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## **Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers — [Source link](#)**

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2 **80 percent in beef steers**

3

4 Seaweed supplementation reduces enteric emissions

5

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15

16

17 **Abstract**

18 The red macroalgae (seaweed) *Asparagopsis spp.* has shown to reduce ruminant enteric methane  
19 (CH<sub>4</sub>) production up to 99% *in vitro*. The objective of this study was to determine the effect of  
20 *Asparagopsis taxiformis* on CH<sub>4</sub> production (g/day per animal), CH<sub>4</sub> yield (g CH<sub>4</sub>/kg dry matter  
21 intake (DMI)), average daily gain (ADG), feed conversion efficiency (FCE), and carcass and meat  
22 quality in growing beef steers. Twenty-one Angus-Hereford beef steers were randomly allocated  
23 to one of three treatment groups: 0% (Control), 0.25% (Low Dose; LD), and 0.5% (High Dose;  
24 HD) *A. taxiformis* inclusion based on organic matter intake. Steers were fed 3 diets: high, medium,  
25 and low forage total mixed ration (TMR) representing typical life-stage diets of growing beef  
26 steers. The LD and HD treatments over 147 days reduced enteric CH<sub>4</sub> yield 45 and 68%,  
27 respectively; however, there was an interaction between TMR type and the magnitude of CH<sub>4</sub> yield  
28 reduction. Supplementing the low forage TMR reduced CH<sub>4</sub> yield 69.8% ( $P < 0.001$ ) for LD and  
29 80% ( $P < 0.001$ ) for HD treatment. Hydrogen (H<sub>2</sub>) yield (g H<sub>2</sub>/DMI) increased significantly  
30 ( $P < 0.001$ ) 336 and 590% compared to Control for the LD and HD treatments, respectively. No  
31 differences were found in carbon dioxide (CO<sub>2</sub>) yield (g CO<sub>2</sub>/DMI), ADG, carcass quality, strip  
32 loin proximate analysis and shear force, or consumer taste preferences. DMI tended ( $P = 0.08$ ) to  
33 decrease 8% in steers in LD treatment but significantly ( $P = 0.002$ ) reduced 14% in steers in HD  
34 treatment. Conversely, FCE tended to increase 7% in steers in LD treatment ( $P = 0.06$ ) and  
35 increased 14% in steers in HD ( $P < 0.01$ ) treatment compared to Control. The persistent reduction  
36 of CH<sub>4</sub> by *A. taxiformis* supplementation suggests that this is a viable feed additive to significantly  
37 decrease the carbon footprint of ruminant livestock and potentially increase production efficiency.

38

39

40 **KEYWORDS**

41 *Asparagopsis*, beef cattle, bromoform, enteric methane, greenhouse gas, seaweed

42

43 **1 INTRODUCTION**

44 Livestock production, particularly ruminants, contributes to anthropogenic greenhouse gas (GHG)  
45 emissions globally. These emissions are estimated to be 7.1 Gt carbon dioxide (CO<sub>2</sub>) equivalents  
46 annually, approximately 14.5% of the global anthropogenic GHG emissions (Gerber et al., 2013).  
47 The majority of GHG emissions from livestock production is mainly in the form of methane (CH<sub>4</sub>),  
48 which is produced largely through enteric fermentation and to a lesser extent manure  
49 decomposition. Enteric CH<sub>4</sub> is a natural by-product of microbial fermentation of feed in the  
50 digestive tract especially in the rumen. Enteric CH<sub>4</sub> emissions not only contribute to GHG but also  
51 represent an energy loss amounting up to 11% of dietary energy consumption (Moraes et al., 2014).  
52 Therefore, reducing enteric CH<sub>4</sub> emissions contributes to the alleviation of climate change through  
53 reduction of GHG emissions from agriculture and can improve productivity through conservation  
54 of feed energy otherwise lost as CH<sub>4</sub>. There is potential for mitigation of enteric CH<sub>4</sub> emissions  
55 through a variety of approaches with a focus on the use of feed additives, dietary manipulation and  
56 forage quality improvement (Hristov et al., 2013).

57 Feed additives used in CH<sub>4</sub> mitigation can either modify the rumen environment or directly  
58 inhibit methanogenesis resulting in lower enteric CH<sub>4</sub> production (g/day per animal) and yield  
59 (g/kg dry matter intake [DMI]). Reductions in CH<sub>4</sub> production of beef cattle through the inhibition  
60 of methanogenesis have been reported for feed additives at 22, 93, and 98% for short-chain nitro-  
61 compounds (3-nitrooxypropanol; 3-NOP; Dijkstra et al. 2018), synthetic halogenated compounds  
62 (Tomkins et al. 2009), and naturally synthesized halogenated compounds in seaweed (Kinley et

63 al. 2020), respectively. The compound 3-NOP inhibits the enzyme methyl-coenzyme M reductase  
64 which catalyzes the final step in methanogenesis in rumen archaea (Duin et al. 2016). Halogenated  
65 CH<sub>4</sub> analogs, such as bromoform, act on the same methanogenesis pathway, but do so by binding  
66 and sequestering the prosthetic group required by methyl-coenzyme M reductase (Smith et al.  
67 1962; Wood et al, 1968; Johnson et al., 1972). Some haloalkanes are structural analogs of CH<sub>4</sub>,  
68 and therefore competitively inhibit the methyl transfer reactions that are necessary in CH<sub>4</sub>  
69 biosynthesis (Ermler et al., 1997; Liu et al., 2011). These CH<sub>4</sub> analogues include  
70 bromochloromethane (BCM), bromoform, and chloroform and have been proven to be the most  
71 effective feed additives for reducing CH<sub>4</sub> production. A 93% reduction of CH<sub>4</sub> was shown in  
72 Brahman cattle with a feed inclusion of BCM at 0.30 g/100 kg LW twice daily for 28 days,  
73 however, there was no improvements on feed intake, weight gain, carcass quality or feed efficiency  
74 (Tomkins et al. 2009). Conversely, Abecia (2012) reported that the inclusion of BCM at 0.30 g/100  
75 kg once per day decreased CH<sub>4</sub> production 33% and increased milk production 36%. The authors  
76 speculated that increased milk production in BCM treated cows could be attributed to a shift to  
77 more propionate production in the rumen, which is a hydrogen (H<sub>2</sub>) sink and provides more energy  
78 compared to other volatile fatty acids. However, long-term efficacy of CH<sub>4</sub> analogues in the rumen  
79 remains to be confirmed. Tomkins et al. (2009), for example, reported a second experiment  
80 resulting in a 57.6% CH<sub>4</sub> reduction after 30 days of treatment which is far less than the reductions  
81 found during the first 28 days. Additionally, chloroform fed to fistulated dairy cows was effective  
82 at reducing enteric CH<sub>4</sub> production through reduced abundance and activity of methanogenic  
83 archaea, but only over a 42 day period (Knight, 2011).

84 Types of feedstuffs can also drive CH<sub>4</sub> production by providing different substrates to  
85 microbial populations which are the drivers of volatile fatty acid (VFA) production in the rumen.

86 There are ways to influence the types of VFA produced in the rumen by changing the types of feed  
87 in the diet (Russell and Wallace, 1997, Van Soest, 1994). This is important for two reasons; first  
88 VFA represent the amount of energy available to the animal for means of animal productivity and  
89 second VFA pathways, such as the production of propionate, are able to utilize reducing  
90 equivalents that normally would be shifted to methanogenesis (Blaxter and Clappton, 1965,  
91 Johnson and Johnson, 1995). Concentrates contain non-structural carbohydrates, such as starch  
92 and sugar, that are rapidly fermented which drives pH down, which negatively impact  
93 methanogenic populations, and are an effective way to increase propionate production (Bannink  
94 et al., 2006, 2008). Forages contain structural carbohydrates, such as neutral detergent fiber (NDF),  
95 and have been linked to CH<sub>4</sub> production (Niu et al, 2018). As NDF in diet increases, rumen pH  
96 also increases resulting in preferential production of acetate over propionate, which generates  
97 reducing equivalents such as H<sub>2</sub> that is shifted toward methanogenesis (Hungate, 1966, Janssen,  
98 2010). Not only can NDF play a significant role in CH<sub>4</sub> production, it has also been suggested to  
99 impact the efficacy of anti-methanogenic compounds added to feed (Dijkstra et al. 2018).

