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Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed

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ABSTRACT

Ruminants are responsible for a large proportion of agricultural greenhouse gas emissions in the form of methane. This can be managed. It is a global initiative to increase productivity of the livestock sector to meet a growing population, but with emphasis on decreasing enteric methane to achieve emissions targets. We investigated the marine red macroalga (seaweed) *Asparagopsis taxiformis* as a feed ingredient to fundamentally eliminate enteric methane in beef cattle fed a high grain diet and provide evidence of improved livestock production performance. *Asparagopsis* was included in the feed of Brahman-Angus cross steers at 0.00%, 0.05%, 0.10%, and 0.20% of feed organic matter. Emissions were monitored in respiration chambers fortnightly over 90 d of treatment, steers were weighed weekly prior to feeding, feed intake monitored daily, rumen fluid samples collected in conjunction with respiration chambers for assessment of rumen function, feces were collected for bromoform residue analysis, and meat, organ, and fat were collected post slaughter for residue analysis and sensory evaluation. Steers receiving 0.10% and 0.20% *Asparagopsis* demonstrated decreased methane up to 40% and 98%, and demonstrated weight gain improvements of 53% and 42%, respectively. There was no negative effect on daily feed intake, feed conversion efficiencies, or rumen function, and no residues or changes in meat eating quality were detected. Commercial production of *Asparagopsis* could create new economies, and with low inclusion rates of this seaweed in ruminant diets the industry has the potential to revolutionize management of greenhouse gas emissions across the ruminant livestock sector with complementary benefits to the environment, and economy of the wider agriculture sector.

1. Introduction

In response to escalating global greenhouse gas (GHG) inventories and subsequent increased pressure on climate change management, it is widely published that food production systems need to solicit highly effective innovative strategies to mitigate the GHG contributions from agriculture (Mayberry et al., 2019; Herrero et al., 2016; Olivier et al., 2005). Improved production efficiency while significantly decreasing GHG emissions from livestock agriculture, is urgent. The largest agriculture GHG contribution is from cattle and sheep production systems that are responsible for up to 18% of total global GHG emissions and mainly in the form of enteric methane (CH_4) (Herrero and Thornton, 2013). Enteric CH_4 is a by-product from anaerobic fermentation of feed organic matter (OM) by a microbial consortium in the rumen. This enteric fermentation produces carbon dioxide (CO_2) and hydrogen (H_2) which is then instrumental in the formation of CH_4 in a reduction pathway by microbial methanogenic archaea (Morgavi et al., 2010). Global concern due to emissions from red meat and dairy production is driving research and development of innovative feed ingredients with the capability to significantly mitigate or preferentially eliminate enteric CH_4 and beneficially redirect feed energy otherwise lost as CH_4 (Patra, 2012). These feed ingredients are diverse with variable efficacy (Maia et al., 2016) and include seaweeds (Kinley

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and Fredeen, 2015).

A hallmark of livestock agriculture is diversity in feed systems and ingredients relative to regional climate, feed stock availability, regulatory guidelines, and pasture types. One such enterprise to offset deficiencies is feeding seaweeds which has long been practiced but has met variable levels of acceptance throughout the history of animal husbandry (Evans and Critchley, 2014). With the application of modern nutritional science for livestock, precision feed formulation has transformed and is open to inclusion of novel ingredients which do not fit conventional feeding practices driven by cost of production. Natural feed ingredients and particularly those that sustainably decrease the environmental impact of food production are increasingly more important to consumers and producers. The focus here is on the seaweed genus *Asparagopsis* which is emerging as an effective tool in innovative and regenerative cleaner production for the wider agriculture sector (Mayberry et al., 2019). Seaweeds are also commonly used in the bioremediation of discharge from marine aquaculture (de Paula Silva et al., 2008) and *Asparagopsis* has been demonstrated as a more efficient biofilter than the most commonly used seaweed biofilters (Mata et al., 2010). When subsequently fed to ruminant livestock it could dramatically reduce the GHG contribution from agriculture and there is indication of potential for improved livestock productivity (growth, lactation, gestation). *Asparagopsis* could create new opportunities using local labor for cultivation and support regional economies while reducing environmental impacts and improving the efficiency of aquaculture and agriculture.

Knowledge of the capability of the red seaweed *Asparagopsis* to inhibit rumen microbial methanogenesis was developed in a stepwise process. Paul et al. (2006) reported a unique cellular structure in *Asparagopsis* capable of retaining bioactive compounds, including bromoform, with antimethanogenic properties (Machado et al., 2016a). A rumen *in vitro* study screened 20 different macroalgae species and *Asparagopsis taxiformis* was identified as the primary candidate for further investigation (Machado et al., 2014). Subsequent *in vitro* work to determine optimum inclusion rates for ruminants has demonstrated no negative impact on fermentation with 99% decrease in CH_4 (Kinley et al., 2016a). Validation of the *in vitro* work was demonstrated *in vivo* with a clear inclusion level response effect and decrease of 80% CH_4 production in sheep (Li et al., 2018). Further validation of the capability of *Asparagopsis in vitro* (Roque et al., 2019a; Kinley et al., 2016b) and *in vivo* as a functional feed ingredient for lactating dairy cattle demonstrated CH_4 decrease of 67% (Roque et al., 2019b). A recent Swedish *in vitro* study evaluated previously demonstrated chemical and plant based dietary strategies for functional and significant enteric CH_4 mitigation (Chagas et al., 2019). The authors reported *Asparagopsis* as the most potent inhibitor and the seaweed had the least impact on rumen fermentation. These studies demonstrated a substantial reduction in effective inclusion level *in vivo* compared to *in vitro*. However, there has been no demonstration in utility with beef feedlot style high grain diets which represent the feeding system that is most practical to penetrate with the seaweed due to fully formulated rations. As a result *Asparagopsis* has become a high priority for further research and development based on its capacity to mitigate enteric CH_4 emissions and potentially generate livestock productivity gains. The discovery of the efficacy of *Asparagopsis* as an antimethanogenic agent for ruminant production systems is tenable as the most promising option to achieve carbon neutrality in the livestock sector in the next decade.

