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Inclusion of *Asparagopsis armata* in lactating dairy cows' diet reduces enteric methane emission by over 50 percent

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ABSTRACT

Livestock production, particularly enteric methane production, contributes to greenhouse gas emissions globally. Various mitigation strategies developed to reduce enteric emissions have limited success. Although *in vitro* studies have shown a considerable reduction in methane emissions using *Asparagopsis* spp., no studies have been conducted to investigate the effect of any species of *Asparagopsis* in dairy cattle. Our objective was to evaluate quantitatively the response of cows consuming *Asparagopsis armata* on methane production (g/kg), yield (g/kg feed intake) and intensity (g/kg milk yield). Twelve post-peak lactating Holstein cows were randomly assigned to three treatments (control, 0.5% and 1% inclusion levels of *A. armata* on organic matter basis) in a 3×3 Latin square design with three 21-day periods. Enteric methane emissions were measured using the GreenFeed system. Methane production by cows decreased significantly by 26.4% at the low (0.5%) level of *A. armata* inclusion and 67.2% at the high (1%) level of inclusion. Feed intake was reduced by 10.8 and 38.0%, in cows fed the low and high level of macroalgae inclusion, respectively. Methane yield decreased significantly by 20.3 and 42.7% in cows fed diet including 0.5% and 1% *A. armata* inclusion levels, respectively ($P = <0.0001$). Methane intensity significantly decreased by 26.8% from cows fed at 0.5% level and 60% at the 1.0% *A. armata* inclusion level. Bromoform concentrations in milk were not significantly different between treatments. Our *in vivo* results showed that *A. armata* has potential to be used as a feed additive to reduce enteric methane emissions.

1. Introduction

The livestock sector contributes 14.5% of global GHG emissions (Gerber et al., 2013), with global methane emissions contributing to about 2.1 Gt CO_2 equivalent in 2010 (Smith et al., 2014). There are considerable differences in contribution of enteric methane in different regions and countries of the world. The main source of anthropogenic methane emissions in the United States is generated by enteric fermentation of livestock (25%; NASEM, 2018). US EPA (2019) estimated the total methane emissions from enteric fermentation in the United States to be 6.46 Tg in 2017, which is equivalent to 27% of the nation's anthropogenic methane emissions. Enteric methane is a natural by-product of microbial fermentation of nutrients in the digestive tract of animals. Enteric methane emissions represent up to 11% of dietary gross energy consumed by ruminants and in North American dairy cattle it is estimated to be about 5.7% of gross energy intake (Moraes et al., 2014).

Hristov et al. (2013) reported that mitigation options including nitrates, ionophores, tannins, direct-fed microbials and vaccines may offer opportunities to reduce enteric methane emissions; however, the results have been inconsistent. Knapp et al. (2014) estimated that nutrition and feeding approaches may contribute to reducing methane emission intensity (i.e., emissions per milk yield) by 2.5–15%, whereas rumen modifiers had little success in sustained methane emissions without compromising milk production. Methane inhibitors may be a more successful approach in reducing emissions from enteric fermentation. For example, 3-nitrooxypropanol (3NOP) has been reported to substantially decrease methane emissions from ruminants (Duin et al., 2016).

Seaweeds have been a traditional part of livestock diet and have been used since the recording of agricultural practices began (Evans

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and Critchley 2014). There have been several studies on seaweeds to characterize their effects as livestock feeds and their potential to manipulate rumen fermentation and methane production. Maia et al. (2016) evaluated several seaweeds and reported that their efficacy is impacted by the formulation of the basal feed of the livestock. The utility of seaweeds as feeds is impacted by the composition of the biomass which in turn is a result of many inherent factors such as species, growth stage, habitat, and external factors such as temperature, light, and nutrient availability. A key feature is the circumstantial production and accumulation of secondary metabolites (Paul et al., 2006) that may have a bioactive impact on the animals or from the perspective of methane production, on the microbial consortium that thrives in a rumen. Many seaweeds have been demonstrated to reduce methane production by rumen methanogens but with variable effects on fermentative health and substrate digestibility (Machado et al., 2014). The effectiveness of the seaweeds has been shown to have a relationship with the level of inclusion in the diet (Kinley et al., 2016; Machado et al., 2016a,b; Li et al., 2018) and only Asparagopsis has been demonstrated to remain effective and dramatically anti-methanogenic without negative impacts on rumen function and at low inclusion levels in animal diets (Kinley et al., 2016; Li et al., 2018).