100 Red seaweeds, particularly the genus *Asparagopsis*, are considered potent anti-methanogenic  
101 organisms due to their capacity to synthesize and encapsulate halogenated CH<sub>4</sub> analogues such as  
102 bromoform and dibromochloromethane within specialized gland cells as a natural defense  
103 mechanism against predation (Paul et al., 2006). Machado et al. (2014) compared a diversity of  
104 tropical macroalgae, including freshwater and marine species, and found that *Asparagopsis*  
105 *taxiformis* at 17% of OM had the largest reduction of CH<sub>4</sub> production *in vitro* with a 98.9% average  
106 reduction. In the stepwise progression of evaluating the seaweeds Kinley et al. (2016a; 2016b)  
107 explored reduced inclusion rates to determine the *in vitro* minimum effective inclusion level of 2%  
108 of OM. In this process only *A. taxiformis* retained anti-methanogenic capability at a very low

109 inclusion. *A. taxiformis* reduced CH<sub>4</sub> production more effectively than synthetic halogenated CH<sub>4</sub>  
110 analogs at equivalent concentrations *in vitro*, largely due to multiple anti-methanogenic bioactives  
111 working synergistically (Machado et al., 2018). Importantly, the concentration of bioactives in  
112 *Asparagopsis spp*, in particular bromoform, has a significant effect on the reduction of CH<sub>4</sub> in  
113 animal trials. For example, Roque et al (2019b) tested the effects of the seaweed *A. armata* (1.3  
114 mg bromoform/g DM) *in vivo* fed to dairy cattle for a two week duration and reported up to 67%  
115 reduction of enteric CH<sub>4</sub> production using a 1% seaweed inclusion rate on organic matter (OM)  
116 basis in a total mixed ration (TMR). Kinley et al. (2020) reported up to 98% CH<sub>4</sub> reduction using  
117 0.2% of OM inclusion of *A. taxiformis* (6.6 mg bromoform/g DM) during a 90-day feeding regime  
118 typical of feedlot TMR. These studies confirm that seaweed quality measured as concentration of  
119 bioactive at feeding and the basal diet formulation have an impact on the efficacy of the seaweed,  
120 and that there is heightened response *in vivo* compared to *in vitro*.

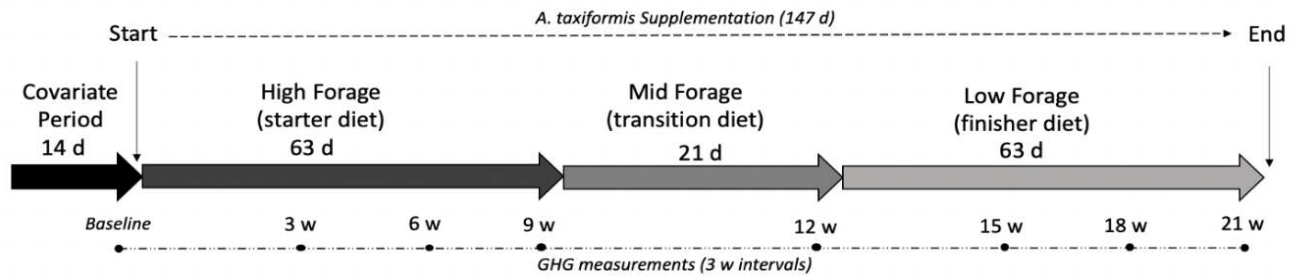
121 For adoption of the seaweed by industry it is crucial that meat quality be maintained or  
122 improved. As with any feed additive, feeding *A. taxiformis* to livestock has the potential for  
123 changes in meat quality, tenderness and taste, and consumer acceptability. Marbling, for instance,  
124 directly impacts flavor and juiciness and it has been shown that marbling can directly influence  
125 consumer preference with some willing to pay a premium (Killinger et. al., 2004).

126 Therefore, the objectives of this study were to (1) measure the long-term effects of *A.*  
127 *taxiformis* over 147 day period, (2) test the efficacy of CH<sub>4</sub> reductions of supplementing *A.*  
128 *taxiformis* to high, medium, and low forage basal TMRs, (3) quantify the effects of *A. taxiformis*  
129 supplementation on production parameters, meat quality (including taste), and bromoform residues  
130 within the meat and liver.

131

## 132 2 MATERIALS AND METHODS

133 This study was approved by the Institutional Animal Care and Use Committee of the University  
134 of California, Davis (Protocol No. 20803).



**FIGURE 1** Experimental timeline including covariate period, total days for *Asparagopsis taxiformis* supplementation, dietary regime, days on diets, and greenhouse gas measurement intervals.

135

### 136 2.1 Study design, animals, and diets

137 Twenty-one Angus-Hereford cross beef steers, blocked by weight, were randomly allocated to one  
138 of three treatment groups: 0% (Control), 0.25% (Low Dose; LD), and 0.5% (High Dose; HD)  
139 inclusion rates of *A. taxiformis* based on OM intake. One steer on HD treatment was injured during  
140 the last 3 weeks of the trial and all data from this steer was removed from statistical analysis. The  
141 experiment followed a completely randomized design, with a 2-week covariate period before  
142 treatment began followed by 3-week data collection intervals for 21-weeks; a total of 147 days of  
143 seaweed treatment (Figure 1). During data collection intervals, alfalfa pellets offered through the  
144 CH<sub>4</sub> measuring device (GreenFeed system, C-Lock, Inc., Rapid City, SD) were included as part  
145 of daily feed intake. The steers were individually housed and were approximately 8 months of age  
146 with an average BW of 352 ± 9 kg at induction to the trial. Steers were fed 3 diets during the study;  
147 high (starter diet), medium (transition diet), and low (finisher diet) forage TMRs, which are typical  
148 life-stage TMRs of growing beef steers (Table 1). Samples from the three diets and alfalfa pellets



149 were collected once a week and bags of *A. taxiformis* were randomly sampled and analyzed (Table  
150 2) for dry matter, acid detergent fiber, NDF, lignin, starch, crude fat, total digestible nutrient and  
151 mineral content (Cumberland Valley Analytical Services, Waynesboro, PA). Steers were offered  
152 water *ad libitum*.

153 **TABLE 1** Ingredients of the experimental diet containing high, medium, and low forage  
154 concentrations (% of DM)

<b>Ingredients</b>	<b>High forage</b>	<b>Medium forage</b>	<b>Low forage</b>
<i>Forage</i>			
Alfalfa hay	35.0	25.0	5.00
Wheat hay	25.0	15.0	6.00
Dry distillers grain	12.0	14.0	6.00
<i>Concentrate</i>			
Rolled corn	20.0	37.0	72.0
Molasses	5.0	5.00	3.00
Fat	1.5	2.00	3.00
Urea	0.35	0.40	1.80
Beef trace salt	0.32	0.32	1.00
Calcium carbonate	0.82	1.15	1.80
Magnesium oxide			0.20
Potassium chloride			0.50

155 The *A. taxiformis* used as a feed additive was provided by Commonwealth Scientific and  
156 Industrial Research Organization (CSIRO) Australia. The seaweed was collected during the  
157 gametophyte phase from Humpy Island, Keppel Bay, QLD (23°13'01"S, 150°54'01"E) by  
158 Center for Macroalgal Resources and Biotechnology of James Cook University, Townsville,  
159 Queensland, Australia. Once collected, it was frozen and stored at -15 °C then freeze dried at  
160 Forager Food Co., Red Hills, Tasmania, Australia and later ground to a size of 2–3 mm. Total  
161 seaweed inclusion ranged from 46.7 to 55.7 g/day for LD and 76.1 to 99.4 g/day for HD  
162 treatment. The seaweed used in the study contained bromoform at a concentration of 7.8 mg/g  
163 dry weight as determined by Bigelow Analytical Services, East Boothbay, ME, USA. To  
164 increase palatability and adhesion to feed, 200 ml of molasses and 200 ml of water was added to

165 *A. taxiformis* then hand mixed into the total mixed ration for each animal. The Control group also  
166 received 200 ml of both molasses and water with their daily feed to ensure *A. taxiformis* was the  
167 only difference between the three treatments. Steers were fed 105% of the previous day's intake  
168 twice daily at 0600 and 1800 hours. Daily feed intake was calculated as the TMR offered minus  
169 feed refusal weights.