The aim of this study was to demonstrate for the first time the effectiveness of high quality *Asparagopsis taxiformis* in beef cattle fed a high grain diet in the systematic *in vivo* characterization of *Asparagopsis* as an antimethanogenic feed ingredient. Based on preceding *in vitro* (Kinley et al., 2016a) and *in vivo* (Li et al., 2018) work it was hypothesized that inclusion of *Asparagopsis* in the total mixed ration (TMR) of beef cattle would significantly decrease enteric CH_4 emissions and improve productivity represented by increased daily weight gain. The objectives of the study were to: (1) demonstrate inclusion level effects of the red seaweed *Asparagopsis* at four feed inclusion levels under intensive beef feedlot finishing conditions over 90 d; (2) quantify the effect of *Asparagopsis* on enteric CH_4 and H_2 production in beef cattle; (3) quantify effect on ruminal metabolites; (4) quantify effect on beef cattle weight gain performance; (5) determine effect on meat eating quality; and (6) determine if residues of the bioactive compound responsible for antimethanogenic effect are present in meat, offal, or feces from steers consuming a high grain diet containing *Asparagopsis taxiformis*.

2. Methods

2.1. Asparagopsis and experimental diet

Asparagopsis taxiformis in the gametophyte lifecycle stage was collected near Humpy Island, Keppel Bay, Qld, AUS (23°13′01″S, 150°54′01″E). The collected biomass was frozen and stored at −15 °C then freeze dried by a commercial food processing company (The Forager Food Company, Red Hills, Tas, AUS) to approximately 95% dry matter (DM) to retain the volatile bromoform *in situ* (Vucko et al., 2017) as the demonstrated active ingredient (Machado et al., 2016a). The freeze dried *Asparagopsis* biomass of approximately 50% organic matter (OM) was milled to 2−3 mm (Hobart D340 mixer, Troy, OH, USA) to ensure a uniform product and stored at −15 °C. The bromoform concentration of the uniform as fed *Asparagopsis* was 6.55 mg/g. The *Asparagopsis* was incorporated into a high grain TMR (Table 1) containing Rhodes grass (*Chloris gayana*) as the primary fiber source and steam rolled barley as the energy dense component of the diet using a horizontal paddle mixer to provide a homogenous preparation with a coefficient of variation <8%. The nutrient composition of the basal TMR (Table 2) was analyzed on four composite samples compiled from series samples collected weekly throughout the project.

Table 1
Composition of the total mixed ration for each Asparagopsis inclusion level.

Ingredient As-Fed Basis, %	Control	0.05% OM (Low)	0.10% OM (Mid)	0.20% OM (High)
Freeze dried *Asparagopsis*	nil	0.09	0.18	0.36
Rhodes Grass Hay	8.00	8.00	8.00	8.00
Steam rolled barley	70.8	70.7	70.6	70.4
Limestone ($CaCO_3$)	1.00	1.00	1.00	1.00
Vegetable Oil	3.20	3.20	3.20	3.20
Whole Cottonseed	9.00	9.00	9.00	9.00
Molasses/Vitamin/Mineral Blend	8.00	8.00	8.00	8.00

Table 2
Nutrient composition of the basal total mixed ration.

Component	% of DM
Rhodes grass hay (% DM)	80.6
Rhodes grass hay NDF (% DM)	72.3
Rhodes grass hay ADF (% DM)	46.9
TMR Dry Matter (% DM as fed)	88.5
Ash (% DM)	8.57
TDN (% DM)	71.9
Organic Matter (% DM)	92.3
ME (Mcal/kg DM)	2.96
NEm (Mcal/kg DM)	1.74
NEg (Mcal/kg DM)	1.12
Starch (% DM)	23.2
Fat (% DM)	8.33
NDF (% DM)	30.6
CP (% DM)	15.5
Ca (% DM)	0.60
P (% DM)	0.35
Mg % DM)	0.23
K (% DM)	0.68
Vitamin A (KIU/kg DM)	3.99
Vitamin E (IU/kg DM)	24.9
Monensin (ppm)	24.9
Salt (% DM)	0.25
Urea % DM)	0.77

2.2. Animals and experimental design

Twenty Brahman-Angus cross (Brangus) steers were maintained at the Commonwealth Industrial and Scientific Research Organization (CSIRO) Lansdown Research Station near Townsville, Qld, AUS (19°39′27.0″S, 146°50′04.6″E). Experimental animals were managed throughout the study period according to guidelines of the Australian code for the care and use of animals for scientific purposes (NHMRC, 2013) and approved by the CSIRO Queensland Animal Ethics Committee (permit A10/2015). After adaptation to the TMR and the day before the initial baseline respiration chamber sessions, steers were weighed and had average live weight (LW) of 477 kg and were allocated to four blocks of five individuals based on ascending LW in a randomized incomplete block design.

Fig. 1 illustrates timewise adaptation of the experimental animals to the feedlot diet and *Asparagopsis*, gas measurements, and sampling events throughout the trial. All treatment group feed formulations were fed *ad libitum*, in one daily feeding (c. 09:00). Throughout the feed adaptation and treatment periods steers were maintained in sheltered and shaded individual pens with dimensions of 2.14 m × 4.15 m providing each steer with 9.1 m^2 as per industry standards and guidelines for cattle (AHA, 2016). Individual external pens were located adjacent to the animal house facility which contained four open circuit respiration chambers. Throughout the 90 d treatment period all steers from each treatment group were managed on 14 d cycles to allow comparable periods in individual pens and open circuit respiration chambers (24 h) to determine daily individual feed dry matter intake (DMI) and CH_4 and H_2 emissions. All treatment groups were accommodated in each measurement period, and all steers passed through each respiration chamber to remove chamber effect. This routine provided a pre-treatment baseline and five independent measures of enteric CH_4 and H_2 production for each steer and allowed determination of efficacy of each treatment level of *Asparagopsis* over time.