In the development of knowledge of *Asparagopsis* spp. effects on rumen microbial production of methane, there has been progression through multiple *in vitro* studies all of which have demonstrated significant if not total reduction of methane emissions at levels of approximately 2% of diet substrates (Dubois et al., 2013; Machado et al., 2016a,b). Even though this dietary level of the seaweed was low and considered feasible for livestock production systems, Li et al. (2018) demonstrated in animals the potential for efficacy at lower intake levels. From their study in sheep using *Asparagopsis taxiformis*, Li et al. (2018) reported up to 80% reduction in methane emissions. Although the project applied *A. taxiformis* offerings of 0.5, 1, 2, and 3% of the diet, the feed formulation provided for voluntary intake of the seaweed product which resulted in partial unavailability or refusal by the sheep. Nevertheless with reduced intake of the seaweed the results showed significant methane reduction and was the first indication that *in vitro* studies had over predicted the levels of *Asparagopsis* intake required for effective methane reduction *in vivo*. This created an exploratory research requirement for subsequent animal studies to characterize the optimal intake of *Asparagopsis* to significantly reduce methane emissions which is proposed to be variable based on diet composition.

In the systematic *in vivo* characterization of *Asparagopsis* as an antimethanogenic feed additive for ruminant livestock this study is the first demonstration of the effects in lactating dairy cattle. Based on previous *in vitro* and *in vivo* work it was hypothesized that application of *Asparagopsis* as a feed additive in a total mixed ration (TMR) would significantly reduce enteric methane emissions and improve productivity represented by increased milk production. The objectives of the study were to: (1) investigate the potential of the macroalgae *Asparagopsis armata* in reducing methane emissions *in vivo*; and (2) quantify methane production (g/day per cow), yield (g/kg dry matter intake (DMI)) and intensity (g/kg milk yield) as a result of inclusion of *A. armata* in the TMR of lactating dairy cattle.

2. Methods

2.1. Animals and experimental design

All animal procedures were approved by the UC Davis Institutional Animal Care and Use Committee. Twelve multiparous Holstein cows with an average weight of 729 ± 24.9 kg, 35.1 ± 2.19 kg/d milk yield and 201 ± 37 days in lactation were housed in a freestall barn equipped with individual animal sensor electronic recognition Calan gates (American Calan, Northwood, NH) to measure individual animal feed intake. As this was the first reported study using dairy cattle, we conducted a pretrial to determine effective levels of *A. armata* inclusion in the ration. The inclusion was gradually introduced from 0.25 to 1% of dietary OM. During the pretrial, there was a linear reduction of methane as the level of inclusion increased. However, at inclusion approaching 1%, the cows demonstrated moderately reduced feed intake. Based on these observations, the inclusion levels were set as follows: no *Asparagopsis*, (control), 0.5% (low) and 1% (high) inclusion levels on OM basis. Cows were randomly assigned to a 3 × 3 Latin square design within the three treatment groups and 3 periods. Cows were fed twice daily at 0600 and 1700 h at 105% of their individual previous day intake and had free access to water at all times. Cows were fed a total mixed ration over the course of the study (Table 1) that was formulated to meet or exceed their growth requirement according to the recommendations of the National Research Council Requirement for Dairy Cattle (NRC, 2001). The nutritional composition of the basal diet is given in Table 1. *Asparagopsis armata* in the sporophyte stage was mixed with 400 ml of molasses and water to increase palatability, then hand mixed in to the total mixed ration. The *A. armata* biomass was harvested from Cloudy Bay, Bruny Island (43.44226S, 147.23773E) near Hobart, Tasmania, Australia. After harvest, the material was blast frozen to −25 °C over approximately 6 h, then freeze dried under vacuum and temperature control for 30 h before packing and shipping. The macroalgae used in the study contained 1.32 mg/g dry weight level of bromoform concentration.