## 170 **2.2 Sample collection and analysis**

171 Methane, CO<sub>2</sub>, and H<sub>2</sub> gas emissions from steers were measured using the GreenFeed system (C-  
172 Lock Inc., Rapid City, SD, USA). Gas emissions were measured during the covariate period and  
173 experimental period during weeks 3, 6, 9, 12, 15, 18, and 21. In each measurement period, gas  
174 emission data were collected during 3 consecutive days as follows: starting at 07:00, 13:00, and  
175 1900 hours (sampling day 1); 0100, 1000, and 1600 hours (sampling day 2); and 2200 and 0400  
176 hours (sampling day 3). Breath gas samples were collected for 3 to 5 minutes followed by a 2-  
177 minute background gas sample collection. The GreenFeed system was calibrated before each  
178 measurement period with a standard gas mixture containing (mol %): 5000 ppm CO<sub>2</sub>, 500 ppm  
179 CH<sub>4</sub>, 10 ppm H<sub>2</sub>, 21% O<sub>2</sub> and nitrogen as a balance (Air Liquide America Specialty Gases, Rancho  
180 Cucamonga, CA). Recovery rates for CO<sub>2</sub> and CH<sub>4</sub> observed in this study were within +/- 3% of  
181 the known quantities of gas released. Alfalfa pellets were offered at each sampling event as bait  
182 feed and was kept below 10% of the total DMI during each 3 day measurement period. The  
183 composition of alfalfa pellets is shown in Table 2. Feed intake and feed costs were recorded daily  
184 and bodyweight (BW) was measured weekly.

185 **TABLE 2** Nutritional composition of experimental diets, *Asparagopsis taxiformis*, and alfalfa  
186 pellets

	<b>High forage</b>	<b>Medium forage</b>	<b>Low forage</b>	<b>Pellets</b>	<b><i>A. taxiformis</i></b>
<i>% Dry matter</i>					
Organic matter	92.1	93.1	94.8	88.6	50.9
Crude protein	17.2	17.4	13.2	17.1	16.8
ADF	22.6	16.7	10.5	28.1	11.5
NDF	33.1	25.8	18.6	35.9	33.7
Lignin	4.08	3.05	1.73	6.16	4.08
Starch	16.9	25.0	46.7	0.90	0.35
Crude fat	4.92	6.04	6.77	3.02	0.63
Calcium	0.77	1.00	0.50	2.06	5.29
Phosphorus	0.33	0.38	0.28	0.24	0.18
Magnesium	0.38	0.38	0.23	0.37	0.81
Potassium	1.74	1.42	0.94	2.10	2.02
Sodium	0.18	0.25	0.30	0.20	6.34
<i>Parts per million</i>					
Iron	438	335	127	1508	8494
Manganese	61.7	56.0	64.0	88.0	142.5
Zinc	43.2	51.50	58.0	19.0	53.5
Copper	8.67	8.00	7.00	10.0	22.5

187 After the feeding trial was completed, all 20 steers were sent to a USDA-inspected  
188 commercial packing plant for slaughter. On the day of slaughter, steers were marked and followed  
189 throughout the process. On the first day, livers were collected and stored on dry ice until placed in  
190 a  $-20^{\circ}\text{C}$  freezer. Carcasses were aged for 48 hours in a large cooler and then graded by a certified  
191 USDA grader. Directly after grading, carcasses were sent to fabrication where the strip loin from  
192 the left side of each carcass was cut and saved for further analysis. All 20 strip loins were placed  
193 on ice and transported back to the University of California, Davis where they were cryovac  
194 packaged and stored at  $4^{\circ}\text{C}$  in dark for 14 days. After 14-day of aging, strip loins were cut into  
195 steaks (2.45 cm thickness) and individually vacuum packaged and stored at  $-20^{\circ}\text{C}$ . Steaks and  
196 livers were analyzed for bromoform concentrations using Shimadzu QP2010 Ultra GC/MS  
197 following a modified protocol described by Paul et al. (2006) (Bigelow Analytical Services, East

198 Boothbay, ME, USA). The limits of bromoform detection and quantification were 0.06 mg/kg and  
199 0.20 mg/kg, respectively.

200 To test for objective tenderness, slice shear force (SSF) and Warner-Brazler shear force  
201 (WBSF) were measured following the protocol described by AMSA (2016). One steak from each  
202 animal was thawed overnight and cooked to an internal temperature of 71°C. Within 1 to 2 minutes  
203 after cooking, the SSF were measured using machine texture analyzer (TMS Pro Texture Analyzer,  
204 Food Technology corporation, Sterling, VA, USA) with a crosshead at the speed of 500  
205 mm/minute. To test WBSF, cooked steaks were cooled at 4°C overnight, and then four cores were  
206 cut using WEN 8-inch 5 Speed Drill Press from one steak from each animal parallel to the muscle  
207 fiber orientation. The WBSF was measured using the TMS Pro texture analyzer with a Warner  
208 Bratzler blade (2.8 mm wide) and a crosshead at speed of 250 mm/minute. The average peak forces  
209 for all four cores were recorded.

210 A consumer sensory panel was conducted at UC-Davis. Strip steaks were thawed at 4°C for  
211 24 hours then cooked to an internal temperature of 71°C using a George Foreman clamshell  
212 (Spectrum Brands, Middleton, WS, USA). Internal temperature was taken from the geometric  
213 center of each steak using a K thermocouple thermometer (AccuTuff 351, model 35100, Cooper-  
214 Atkins Corporation, Middlefield, CT, USA). Following cooking, steaks were rested for 3 minutes  
215 then cut into 1.5 cm<sup>3</sup> pieces. Each steak was randomly assigned a unique three digit number, placed  
216 into glass bowls covered in tin foil then stored in an insulated food warmer (Carlisle model  
217 PC300N03, Oklahoma, OK, USA) for longer than 30 minutes prior to the start of each sensory  
218 evaluation session. A total of 112 participants evaluated steak samples during one of the 5 sessions  
219 held over a 4-day period. Each participant evaluated a total of three steak samples, one from each  
220 treatment group, with a minimum of two 1.5 cm<sup>3</sup> pieces per steak. Each participant was asked to

221 evaluate tenderness, flavor, juiciness, and overall acceptance using a 9-point hedonic scale (1 =  
222 Dislike extremely and 9 = Like extremely).

223

### 224 **2.3 Statistical analysis**

225 Statistical analysis was performed using R statistical software (version 3.6.1; The R Foundation  
226 for Statistical Computing, Vienna, Austria). The linear mixed-effects models (lme) procedure was  
227 used with the steer as the experimental unit. GreenFeed emission data were averaged per steer and  
228 gas measurement period, which was then used in the statistical analysis. Dry matter intake and cost  
229 per kg of gain (CPG) data was averaged by week and used in the statistical analysis. Average daily  
230 gain (ADG) was calculated by subtracting initial BW from final BW then dividing by the number  
231 of experimental days for each diet regimen and the duration of the study (i.e. 63 days on high  
232 forage (starter) TMR, 21 days on medium forage (transition) TMR, then 63 days on low forage  
233 (finisher) TMR with total study duration of 147 days). Feed conversion efficiency (FCE) was  
234 calculated by dividing ADG by DMI for each diet regimen and the duration of the study.

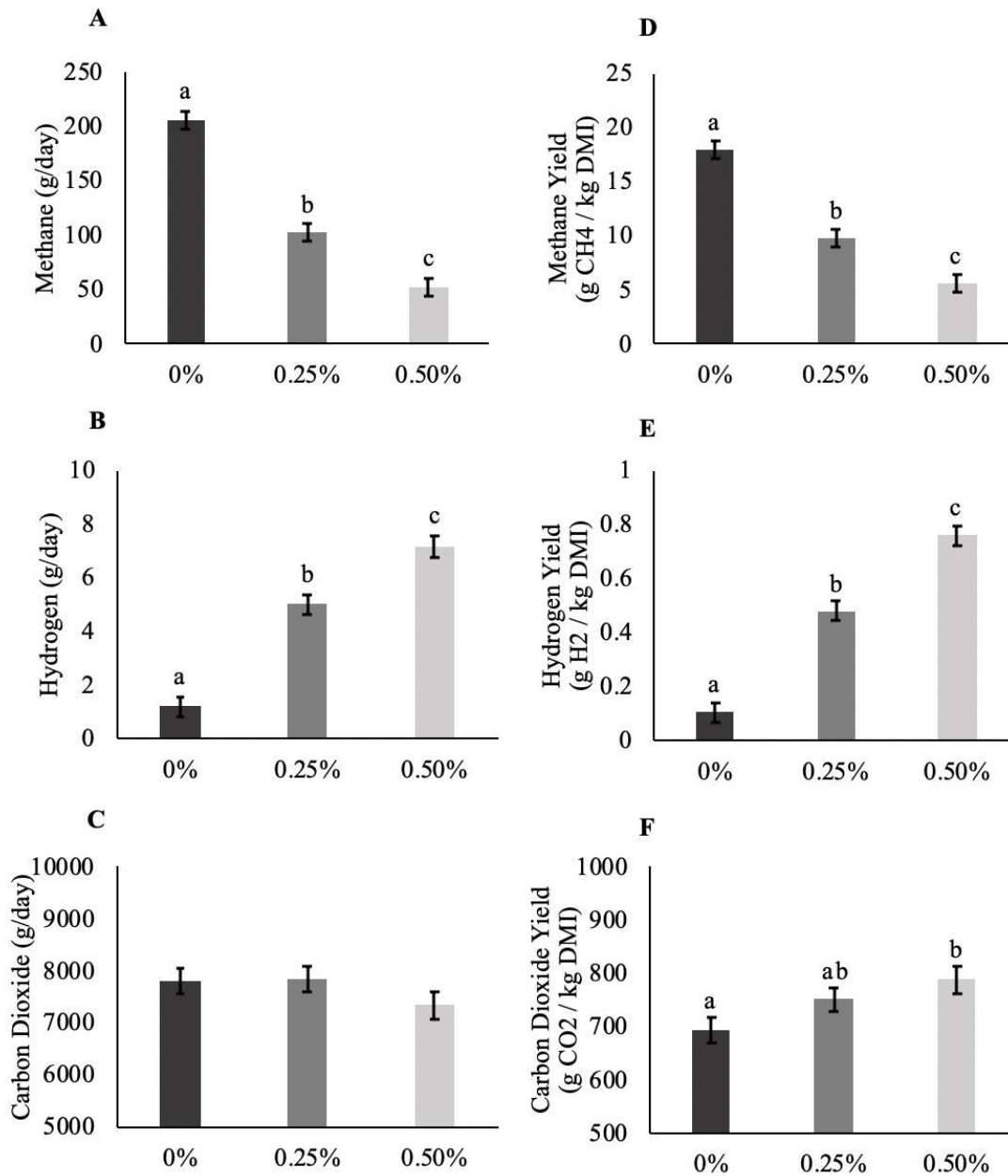
235 The statistical model included treatment, diet, treatment  $\times$  diet interactions, and the covariate  
236 term, with the error term assumed to be normally distributed with mean = 0 and constant variance.  
237 Individual animal was used as random effect, whereas all other factors were considered fixed. Data  
238 was analyzed as repeated measures with an autoregressive 1 correlation structure. Statistical  
239 significance was established when  $P \leq 0.05$  and a trend at  $0.05 > P \leq 0.10$ . The consumer sensory  
240 evaluation data were analyzed using the Kruskal-Wallis test. The Dunn's test with  $P$ -value  
241 adjustment following Bonferroni methods was used for post-hoc pair-wise comparisons.

242

## 243 **3 RESULTS**

### 244 3.1 Gas parameters

245 The emissions as production (g/day per animal) and yield (g/DMI kg) of CH<sub>4</sub>, H<sub>2</sub>, and CO<sub>2</sub> gases  
246 from the steers in the three treatment groups (Control, LD, HD) are presented in Figure 2 (for the  
247 duration of the trial) and Table 3 (divided by the three diet regimes). Where *P* values for significant  
248 effects are not given, they are *P* < 0.01. Inclusion of *A. taxiformis* in the TMR had a significant  
249 linear reduction in enteric CH<sub>4</sub> production and yield. Methane production for the collective feeding  
250 stages of experimental period resulted in a reduction of 50.6 and 74.9% for LD and HD treatments,  
251 respectively, compared to Control. Methane yields for LD and HD were 45 and 68% lower,  
252 respectively, compared to Control. However, there was an interaction between diet formulation  
253 and magnitude of CH<sub>4</sub> production and yield reduction. Methane production in steers on the high  
254 forage TMR with *A. taxiformis* inclusion was significantly reduced 36.4% for LD and 58.7% for  
255 HD and CH<sub>4</sub> yield was significantly reduced 32.7% for LD and 51.9% for HD compared to the  
256 Control steers. Methane production during the medium forage TMR phase was significantly  
257 reduced 51.8 and 86.8%, for LD and HD treatments compared to Control, respectively, whereas  
258 CH<sub>4</sub> yield was significantly reduced 44.6 and 79.7%, respectively. Steers fed low forage TMR in  
259 LD and HD treatments produced 72.4 and 81.9% lower CH<sub>4</sub> compared to Control, respectively.  
260 Similarly, their CH<sub>4</sub> yield was significantly reduced 69.8 for LD and 80.0% for HD.



**FIGURE 2** Means, standard deviations, and statistical differences of methane, hydrogen, and carbon dioxide production (g/d) (A,B,C), and yield (g/kg dry matter intake (DMI)) (D,E,F) for 0%, 0.25%, and 0.50% *Asparagopsis taxiformis* inclusion. Means within a graph with different alphabets differ ( $P < 0.05$ )

262 **TABLE 3** Effect of *Asparagopsis taxiformis* inclusion levels of 0%, 0.25%, and 0.5% of feed organic matter to three stages of beef  
 263 cattle diets on gas parameters

		Diet											
		High forage				Medium forage				Low forage			
		0%	0.25%	0.50%	SE	0%	0.25%	0.50%	SE	0%	0.25%	0.50%	SE
<i>Gas Emission Data</i>													
Methane production	(g/day)	237 <sup>a</sup>	151 <sup>b</sup>	98.0 <sup>c</sup>	11.4	241 <sup>a</sup>	116 <sup>b</sup>	31.9 <sup>c</sup>	15.3	139 <sup>a</sup>	38.4 <sup>b</sup>	25.2 <sup>b</sup>	11.4
Methane yield	(g/DMI kg)	22.1 <sup>a</sup>	14.9 <sup>b</sup>	10.6 <sup>c</sup>	1.02	19.2 <sup>a</sup>	10.6 <sup>b</sup>	3.9 <sup>c</sup>	1.36	12.4 <sup>a</sup>	3.8 <sup>b</sup>	2.5 <sup>b</sup>	1.02
Hydrogen production	(g/day)	1.25 <sup>a</sup>	3.48 <sup>b</sup>	5.77 <sup>c</sup>	0.44	1.38 <sup>a</sup>	5.88 <sup>b</sup>	8.76 <sup>c</sup>	0.56	0.97 <sup>a</sup>	5.71 <sup>b</sup>	6.94 <sup>b</sup>	0.44
Hydrogen yield	(g/DMI kg)	0.12 <sup>a</sup>	0.35 <sup>b</sup>	0.68 <sup>c</sup>	0.05	0.11 <sup>a</sup>	0.55 <sup>b</sup>	0.93 <sup>c</sup>	0.06	0.09 <sup>a</sup>	0.53 <sup>b</sup>	0.66 <sup>b</sup>	0.05
Carbon dioxide production	(g/day)	7422	7399	7035	324	8393	8335	7185	365	7577	7770	7795	324
Carbon dioxide yield	(g/DMI kg)	706	742	815	27.3	694	779	806	32.9	678 <sup>a</sup>	731 <sup>ab</sup>	744 <sup>b</sup>	27.3

<sup>a,b,c</sup> superscripts; *P*-value<0.05



265 Hydrogen production significantly increased 318 and 497% and H<sub>2</sub> yield also increased  
266 significantly 336 and 590% compared to Control for the LD and HD treatments for the collective  
267 feeding stages of the experiment, respectively. Hydrogen production in steers receiving *A.*  
268 *taxiformis* to high forage TMR in the LD and HD treatments significantly increased 177 ( $P = 0.03$ )  
269 and 360%, respectively. Hydrogen yield increased 198 ( $P = 0.04$ ) and 478% in steers on LD and  
270 HD treatments, respectively. Hydrogen production from steers on the medium forage TMR  
271 increased 326 ( $P = 0.03$ ) and 535% ( $P < 0.01$ ), for LD and HD treatments, respectively, whereas  
272 H<sub>2</sub> yield significantly increased 404 and 753%, respectively. Supplementation of *A. taxiformis* to  
273 the low forage TMR fed to steers significantly increased H<sub>2</sub> production 419 and 618% and reduced  
274 H<sub>2</sub> yield 503 and 649% in LD and HD treatments, respectively. Carbon dioxide (CO<sub>2</sub>) production  
275 was not affected by either LD or HD treatments compared to Control. However, CO<sub>2</sub> yield was  
276 significantly greater in HD group compared to Control ( $P = 0.03$ ).

### 277 **3.2 Animal production parameters**

278 Dry matter intake, ADG, feed conversion efficiency (ADG/DMI; FCE) and cost per gain  
279 (\$USD/kg weight gain; CPG) as impacted by treatment groups (Control, LD, HD) for the  
280 collective feeding stages is presented in Table 4 and for the individual stages and TMRs in Table  
281 5. Initial BW, final BW, carcass weight and total weight gained are shown in Table 4. Considering  
282 all feeding stages, steers on the LD treatment tended ( $P = 0.08$ ) to decrease their DMI 8% and was  
283 significantly reduced 14% in steers on the HD ( $P < 0.01$ ) treatment compared to Control. Steers  
284 fed the high and medium forage TMR in the HD treatment decreased their DMI 18.5 ( $P = 0.01$ )  
285 and 18.0% ( $P < 0.01$ ), respectively, compared to Control. No significant effects were observed in  
286 ADG by the LD or HD treatment groups. With the reduction of DMI in LD and HD treatments  
287 and similar ADG among all 3 treatments, FCE tended to increase 7% in LD ( $P = 0.06$ ) treatment

288 and significantly increased 14% in steers in HD ( $P < 0.01$ ) treatment. When averaged throughout  
 289 the experiment as well as within the 3 TMR stages, CPG was not statistically significant. However,  
 290 over the collective feeding stages, CPG was consistently lower in HD and LD groups compared to  
 291 Control with approximately \$0.37 USD/kg gain differential between HD and Control and \$0.18  
 292 USD/kg gain differential between LD and Control. Additionally, cost differentials for HD were  
 293 \$0.29, \$0.40, and \$0.34 USD/kg gain and for LD were \$0.15, \$0.49, and \$0.34 USD/kg gain for  
 294 the high, medium, and low forage TMRs, respectively.

295 **TABLE 4** Effect of *Asparagopsis taxiformis* inclusion levels of 0%, 0.25%, and 0.5% of feed  
 296 organic matter on animal parameters over 21 weeks  
 297

		0%	0.25%	0.50%	SE
<i>Animal Parameters</i>					
Dry matter intake	(kg/day)	11.32 <sup>a</sup>	10.42 <sup>ab</sup>	9.69 <sup>b</sup>	0.29
Average daily gain	(kg/day)	1.60	1.52	1.56	0.06
Feed conversion efficiency	(ADG/DMI)	0.14 <sup>a</sup>	0.15 <sup>a</sup>	0.16 <sup>b</sup>	0.01
Cost per gain	(\$/kg gain)	2.25	2.07	1.88	0.18
Initial body weight	(kg)	357	348	350	9.21
Final body weight	(kg)	589	572	587	11.1
Carcass weight	(kg)	370	361	350	13.4
Total gain	(kg)	232	224	236	6.09

<sup>a,b,c</sup> superscripts;  $P$ -value  $< 0.05$

298 **TABLE 5** Effect of *Asparagopsis taxiformis* inclusion levels of 0%, 0.25%, and 0.5% feed organic matter to beef cattle diets on  
 299 animal parameters

		Diet											
		High forage				Medium forage				Low forage			
		0%	0.25%	0.50%	SE	0%	0.25%	0.50%	SE	0%	0.25%	0.50%	SE
<i>Animal Parameters</i> *													
Dry matter intake	(kg/day)	10.31 <sup>a</sup>	9.69 <sup>ab</sup>	8.40 <sup>b</sup>	0.33	12.18 <sup>a</sup>	10.81 <sup>ab</sup>	9.99 <sup>b</sup>	0.33	11.47 <sup>a</sup>	10.77 <sup>ab</sup>	10.67 <sup>b</sup>	0.33
Average daily gain	(kg/day)	1.58	1.61	1.53	0.11	1.62	1.50	1.38	0.11	1.60	1.44	1.75	0.11
Feed conversion	(ADG/DMI)	0.15	0.17	0.18	0.01	0.13	0.14	0.14	0.01	0.14	0.13	0.17	0.01
Cost per gain	(\$/kg)	1.83	1.68	1.54	0.25	2.67	2.18	2.20	0.26	2.24	2.35	1.90	0.27

<sup>a,b,c</sup> superscripts; *P*-value<0.05

### 301 **3.3 Carcass quality parameters**

302 There was no statistical difference between treatment groups for rib eye area (Table 6). No effects  
303 were found between Control, LD, and HD in moisture, protein, fat, ash, carbohydrates, or calorie  
304 content of strip loins (Table 6). The average WBSF values for the Control, LD and HD groups  
305 were 2.81, 2.66 and 2.61 kg, respectively. Additionally, the SSF averages were measured as 17.1  
306 for Control, 16.75 for LD and 17.4 kg for HD treatments. No significant differences ( $P > 0.05$ )  
307 were found in shear force resistance among treatment groups. Mean scores of all sensory attributes  
308 (tenderness, juiciness, and flavor) by consumer panels were not significantly different ( $P > 0.05$ )  
309 among treatment groups (Table 6). The taste panel considered all steaks, regardless of treatment  
310 group, to be moderately tender as well as moderately juicy. This was consistent with the taste panel  
311 stating that they moderately liked the flavor of all steaks regardless of treatment group. There was  
312 no difference ( $P > 0.05$ ) in overall acceptability among treatment groups.

313 There was a linear increase in iodine concentrations in both LD ( $P < 0.01$ ) and HD ( $P < 0.01$ )  
314 compared to Control. Iodine concentrations for the Control treatment group were below detection  
315 levels, which was set at 0.10 mg/g (Table 6). However, 5 out of 7 steers in LD treatment group  
316 had iodine levels above the detection level with a treatment average of 0.08 mg/g ( $P < 0.01$ ). All  
317 6 steers in the HD treatment group were found to contain iodine levels above the detection level  
318 with concentration levels ranging between 0.14 – 0.17 mg/g with a mean of 0.15 mg/g ( $P < 0.01$ ).  
319 Bromoform concentrations for all treatment groups were below detection levels, which were 0.06  
320 mg/kg.

321 **TABLE 6** Effects of *Asparagopsis taxiformis* supplementation on carcass quality, proximate  
 322 analysis, shear force, and consumer panel preference.

	Level of <i>Asparagopsis taxiformis</i> inclusion			SE
	0%	0.25%	0.50%	
<i>Carcass Quality</i>				
Rib eye area (inches)	11.3	11.2	10.6	0.37
<i>Proximate Analysis</i>				
Moisture (g/100g)	53.9	55.4	55.3	1.7
Protein (g/100g)	16.1	17.1	17.2	0.7
Fat (g/100)	29.1	26.1	26.3	2.2
Ash (g/100g)	0.73	0.86	0.88	0.05
Carbohydrates (g/100g)	0.24	1.01	0.42	0.38
Calories	327	307	307	17.7
Iodine (PPM)	0.00 <sup>a</sup>	0.08 <sup>b</sup>	0.15 <sup>c</sup>	0.02
<i>Shear Force</i>				
Warner- Bratzler (kgf)	2.81	2.66	2.61	0.24
Slice Shear Force (kgf)	17.1	16.8	17.4	1.87
<i>Consumer Panel<sup>1</sup></i>				
Tenderness	6.72	6.68	6.45	0.17
Juiciness	6.35	6.33	6.07	0.17
Flavor	6.63	6.34	6.24	0.15
Overall	6.66	6.36	6.46	0.16

<sup>a,b,c</sup> superscripts; *P*-value <0.05

<sup>1</sup>A 9-point hedonic scale was used

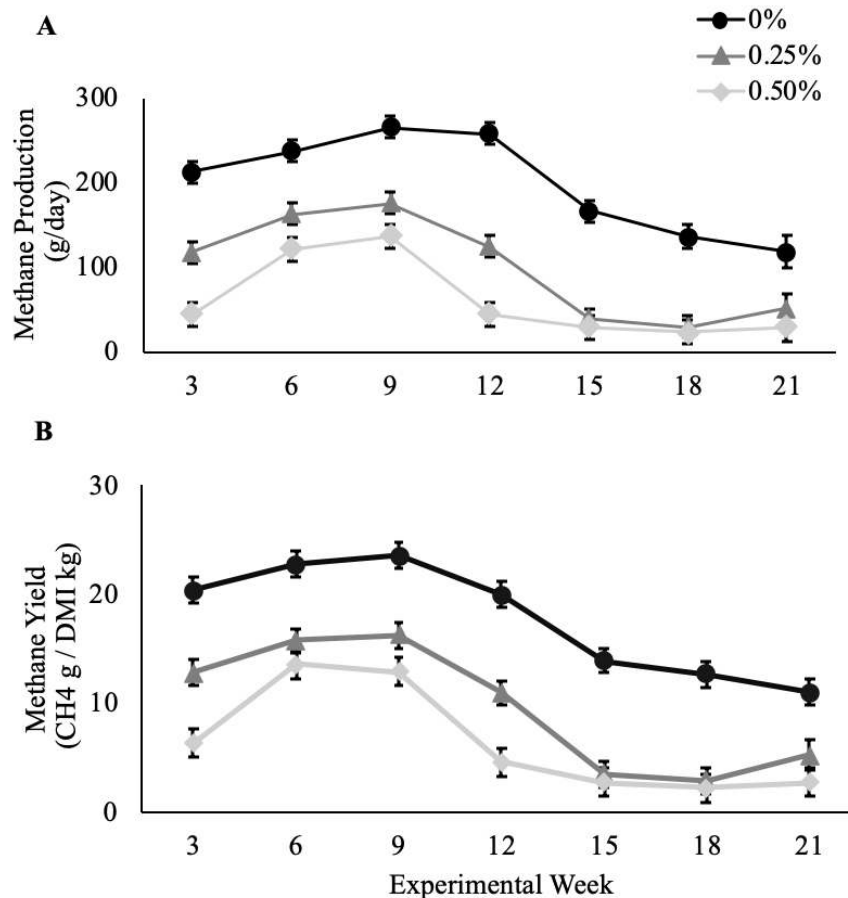
(1 = Dislike extremely and 9 = Like extremely).

## 323 **4 DISCUSSION**

### 324 **4.1 Enteric methane production and yield**

325 This study demonstrated that dietary inclusion of *A. taxiformis* induces a consistent and  
 326 considerable reduction in enteric CH<sub>4</sub> production from steers on a typical feedlot style diet. Enteric  
 327 CH<sub>4</sub> is the largest contributor to GHG emissions from livestock production systems. Similar  
 328 reductions in CH<sub>4</sub> yield, which is standardized by DMI, has also been established. There is a  
 329 concern that feed additives and other CH<sub>4</sub> reducing agents decrease in efficacy over time (Knight  
 330 et al., 2011, Rumpler et al., 1986). This study provided evidence that the seaweed inclusion was  
 331 effective in reducing CH<sub>4</sub> emissions, which persisted for the duration of the study of 147 days

332 (Figure 3). Notably, until this study the longest exposure to *A. taxiformis* had been demonstrated  
333 for steers in a study ending after a 90-d finishing period (Kinley et al. 2020). To date, only three  
334 *in vivo* studies have been published using *Asparagopsis spp* to reduce enteric CH<sub>4</sub> emissions in  
335 sheep (Li et al. 2018), lactating dairy cattle (Roque et al., 2019b), and feedlot Brangus steers  
336 (Kinley et al., 2020). All studies show considerable if not variable reduction in enteric CH<sub>4</sub>  
337 emissions. The differences in efficacy are likely due to levels of seaweed inclusion, formulation  
338 of the diets, and differences in seaweed quality based on bromoform concentrations.



**FIGURE 3** Methane production [g CH<sub>4</sub>/day] (A) and methane yield [g CH<sub>4</sub>/kg DMI] (B) from beef steers supplemented with *Asparagopsis taxiformis* at 0%, 0.25%, and 0.5% of basal total mixed ration on an organic matter basis during the 21 week experimental period. Data points are treatment means for each gas collection timepoint and error bars represent standard errors.

339           It has been previously hypothesized that NDF levels can also influence the rate at which  
340 CH<sub>4</sub> is reduced with the inclusion of inhibitors (Dijkstra et al. 2018). In the current study, the  
341 magnitude of reductions in CH<sub>4</sub> production were negatively correlated ( $r^2 = 0.89$ ) with NDF levels  
342 in the 3 diet regimens that contained 33.1% (high forage), 25.8% (medium forage), and 18.6%  
343 (low forage) NDF levels. Enteric CH<sub>4</sub> production was reduced 32.7, 44.6 and 69.8% in steers on  
344 the LD treatment and 51.9, 79.7, and 80.0% on HD treatment with high, medium and low forage  
345 TMRs, respectively. The low forage TMR, containing the lowest NDF levels, was the most  
346 sensitive to the inclusion of *A. taxiformis* with CH<sub>4</sub> reductions above 70% at equivalent inclusion  
347 levels compared to the higher forage TMRs. Li et al. (2018) reported an 80.6% reduction of CH<sub>4</sub>  
348 yield in sheep fed diets containing 55.6% NDF, however, the level of *A. taxiformis* intake by the  
349 sheep was unclear but was offered at 6 times greater levels than the HD treatment in our study.  
350 Roque et al. (2019b) showed 42.7% reduction in CH<sub>4</sub> yield in lactating dairy cattle fed a diet  
351 containing 30.1% NDF at 1% inclusion rate of *A. armata*. The high forage TMR in our study had  
352 a similar NDF level to Roque et al. (2019b), however, had approximately double the reduction of  
353 CH<sub>4</sub>, even when consuming 50% less seaweed. These differences relate to a large degree to the  
354 quality of seaweed in terms of the concentration of bromoform, which was 1.32 mg/g in Roque et  
355 al. (2019b) compared to 7.82 mg/g in the current study. Kinley et al. (2020) conducted an *in vivo*  
356 study focused on feedlot steers using the same collection of *A. taxiformis* as sub-sampled and used  
357 in this study. This seaweed had bromoform concentration of 6.55 mg/g, which was marginally  
358 lower than our study and may be due to variation in the collection, sampling or analysis techniques.  
359 Despite the lower bromoform concentration in the seaweed and using 0.20% inclusion rate of *A.*  
360 *taxiformis* on OM basis, the CH<sub>4</sub> yield was reduced by up to 98% in Brangus feedlot steers. The  
361 diet used by Kinley et al. (2020) included 30.6% NDF, which was similar to our high fiber diet.

362 The greater efficacy of *A. taxiformis* in that study could be due to collective feed formulation  
363 differences such as the energy dense component of barley versus corn, which is typical of  
364 Australian and American feedlots, respectively. Additionally, it could be due to beneficial  
365 interaction with the ionophore, monensin, that was used in the Australian study. Monensin has not  
366 been used in any other feed formulation in other *in vivo* studies with the inclusion of *Asparagopsis*  
367 species. Use of monensin in diets has shown to decrease CH<sub>4</sub> yields by up to 6% in feedlot steers  
368 while also having an enhanced effect in diets containing greater NDF levels (Appuhamy et al.,  
369 2013). A potential enhancing interaction of the seaweed with monensin is of great interest and  
370 further investigation will elucidate this potential that could have significant beneficial economic  
371 and environmental impact for formulated feeding systems that use monensin.

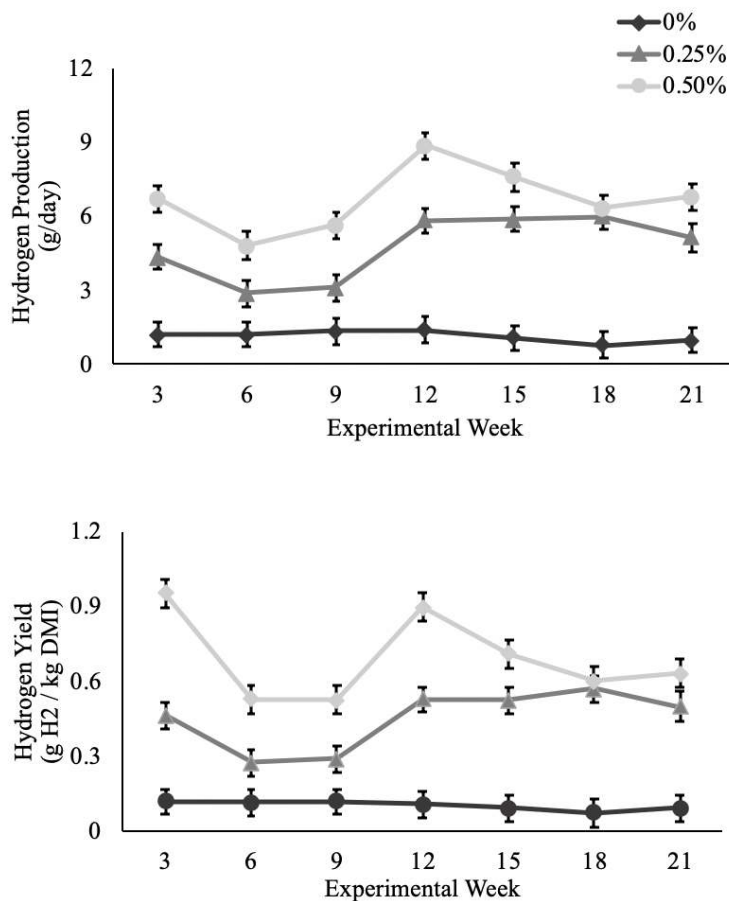
372

#### 373 **4.2 Enteric hydrogen and carbon dioxide emissions**

374 Increases in H<sub>2</sub> yield have typically been recorded when anti-methanogenic feed additives are  
375 used, and with the addition of *Asparagopsis* species in dairy cattle (1.25 to 3.75 fold; Roque et al.,  
376 2019b) and Brangus feedlot steers (3.8-17.0 fold; Kinley et al., 2020). Similar increases in H<sub>2</sub> yield  
377 have been reported in feed additives that reduce enteric CH<sub>4</sub> emissions targeting methanogens. For  
378 example, in lactating dairy cows supplemented with 3-NOP, H<sub>2</sub> yield increased 23 – 71 fold  
379 (Hristov et al., 2015). Bromochloromethane (BCM) fed to goats increased H<sub>2</sub> (mmol/head per day)  
380 5 – 35 fold, while chloroform fed to Brahman steers increased H<sub>2</sub> yield 316 fold (Mitsumori et al.,  
381 2012; Martinez-Fernandez et al., 2016). Although feeding *Asparagopsis spp.* increased overall H<sub>2</sub>  
382 yield (Figure 4), the magnitude was considerably lower (1.25 to 17 fold) compared to alternative  
383 CH<sub>4</sub> reducing feed additives (5 to 316 fold), with similar levels of reductions in CH<sub>4</sub>. This indicates  
384 that there may be a redirection of H<sub>2</sub> molecules that would otherwise be utilized through the



385 formation of CH<sub>4</sub> and redirected into different pathways that could be beneficial to the animal. For  
386 example, increased propionate to acetate concentrations have been recorded in *in vitro* studies  
387 using *A. taxiformis* (Machado et al., 2016a; Roque et al., 2019a) during inhibition of CH<sub>4</sub> which  
388 could indicate that some of the excess H<sub>2</sub> is being utilized for propionate production. Consistent  
389 with this theory, similar results have been reported using other CH<sub>4</sub> analogues such as BCM  
390 (Denmen et al., 2015) and chloroform, (Martinez-Fernandez et al., 2016) both of which showed  
391 increases in propionate production. In general, significant reduction of CH<sub>4</sub> production in the  
392 rumen without detriment to rumen function is typically associated with reduction of acetate,  
393 increase in propionate, and reduction of the acetate:propionate ratio (Kinley et al., 2020).  
394



**FIGURE 4** Hydrogen production [g H<sub>2</sub>/day] (A) and Hydrogen yield [g H<sub>2</sub>/kg DMI] (B) from beef steers supplemented with *Asparagopsis taxiformis* at 0% , 0.25% , and 0.5% of basal total mixed ration on an organic matter basis during the 21 week experimental period. Data points are treatment means for each gas collection timepoint and error bars represent standard errors

395 In contrast to the lactating dairy cattle study in which CO<sub>2</sub> yield increased significantly in  
396 dairy cattle fed 1% *A. armata* (Roque et al., 2019b), there was no significant difference in CO<sub>2</sub>  
397 emissions or yield in the current study. This could be due to the relationship between the amount  
398 of seaweed fed and DMI intake.