Throughout the study period all steers in each treatment group (Control, Low, Mid, High) were fed the TMR *ad libitum*. The steers were adapted to the basal Control diet over 45 d by gradually increasing the grain component using starter-split-intermediate-split-final diet steps with slow increments between each feeding stage. Following adaptation to the basal TMR, steers in representative groups of four were sequentially (staggered start) placed in one of four open circuit respiration chambers for pre-treatment baseline measurements then began introduction to their respective *Asparagopsis* treatment TMR. Steers were accustomed and adjusted to their final respective inclusion levels of *Asparagopsis* in an exploratory process over 30 d to characterize the inclusion range for the experiment. Inclusion levels were determined by placing steers in respiration chambers for 2 h monitoring periods to identify the minimum inclusion level that induced CH_4 reduction and from that process treatment groups were set as *Asparagopsis* range of 0.00, 0.05, 0.10, and 0.20% (OM basis) of the TMR for the Control, Low, Mid, and High inclusion levels, respectively. Steers remained on their respective *Asparagopsis* treatment level for a further 60 d. All steers finished their dietary treatment with *Asparagopsis* on the same day following the final chamber session of the last group to ensure they were slaughtered with an equivalent withholding period (2 d).

2.3. Feed and feces analysis

Dry matter content of the TMR and *Asparagopsis* was determined on each feed batch by drying at 105 °C to constant weight. Organic matter (OM) was determined by loss on ignition at 600 °C for 8 h (Horwitz, 2000). Feces were collected from the rectum of the steers following their respective respiration chamber sessions. Bromoform content of the *Asparagopsis* and feces was quantified as previously described (Paul et al., 2006) and determined by extraction in methanol with naphthalene as internal standard and the extract analyzed using gas chromatography and mass spectrometry (GC-MS). Routine feed analyses were completed on composite samples through commercial feed analyses services applying near infrared reflectance spectroscopy (NIRS) (González-Martín et al., 2006).

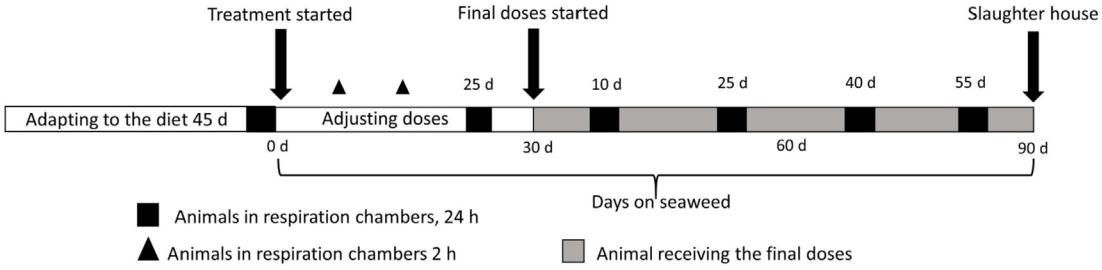

Fig. 1. Experimental timeline including adaptation, management of *Asparagopsis* inclusion, *ad libitum* feeding and treatment periods, respiration chamber sessions, and total days on *Asparagopsis* prior to slaughter.

2.4. Enteric methane and hydrogen production

Technical parameters of the open circuit respiration chambers have been reported in detail (Martinez-Fernandez et al., 2016). In brief, dimensions of the chambers were 4.0 m × 2.4 m × 2.4 m resulting in an internal volume of 23 m^3. Each chamber was constructed of a galvanized steel frame covered with 4.5 mm clear polycarbonate and sealed to be gas tight yet allowed for full visibility of steers during measurement periods. Each chamber was equipped with drinking water and a feed bin containing the daily ration and fitted with a door (1.05 m × 2.10 m) at either end for entry and exit. Measurements for total CH_4 and H_2 production were taken over 24 h. Exact flow rates were corrected to measured conditions for temperature and pressure for each individual chamber and were used in calculations for CH_4 and H_2 production (Williams et al., 2007). The atmosphere inside the chambers was maintained at 2 °C below ambient temperature. Atmospheric pressure was held at approximately −10 Pa with relative humidity in the range of 50%–75% throughout the 24 h measurement periods. Chamber exhaust samples passed through the membrane drier and through independent rotameters before analysis for CH_4 (Servomex 4100, Servomex Group Ltd, Egham, Surrey, GBR) and H_2 (Servomex Chroma). System calibration and gas recoveries were accomplished by releasing standard CH_4 (99.9% purity) at known rates (g/min) and regressed against chamber readings.

2.5. Feed intake

Each day prior to feeding, the daily intake of the respective treatment TMRs was determined by measurement of difference between offered feed and feed remaining after 24 h. From this value the feed offered each day was adjusted based on consistency over several days and either increased or decreased in 500 g increments (as fed). Individual daily intakes were recorded throughout the study to determine treatment group DMI. These values were used to express experimental variables on a per kg DMI basis.

2.6. Daily weight gain and feed conversion

Average daily weight gain (ADWG, kg/d) for individual steers was determined by recording LW measurements every seven days in the morning prior to daily feeding. This provided for tracking and demonstration of treatment effect on ADWG between individuals and treatment groups during the experimental period. The feed conversion ratio (FCR) was calculated as DMI/ADWG.

2.7. Ruminal volatile fatty acid production

The protocol and chromatography parameters for volatile fatty acid (VFA) analysis used in this study have been reported in detail (Kinley et al., 2016a). In brief, VFA concentration was measured in rumen fluid collected by esophageal tubing from each steer on the day of exit from the respiration chambers. Collected rumen fluid was mixed at a ratio of 4 mL of rumen fluid to 1.0 mL of fresh 20% metaphosphoric acid prior to being stored at −20 °C. When thawed for analysis of acetate, propionate, n-butyrate, iso-butyrate, isovalerate and n-valerate content the mixture was vortexed, and a 1.5 mL subsample was centrifuged (Labnet Prism R; Edison, NJ, USA) for 15 min at 13,500 *g* and 4 °C (Kinley et al., 2016a). Then 0.50 mL of clear supernatant was extracted by pipette, spiked with 0.05 mL of 11 mM 4-methylvaleric acid (Sigma-Aldrich; Castle Hill, NSW, Australia) as internal standard and analyzed using a Shimadzu GC-2010 equipped with a Restek Stabilwax (30 m × 0.25 mm × 0.25 mm) fused silica column and flame ionization detector.