2.2. Sample collection and analyses

Methane, carbon dioxide, and hydrogen gases from each cow were measured using the automated emissions measurement of the GreenFeed Large Animal System (C-Lock, Inc., Rapid City, SD). Gases were measured for 7 days to develop a baseline followed by 14 days with each cow visiting the system at least 3 times a day. Breath gas samples were collected for 8–10 min followed by a 2-min background gas sample collection. Calibration of the GreenFeed gas monitor was performed once per week throughout the trial. Bait feed consisting of alfalfa pellets was offered at each sampling event and kept below 5% of the total DMI during each

Table 1
Ingredients and chemical composition of the basal experimental diet and *A. armata* (% of dry matter).

Ingredients	Basal diet	Asparagopsis armata
% Dry matter		
Organic matter	91.3	49.6
Crude protein	17.6	18.3
Neutral detergent fiber	30.1	27.2
Acid detergent fiber	21.2	10.9
Fat	4.65	0.32
Total digestible nutrients	70.9	30.5
Lignin	5.04	2.83
Calcium	1.14	4.47
Phosphorus	0.44	0.27
Sodium	0.24	9.36
Magnesium	0.33	1.38
Parts per million		
Iron	415	1188
Manganese	60.0	62.3
Zinc	68.0	66.3
Copper	13.0	13.3

sampling period. Diets were formulated to account for bait feed consumption offered by the GreenFeed system. Milk production, bodyweight, blood, feces, orts, and milk components were measured for 14 days daily during the treatment period and 7 days daily post treatment. Milk production was recorded twice daily representing morning and afternoon milking throughout the trial. Milk bromoform concentrations were analyzed using an Agilent 7890B GC applied to Agilent 7000C triple quad Mass Spectrometer equipped with a ZB-5ms column (Agilent Technologies, Inc. Santa Clara, CA). The limit of detection and limit of quantification were 0.01 μg/L and 0.05 μg/L, respectively.

2.3. Statistical analyses

All statistical analyses were performed using R statistical software (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria). Gas measurements were averaged per cow and treatment period and the averaged data was used for statistical analysis. Additionally, gas measurements were presented (normalized) as emissions per kg DM intake (g/kg DMI). Dry matter intake, body weight, milk production, milk components, and feed conversion efficiency (milk production/DMI) were averaged per treatment period prior to statistical analysis. Statistically significant differences were declared at $P < 0.05$. Tendencies were declared at $0.05 < P < 0.10$.

3. Results

3.1. Gas parameters

The average methane, hydrogen, and carbon dioxide production for the three treatment groups is given in Fig. 1. Methane production (g/d) by cows decreased significantly by 26.4% at the low (0.5%) level of *A. armata* inclusion and 67.2% at the high (1%) level (Fig. 1A). Hydrogen production increased 163 and 236% by cows fed diets with low and high levels of macroalgae inclusion. Carbon dioxide production was similar between control and low level of inclusion; however, there was a significant 13.9% decrease in total carbon dioxide production between control and high level of inclusion. When normalized for amount of feed intake, methane yield (g/kg DMI) decreased significantly by 20.3% in cows offered 0.5% *A. armata* and at 1% inclusion level decreased further to 42.7% ($P < 0.0001$). Hydrogen yield significantly increased with the addition of *A. armata* with the average hydrogen yield for cows fed 0.5% *A. armata* increasing by 55.5% and further increasing by 78.9% at the highest macroalgae inclusion level ($P = <0.0001$). Similar to hydrogen yield, carbon dioxide yield significantly increased with the addition of *A. armata*. Average carbon dioxide yield increased by 12.8 and 36.5%, at low and high levels of *A. armata* inclusion, respectively ($P = 0.0001$). Fig. 2 shows the methane, hydrogen and carbon dioxide intensity in cows fed seaweed compared to control. When standardized by level of milk yield (MY), methane intensity (g/kg MY) decreased by 18.2 and 60.1%, at low and high levels of *A. armata* inclusion, respectively ($P = 0.0001$). Average hydrogen intensity increased by 33.3% for cows consuming diet with 0.5% *A. armata*, and 61.7% for cows consuming diet with 1% *A. armata*. