are consistent with previous work (Kinley et al., 2020; Cowley et al., 2024). This is the most extensive data set with 30 treated and 30 control animals tested, demonstrating a much more thorough level of evidence than most toxicology studies. Since methanogens in the rumen metabolize bromoform through reductive dehalogenation, these results were expected (Glasson et al., 2022). Bromine levels were 6.92 mg/kg higher in the kidneys of cattle fed SeaFeed as compared to controls. Bromine levels were 2.2 mg/kg higher in the muscle of cattle fed SeaFeed as compared to controls. These elevated levels of bromine in cattle fed bromoform are consistent with previous publications (Cowley et al., 2024). Bromine is excreted via the urinary system and hence the elevated levels in the kidneys were expected. Importantly, the levels of bromine observed in the muscle and kidney products are well below the acceptable daily intakes for bromine given expected serving sizes and hence do not represent a human food safety risk (Cowley et al., 2024).

CONCLUSION

This 200-d study completed in a commercial feedlot demonstrates that supplementing a steam-flaked wheat and barley diet with SeaFeed (25 mg bromoform/kg DMI) reduced methane emissions by 51.7%, improved performance with an additional 19.7 kg of live exit weight, and produced safe beef with similar eating quality to conventional beef.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Conflict of interest statement

None declared.

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