2.8. Meat eating quality

From every carcass, one LD (M. Longissimus dorsi) was boned-out after 24 h of chilling at less than 7 °C and separated into cranial and caudal portions. Caudal portions were vacuum packed and chilled to −0.5 °C over a 5 h period. All samples were aged for 7 d prior to storage at −20 °C until sensory evaluation was completed. Caudal portions of the LD were used for consumer sensory evaluation of flavor, tenderness, juiciness and overall acceptance to generate MQ4 scores. Objective measures of beef quality; color, peak force (PF) and initial yield (IY), of cooked samples were conducted on the cranial portions of each LD.

The subjective testing of each LD portion was completed according to the Meat Standards Australia (MSA) Grill Protocol (Watson et al., 2008) with consumers receiving the treatment samples nested within other variable quality meat samples from different steers and cuts (Thompson et al., 2005). Every cooked meat sample was tasted by ten consumers who ranked the eating experience for tenderness, juiciness, flavor and overall acceptance. The two highest and two lowest responses were removed and the mean of the remaining six consumer scores produced the clipped scores which eliminates any outlier effect. The MQ4 score is formulated as:

$$MQ4 = (\text{Tenderness} \times 0.3) + (\text{flavor} \times 0.3) + (\text{Overall} \times 0.3) + (\text{Juiciness} \times 0.1)$$

2.9. Residues of bromoform in animal tissues

Samples of depot fat (brisket) and kidney were collected on the processing line as the hot carcasses passed through the evisceration process. After 24 h of chilling at less than 7 °C meat samples (M. Longissimus dorsi) were collected from all carcasses in each treatment group. Samples were placed on dry ice and transferred to −80 °C storage until residue analysis for bromoform. The analysis protocols for bromoform in meat and organs used in this study have been reported (Tomkins et al., 2009). Samples of fat, kidney, and meat were analyzed using purge and trap gas GC-MS by the Australian National Measurement Institute (Melbourne, Vic, AUS) using method VL-234 with a limit of detection of 0.05 mg/kg. In this method 500 g samples of meat, kidney or fat were homogenized and then sub-samples of 5.0 g (±0.10 g) were analyzed. Samples were incubated at 100 °C for 15 min in headspace sample digestion vessels and the generated vapor was then analyzed by GC-MS.

2.10. Statistical analysis

The effect of *Asparagopsis* inclusion (Control, Low, Mid, High) was analyzed for CH_4 and H_2 production, DMI, LW, ADWG, FCR, rumen VFA concentrations, and parameters of carcass and meat eating quality in a randomized incomplete block design. Control and treatment levels were analyzed as a linear-mixed model using the MIXED procedure of SPSS (IBM Corp, 2015). The treatment level was considered the fixed effect and body weight blocks were the random effect with the steers as experimental units. Linear and quadratic components of the response to incremental levels of *Asparagopsis* in the TMR were evaluated using polynomial contrasts. Effects were declared significant at $P < 0.05$ and P-values

between 0.05 and 0.10 were considered as a trend. When significant differences were detected, differences among means were tested by pairwise comparisons (LSD test).

3. Results

3.1. Impact on methane and hydrogen emissions

The average CH_4 and H_2 emissions for the four treatment groups is given in Fig. 2. As the seaweed inclusion increased the production of CH_4 decreased (g/kg DMI) significantly ($P = 0.008$) both linearly and quadratically. Compared to the Control group, which received no seaweed, the decrease in CH_4 production was apparent for the Low (0.05%) level of inclusion and significant for the Mid (0.10%) and High (0.20%) levels with decreases of 9%, 38%, and 98% for the incremental inclusion levels, respectively. As the seaweed inclusion increased the enteric emission of H_2 increased (g/kg DMI) significantly ($P = 0.006$) both linearly and quadratically. Compared to the Control group, the there was no increase in H_2 emission for the Low (0.05%) level of inclusion and significant increases for the Mid and High levels with increases of 0%, 380%, and 1700%, respectively. The result was a 17-fold rise which equates to an H_2 increase of 1.7 g/kg DMI for the High level of inclusion.

3.2. Productivity and feed efficiency

The response in ADWG and FCR for both the full 90 d treatment period and the concluding 60 d following adaptation to the final inclusion levels of *Asparagopsis* are given in Fig. 3A and Fig. 3B. The Inclusion of *Asparagopsis* induced significant ADWG (kg/d) increase after the 90 d treatment period ($P = 0.010$). The increase in ADWG compared to the Control was not significant for the Low inclusion level. There were significant LW increases of 137 kg and 130 kg for the Mid and High inclusion levels compared with LW increase of 113 kg for the Control steers, resulting in an increased ADWG of 26% and 22%, respectively (Fig. 3A). Improvements in FCR approached a statistical trend after the full 90 d treatment period ($P = 0.124$) but were not significant.

After adaptation to the final inclusion levels and during the concluding 60 d of the treatment period the increase in ADWG was significant ($P = 0.027$). There was no ADWG increase for the Low inclusion level during the final 60 d. There were significant LW increases of 81 kg and 75 kg for the Mid and High inclusion levels compared with LW increase of 53 kg for the Control steers, resulting in an increased ADWG of 51% and 42%, respectively (Fig. 3B). Improvements in FCR was not significant after the final 60 d following achieving final inclusion *Asparagopsis* levels treatment period ($P = 0.210$).