399

#### 400 **4.3 Animal production parameters**

401 Dry matter intake reductions observed in this study were consistent with previous studies in  
402 lactating dairy cows where decreases in DMI were found to be 10.7 and 37.9% at 0.50 and 1.0%  
403 inclusion rate of *A. armata* (Roque et al., 2019b), respectively. Decreases in DMI have also been  
404 reported in cattle fed other anti-methanogenic feed additives in a linear dose-response fashion. For  
405 example, Tomkins et al. (2009) reported 3 to 19% reductions in DMI in steers supplemented with  
406 BCM at dosages between 0.15 and 0.60 g/100 kg live weight. Additionally, Martinez-Fernandez  
407 (2016) found 1.7 to 15% reductions in DMI when feeding steers chloroform at dosages between 1  
408 to 2.6 g/100g liveweight. In contrast, Kinley et al. (2020) reported no significant differences at the  
409 highest *A. taxiformis* level of 0.20%. However, the inclusion level was less than our study's lowest  
410 inclusion rate, so based on previous experiment's observation of reduced DMI in a dose-response  
411 manner (Roque et al., 2019b), it was expected to have lower effect on DMI. Decreases in DMI are  
412 normally associated with lower productivity due to lower levels of nutrients and dietary energy  
413 consumed. However, there was no significant difference in ADG between steers in the HD  
414 treatment and Control (average 1.56 kg/day) groups despite consuming 14% less feed. The results  
415 were in agreement with a previous study (Roque et al., 2019b), in which milk production was not  
416 compromised at a 0.5% OM inclusion level despite reductions in DMI. The FCE (ADG/DMI)  
417 increased significantly in HD treatment group, suggesting that inclusion rates of *A. taxiformis* at

418 0.5% improves overall feed efficiency in growing beef steers. Since a large proportion of on farm  
419 costs is the purchase of feed, an improved feed efficiency is particularly exciting for producers to  
420 decrease feed costs while also producing the same amount of total weight gains. Total gains were  
421 between 224 kg (LD) to 236 kg (HD) combined with an average cost differential of ~\$0.18 USD/kg  
422 gain (LD) and ~\$0.37 USD/kg gain (HD). A producer finishing 1000 head of beef cattle has the  
423 potential to reduce feed costs by \$40,320 (LD) to \$87,320 (HD) depending on seaweed dosage.  
424 While the CPG in this study were not statistically significant, this may be due to low animal  
425 numbers in each treatment and warrants further investigation on a larger feedlot setting to reduce  
426 animal variability.

427

#### 428 **4.4 Bromoform and iodine residues**

429 Bromoform is considered to be the active ingredient responsible for CH<sub>4</sub> reduction when fed to  
430 cattle (Machado et al., 2016b). However, high levels of bromoform is considered to be hazardous  
431 for humans and mice. While bromoform intake limits are yet to be defined for cattle specifically,  
432 the United States Environmental Protection Agency (2017) has suggested a reference dose for  
433 bromoform, an estimated level of daily oral exposure without negative effects, to be 0.02 mg/kg  
434 BW/day. It is essential that food products from livestock consuming the seaweed are confirmed as  
435 safe for consumption and that bromoform residues are not transferred to the edible tissues and offal  
436 of bovines at levels detrimental to food safety. Previous studies have demonstrated that bromoform  
437 was not detectable in the kidney, muscle, fat deposits, blood, feces, and milk in either sheep (Li et  
438 al., 2018), dairy cows (Roque et al., 2019b), or feedlot steers (Kinley et al., 2020). Strip loin and  
439 liver samples from steers were collected and in agreement with previous studies, no bromoform  
440 was detected in this study.

441 The National Academies of Sciences, Engineering, and Medicine (2016) recommendations  
442 for daily iodine intake in growing beef cattle is 0.5 ug/g DMI and maximum tolerable limit is 50  
443 ug/g DMI. In this study, recommended daily iodine intake levels were 5.2 mg/day and 4.85 mg/day  
444 and maximum limits are 521 mg/day and 485 mg/day for LD and HD treatment groups,  
445 respectively. The iodine level in the *A. taxiformis* fed in the current study contained 2.27 mg/g,  
446 therefore, maximum daily intake of seaweed iodine was 106 – 127 mg/day and 173 – 225 mg/day  
447 for the LD and HD treatment groups, respectively. While these levels do not exceed maximum  
448 tolerable limits, they exceed daily iodine intake recommendations, therefore it was appropriate to  
449 test for iodine residue levels in meat used for human consumption. The US Food and Nutrition  
450 Board of the National Academy of Sciences has set a tolerable upper intake level (UL) for human  
451 consumption of foods, which is defined as the highest level of daily intake that poses no adverse  
452 health effects (Trumbo et al., 2001). The iodine UL ranges between 200 ug/day to 1,100 ug/day  
453 depending on age, gender, and lactation demographics. Strip loins tested for iodine residues had  
454 levels of 0.08 and 0.15 ug/g from steers in treatments LD and HD, respectively. These iodine  
455 residues are far under the UL limits for human consumption. For example, UL for a person under  
456 3 years of age is 200 ug/day meaning that this person would have to consume more than 2,500  
457 g/day and 1,330 g/day of meat from a LD and HD steers, respectively, to reach the UL. An adult  
458 over the age of 18 has an UL of 1,100 ug/day and would have to consume more than 13.8 kg/day  
459 and 7.3 kg/day of meat from a LD and HD steers, respectively, to reach their UL of iodine intake.  
460 At the inclusion levels and iodine concentration of *A. taxiformis* used in this study the margin of  
461 safety is extremely high and the likelihood of iodine toxicity from consuming the meat is extremely  
462 low.  
463

#### 464 **4.5 Carcass quality parameters:**

465 Marbling scores ranged from 410 – 810 while all carcasses, regardless of treatment, graded as  
466 either choice or prime. The value placed on tenderness in the marketplace is high and has even  
467 been found that consumers are likely to pay premiums for more tender beef (Miller et. al, 2001).  
468 Rodriguez-Herrera et al (2016) found that cattle supplemented with a high amount of  
469 *Schizochytrium spp.* (30 g/kg of feed intake) had improved tenderness compared to the control  
470 treatment and a low dose treatment group. Conversely, Phelps et al (2016) reported that  
471 supplementation of *Schizochytrium spp.* in cattle at 0 to 150 g/day per animal, a lower inclusion  
472 rate than Rodriguez-Herrera et al (2016), did not have a significant impact on objective tenderness.  
473 While no significant differences in meat tenderness were observed for either WBSF or SSF values  
474 among the three treatment groups in this study, it may be due to low inclusions rates and warrants  
475 further investigation. If the macroalgae *A. taxiformis* has the potential to perform similarly to the  
476 microalgae *Schizochytrium spp.*, this could increase consumers' preferences for algae-fed beef  
477 while also providing producers with a tenderness premium for their product.

478 The current study showed no statistical differences in consumer sensory evaluation or  
479 preference toward steaks from any of the treatment groups in agreement with Kinley et al. (2020)  
480 in which steaks from beef cattle fed *A. taxiformis* were evaluated for taste, tenderness, juiciness,  
481 or overall flavor. Another study demonstrated that trained panelists were unable to detect a  
482 difference in tenderness in beef steaks from cattle fed up to 150 g of *Schizochytrium spp.* per day  
483 (Phelps et al, 2016). Conversely, some studies reported that the supplementation of  
484 *Schizochytrium* fed at a 3.89% inclusion rate of DMI in lamb diets and up to 30 g/kg of feed intake  
485 to cattle resulted in meat samples as having a “seaweed” or “fishy” flavor in which the authors  
486 attributed to increased docosahexaenoic acid levels (Rodriguez-Herrera et. al, 2018; Urrutia et. al.,

487 2016). However, these two studies feed a substantially greater amount of seaweed in their diets  
488 than the current study, which could be the reason for the alteration in overall taste. The current  
489 study indicates that the supplementation of *A. taxiformis* at 0.25% or 0.5% to cattle does not  
490 significantly impact overall meat quality nor alter the sensory properties of the steaks.

491 In summary, this study showed that the use of *A. taxiformis* supplemented to beef cattle diets  
492 reduced enteric CH<sub>4</sub> emissions for a duration of 21 weeks without any loss in efficacy. The efficacy  
493 was highly correlated with the proportion of NDF in the diet. Additionally, *A. taxiformis* has no  
494 measurable residual effect in the product and did not alter meat quality or sensory properties.  
495 Importantly, the use of *A. taxiformis* impacts DMI and not ADG, therefore increasing overall feed  
496 efficiency (FCE) in growing beef steers. There may also be potential to reduce the cost of  
497 production per kg of weight gain. These feed cost reductions in combination with significantly  
498 reduced CH<sub>4</sub> emissions have a potential to transform beef production into a more financially and  
499 environmentally sustainable and product efficient industry.

500

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