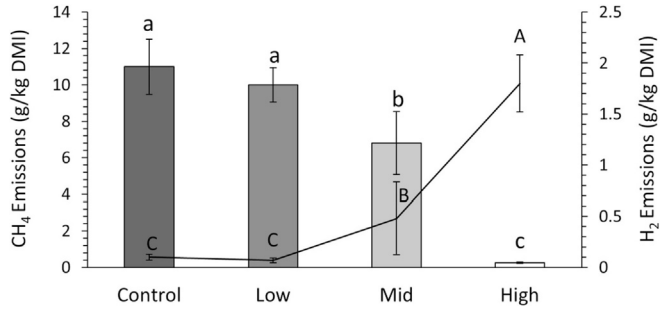

Fig. 2. Enteric methane (CH_4) (columns, lowercase letters) and hydrogen (H_2) (line points, uppercase letters) production from Brangus steers consuming a high grain total mixed ration with increasing *Asparagopsis taxiformis* inclusion at 0.00 (Control), 0.05 (Low), 0.10 (Mid), and 0.20% (High) of organic matter intake ($n = 5$ per treatment group). Columns and line points identified with different letters were significantly different at $P < 0.05$.

3.3. Feed intake

The average DMI (kg/d) for the four treatment groups is given in Fig. 4. The inclusion of *Asparagopsis* in the feedlot ration of Brangus steers at the levels used in this study had little effect on DMI during the treatment period. The only significant difference was between the steers receiving the Mid inclusion level which had greater DMI than the Low inclusion steers. Compared to the Control steers the DMI was only marginally lower (10.8%) in Low inclusion level steers and marginally higher (7.5%) in the Mid inclusion level and was similar to the DMI of the steers receiving the High inclusion level.

3.4. Rumen volatile fatty acid concentrations

The total VFA (tVFA) for the four treatment groups is given in Fig. 5. At the inclusion levels applied in the present study there was no significant change in ruminal tVFA due to inclusion of *Asparagopsis* in the diet of Brangus steers ($P = 0.176$). Acetate tended to be reduced ($P = 0.054$) compared to the Control group with 4%, 14%, and 20% reduction for the Low, Mid, and High inclusion levels, respectively, while the propionate proportion tended to increase ($P = 0.051$). Both acetate and propionate only marginally missed significant decrease and increase, respectively. The acetate:propionate ratio (A:P) was significantly and linearly reduced ($P = 0.026$)

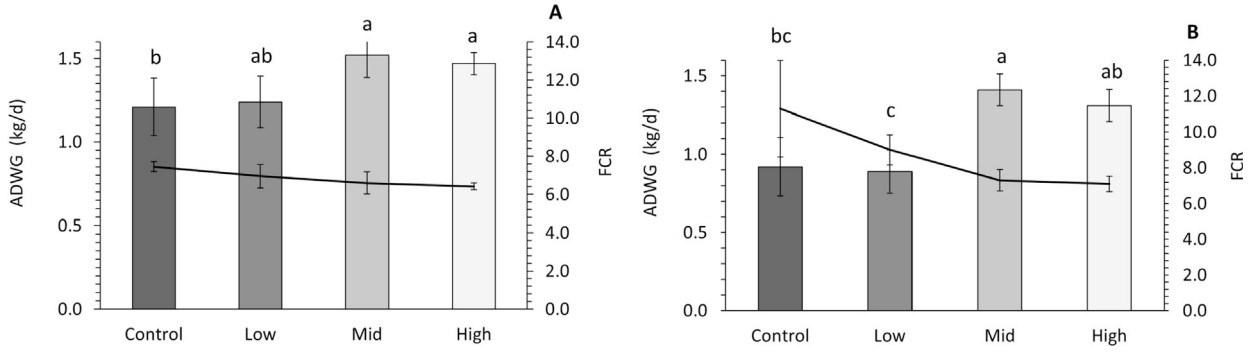

Fig. 3. A Changes induced over a 90 d treatment period in average daily weight gain (ADWG) (columns) and feed conversion ratio (FCR) (line points) in Brangus steers consuming a total mixed ration with different inclusion levels of *Asparagopsis taxiformis* at 0.00 (Control), 0.05 (Low), 0.10 (Mid), and 0.20% (High) of organic matter intake ($n = 5$ per treatment group). Columns identified with different letters were significantly different at $P < 0.05$. Fig. 3B The same changes induced in the concluding 60 d of the treatment period ($n = 5$ per treatment group).

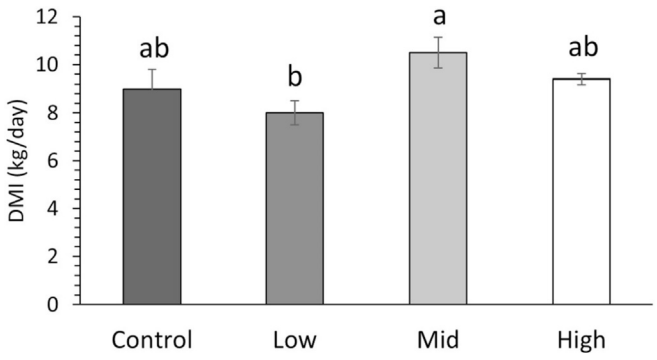

Fig. 4. Dry matter intake (DMI, kg/d) during the final 60 d of the study for Brangus steers consuming a total mixed ration with different levels of *Asparagopsis taxiformis* of 0.00 (Control), 0.05 (Low), 0.10 (Mid), and 0.20% (High) of organic matter intake (n = 5 per treatment group). Columns identified with different letters are significantly different at $P < 0.05$.

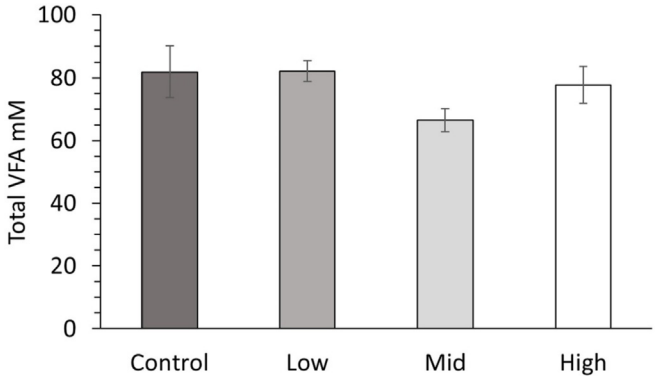

Fig. 5. Total volatile fatty acid (tVFA) concentration during the concluding 60 d of the study for Brangus steers consuming a total mixed ration with different levels of *Asparagopsis taxiformis* of 0.00 (Control), 0.05 (Low), 0.10 (Mid), and 0.20% (High) of organic matter intake (n = 5 per treatment group). Columns identified with different letters are significantly different at $P < 0.05$.

by 14%, 29%, and 35% with increasing *Asparagopsis* inclusion in the diet of the steers. Even with tendency for changes in acetate, propionate, and A:P, the butyrate VFA species remained consistent without effect from *Asparagopsis*.

3.5. Meat eating quality

All carcasses from this study were graded by Meat Standards Australia (MSA) inspectors. Average grades of MSA 3-Star indicated excellent meat eating quality. There was no difference in grading between treatment groups. The inclusion of *Asparagopsis* in TMR did not influence the tenderness, juiciness, flavor, consumer satisfaction, or overall liking of the meat as demonstrated by MQ4 scores generated from consumer sensory evaluations.

Bromoform, which is synthesized and stored within gland cells of *Asparagopsis* (Paul et al., 2006), is the principal bioactive that induces the mitigation of enteric methanogenesis (Machado et al., 2016a). Residues of bromoform were not detected in any meat, kidney, fat, or feces collected from steers in this study.

4. Discussion

4.1. Impact on enteric methane emissions

As an inclusion in the feedlot style diets of Brangus steers, *Asparagopsis* had an unprecedented effect on decreasing CH_4 emissions. In an exploratory 30 d period the experimental *Asparagopsis* inclusion levels were determined to be 0.00%, 0.05%, 0.10%, and 0.20% on an organic matter (OM) intake basis which is substantially lower than that hypothesized based on the effective 2.0% from previous *in vitro* studies (Kinley et al., 2016a). The steers remained at these inclusion levels for a further 60 d to complete a feedlot finishing period where *Asparagopsis* was included in the TMR. This work has now demonstrated that as an inclusion in a high grain TMR at only 0.20% of OM intake, *Asparagopsis* can fundamentally eliminate enteric CH_4 production, and at 0.10% there is close to 40% decrease compared to the basal TMR (Fig. 2). The significant increase in efficacy to decrease CH_4 with small changes in inclusion quantity is demonstrated by the sharp rise in efficacy between the 0.1% and 0.2% inclusion levels. The quantity of *Asparagopsis* is small and is a key reason why this seaweed is a preeminent tool for cleaner cattle production. However, the increase between these levels represents a 100% increase in the seaweed inclusion. The point of optimal inclusion has been reached and efficacy increases rapidly with increasing inclusion up to the optimal level, after which further increase in seaweed inclusion would be uneconomical.

Conservatively, if *Asparagopsis* is adopted at only 20% market penetration across the major OECD (Organization for European Economic Co-operation) nations in beef and dairy it could remove CH_4 emissions of approximately 230 $MtCO_2e/y$. According to the review of Herrero et al. (2016) this is up to 15% of total global enteric CH_4 emissions. As demonstrated in this study, feedlot beef production could benefit most dramatically with near elimination of enteric CH_4 emissions if fully adopted. This is mainly a result of the highly effective response in the high grain diet but also a factor of accessibility and managing of feed formulations in feedlots.

Variation has emerged between *in vivo* studies when comparing the efficacy of *Asparagopsis* for ruminants. A study with Merino sheep (Li et al., 2018) reported 80% decrease in enteric CH_4 production with *Asparagopsis* offered separately at 3% of intake (OM basis), and in a study with lactating Holstein dairy cows (Roque et al., 2019b) using a TMR containing the seaweed at 1% of intake a 67% decrease has been reported. In the current study with Brangus beef steers, an intake of *Asparagopsis* at only 0.2% induced more than 98% CH_4 inhibition. Contributing to the range of ruminal response, the as fed *Asparagopsis* products were different with a range of bromoform content (mg bromoform/g dry biomass) at 1.72, 1.75, 1.32, and 6.55 mg/g for the *in vitro*, sheep, dairy, and the current study, respectively. In the sheep study (Li et al., 2018) the *Asparagopsis* was dried in a solar kiln (55 °C). Processing *Asparagopsis* using higher temperatures is now known to reduce bromoform content (Vucko et al., 2017). Freeze drying remains the optimal method to generate a stable product and retain bromoform content in dried *Asparagopsis* (Vucko et al., 2017). Offered at 3% of intake (OM basis) the kiln dried product was still a potent antimethanogenic agent, even where consumption of *Asparagopsis* plateaued. In that sheep feeding study the dried *Asparagopsis* was not incorporated in a TMR but was offered separately with crushed lupins contributing to moderated consumption of the seaweed. Both the dairy study (Roque et al., 2019b) and the current study used freeze dried *Asparagopsis*. The dairy study used *A. armata* in the sporophyte stage of its lifecycle that was inherently lower in bromoform. It is not clear if lifecycle stage or species contributed to lower bromoform content compared to the *A. taxiformis* in the gametophyte stage used in this study. All *Asparagopsis* biomass collected for the studies were wild harvest and inherent natural variation is also likely to drive differences in efficacy when used to

mitigate enteric methanogenesis. Exploration of natural variation is recommended to characterize the bromoform content of *A. taxiformis* and *A. armata* at all stages of the lifecycle and relative to strains and growing conditions to optimize cultivation systems for future production.

4.2. Impact of increasing ruminal hydrogen

Hydrogen is produced during rumen fermentation of feed by fungi, protozoa and bacteria. The H_2 is mainly consumed by rumen methanogenic archaea due to a more thermodynamically favorable pathway that generates CH_4 by reducing single carbon compounds and maintaining a low H_2 partial pressure in the rumen (Janssen, 2010). However, it has been shown that when rumen microorganisms cannot capture the excess H_2, which can occur under substantial methanogenesis inhibition, it is eructated by the ruminant (Martinez-Fernandez et al., 2016), probably as a substitute mechanism to partially maintain a low H_2 pressure in the rumen.

In the present study as CH_4 decreased, H_2 production increased and this partially explains the redirection of rumen metabolic hydrogen [H]. It has been reported previously that H_2 pressure in the rumen may impact DMI and impair rumen fermentation and negatively influence animal productivity (Wolin et al., 1997; Janssen, 2010). More recent studies suggest that methanogenesis suppression of up to 80% (Mitsumori et al., 2012) and at 58% (Martinez-Fernandez et al., 2016) will not have a detrimental impact on DMI and rumen fermentation. Published studies (Martinez-Fernandez et al., 2017, 2018) have also reported an increase in ADWG when enteric CH_4 was decreased by using synthetic antimethanogenic agents confirming that the redirection of H_2 from CH_4 production into alternative reduced end-products is beneficial to the ruminant. There was no detrimental effect on DMI or rumen function by increased ruminal H_2 pressure induced by *Asparagopsis* in this study. There were nominal increases in DMI for steers with significant decreases in enteric CH_4 and increases in H_2 production.

In a study of Brahman steers eating a high concentrate diet containing chloroform, a CH_4 decrease of 58% was induced concomitant with H_2 emissions of 3.16 g/kg DMI (Martinez-Fernandez et al., 2016). A study using 3-nitrooxypropanol (Vyas et al., 2016) likewise reported higher emissions of H_2 of 2.0 g/kg DMI with lower CH_4 inhibition at 80%, compared to this study. When feeding *Asparagopsis*, the H_2 emissions from steers with a 98% decrease in CH_4 was only 1.8 g/kg of DMI suggesting an improved redirection of [H] away from CH_4 formation. The implication is that using *Asparagopsis* delivers greater CH_4 mitigation with less enteric H_2 emissions compared with previously demonstrated antimethanogenic feed additives.

4.3. Impact on rumen volatile fatty acids

Volatile fatty acids in the rumen are the result of anaerobic fermentation of feed by a consortium of microorganisms. The major VFA ions are acetate ($C_2H_3O_2^-$), propionate ($C_3H_5O_2^-$), and butyrate ($C_4H_7O_2^-$) which are energetic molecules used in ruminant animal metabolism that play an important role in ruminant livestock productivity. Stability in tVFA production is desirable due to the importance of these substrates on overall animal performance. It has been demonstrated previously that with *in vitro* inclusion of up to 5% (OM basis) *Asparagopsis* there was little change in tVFA (Kinley et al., 2016a). In an *in vivo* study with sheep an inclusion of up to 3% (OM basis) was observed to decrease tVFA (Li et al., 2018). In the present study with beef cattle tVFA was unaffected and even within the 10-fold lower *Asparagopsis* inclusion level compared with the sheep study, the present study resulted in a greater decrease in CH_4.

Although rumen archaea are the main H_2 users, there are other rumen microorganisms, which can use H_2 to produce alternative end products including VFA (Leng, 2014). Rumen bacteria, such as *Prevotella*, can use H_2 to produce propionate (Denman et al., 2015), which is a glucose precursor (gluconeogenic) in ruminants (Newbold et al., 2005). Although propionate has been considered the major H_2 sink when methanogenesis is inhibited in the rumen, other alternative H_2 sinks identified are towards butyrate, formate (CHO_2^-), microbial biomass production, and reductive acetogenesis (Ungerfeld, 2015). This partial conservation of the up to 12% of feed energy otherwise lost as CH_4 (Johnson and Johnson, 1995) could contribute to improved animal productivity. A meta-analysis reported that redirecting the rumen metabolic [H] away from CH_4 and towards VFA or other metabolites can improve ruminant efficiency and decrease environmental impact (Ungerfeld, 2015). In this study, the noteworthy change in rumen fermentation patterns due to *Asparagopsis* inclusion was decreased acetate and increased propionate resulting in a beneficial decrease in the A:P. This has been consistently demonstrated when CH_4 emissions are inhibited with increasing levels of *Asparagopsis* in the diet (Machado et al., 2016b; Kinley et al., 2016a; Li et al., 2018).

4.4. Impact on feedlot beef cattle productivity and products

Improved ADWG and FCR concomitant with significantly decreased CH_4 emissions induced by dietary inclusion of *Asparagopsis* supports the theory of beneficial redistribution of energy otherwise lost as CH_4. The Mid and High *Asparagopsis* inclusion level steers demonstrated ADWG of 1.5 kg/d which is in the high end of the range reported for Australian commercial feedlots of 1.1–1.7 kg/d (DAF, 2018). The Control steers demonstrated 1.2 kg/d which is in the low end of the range and contributes to apparent significant ADWG improvement of the *Asparagopsis* inclusion steers.

The productivity gains (ADWG) observed in steers consuming a TMR containing *Asparagopsis* is a promising outcome. This requires further validation as even though ADWG was demonstrated to be significant, the FCR was not, although a greater than 35% improvement in FCR for the Mid and High inclusion levels was observed. Although the increases are numerically substantial, they are based on a small sample size and there is a large underlying variability in ADWG between individual steers, including steers that did not receive the seaweed. The statistically significant impact on enteric CH_4 and animal productivity (ADWG) justifies demonstration of *Asparagopsis* as a feed ingredient in a commercial feedlot setting to accommodate individual animal variability, and to validate and quantify productivity gains associated with substantial reduction of enteric CH_4.

Carcass characteristics and meat eating quality were not compromised by including *Asparagopsis* in the TMR over 90 d. All steers were MSA graded and MQ4 scores demonstrated high meat eating quality. No bromoform could be detected in any meat or edible offal collected from steers consuming *Asparagopsis*. This was also the case with sheep consuming *Asparagopsis* (Li et al., 2018). Due diligence requires continued monitoring if inclusion periods are extended and the cumulative intake levels are increased which may be the case in some dairy systems. However, a study with dairy cattle demonstrated that there was no difference in bromoform content in the milk of cows without *Asparagopsis* in the TMR compared with cows consuming *Asparagopsis* at 0.5% and 1.0% of DMI (Roque et al., 2019b).

4.5. Impact and integration in the red meat industry

In response to concerns over GHG inventories, the red meat and dairy industries have been working to develop technologies that will mitigate emissions from livestock (Mayberry et al., 2019). While many potential solutions at variable levels of efficacy are on the horizon, limited effective measures have been put into practice on farm. Current and horizon technologies, although collectively important, do not promise the level of GHG emissions abatement for ruminant livestock demonstrated in this study. When integrated in ruminant production systems *Asparagopsis* would have the potential to progress the red meat industry towards carbon neutrality (Mayberry et al., 2019), particularly with concomitant improvement in beef cattle performance.

It is recommended that the efficacy of *Asparagopsis* in decreasing enteric CH_4 emissions be investigated as an ingredient in diets of variable fiber content. Feed digestibility differences between high grain and high forage diets are expected to impact the inclusion level of *Asparagopsis* required to achieve comparable decreases in enteric CH_4. To widely utilize *Asparagopsis*, this knowledge is essential to accommodate regional and global variability of cattle feeding systems. Increasing the inclusion level of *Asparagopsis* also requires attention to trace elements and minerals that may accumulate in seaweeds. *Asparagopsis,* like many other seaweeds, may contain iodine at concentrations that may exceed maximum tolerable levels in feeding systems of high DMI if not accommodated in diet formulation. To this end a seaweed cultivation industry targeting *Asparagopsis* should strive to maximize bromoform content, and provide a certified product, thereby minimizing the amount of seaweed product required to maximize CH_4 mitigation and animal productivity outcomes.

The quality of as fed *Asparagopsis* defined by its bromoform content will be the primary determinant for inclusion level in a ruminant diet. A high quality product with bromoform content similar or higher than the product used in this study (>6.55 mg/g) will enhance all aspects of its utility through reducing the inclusion level. This would have environmental, economic, and management impact throughout the supply chain thereby reducing cost and increasing adoption. This also minimizes intake requirement of the product to achieve optimal CH_4 mitigation effect. The feed formulation and mechanism of delivery to the animal would likewise be simplified. This would create opportunities for the product across different feeding systems including extensive grazing production systems where emission intensities are typically higher because of the diet variability (Charmley et al., 2008).

The small level of *Asparagopsis* inclusion for effective mitigation of enteric CH_4 is a hallmark of this seaweed and will be advantageous in development of a consistently high quality supply to make sizeable changes in the agriculture GHG emissions inventory. Lack of large scale cultivation remains a barrier to wide scale adoption. Solutions for the supply chain continue to evolve as global research in *Asparagopsis* cultivation and processing techniques expand in response to consistent and unprecedented potency of *Asparagopsis* in enteric CH_4 mitigation, public desire for improved and cleaner food production, and immense economic opportunities throughout the *Asparagopsis* supply chain.

Research into the utility of seaweeds as feed ingredients in ruminant diets continues to increase (Maia et al., 2016; Machado et al., 2015) and *Asparagopsis* is the standout candidate with potential to substantially decrease the environmental impact of ruminant livestock while increasing animal productivity in more efficient and cleaner production systems. The results from this study have demonstrated that the inclusion of *Asparagopsis* in a TMR is the most promising antimethanogenic agent to date for ruminant production systems to achieve emissions targets and carbon neutrality.

5. Conclusions

The potential of *Asparagopsis* as a tool to minimize enteric CH_4 emissions as demonstrated in *in vitro* studies and in sheep and dairy studies was investigated in feedlot beef cattle. *Asparagopsis* included in the high grain TMR at 0.05%, 0.10%, and 0.20% of diet OM resulted in decrease of CH_4 production (g/kg DMI) of 9%, 38% and 98%, respectively. Enteric H_2 emissions increased with increasing *Asparagopsis* inclusion by 0%, 380%, and 1700% without compromising DMI. Growth rate of the steers was enhanced by the 0.10% and 0.20% inclusion levels after a 90 d finishing period with ADWG increases of 26% and 22%, respectively. In the concluding 60 d the ADWG was enhanced by 51% and 42%, respectively. The tVFA were not affected and the VFA species acetate was decreased in favor of propionate resulting in a favorable decrease in A:P of 14%, 29%, and 35% with increasing *Asparagopsis*. Meat eating quality was not affected by the seaweed inclusion in either meat quality grading or consumer sensory evaluations. The *Asparagopsis* derived antimethanogenic compound bromoform was not detected in meat, fat, organs, or feces of any of the steers. This study exposed steers to *Asparagopsis* for 90 d which represents a typical feedlot finishing period. Improvements in *Asparagopsis* quality through optimal cultivation and processing would further reduce effective inclusion level, exposure to excess minerals, and optimize the supply chain. The implication of this study is that enteric CH_4 could be virtually eliminated while using *Asparagopsis* as a feed ingredient in the high grain TMR of feedlot beef cattle.

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CRediT authorship contribution statement

Robert D. Kinley: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Gonzalo Martinez-Fernandez:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing - original draft, Writing - review & editing. **Melissa K. Matthews:** Formal analysis, Investigation, Methodology, Resources, Supervision. **Rocky de Nys:** Conceptualization, Formal analysis, Methodology, Resources, Visualization, Writing - review & editing. **Marie Magnusson:** Formal analysis, Methodology, Writing - review & editing. **Nigel W. Tomkins:** Conceptualization, Funding acquisition, Methodology, Resources, Visualization, Writing - review & editing.

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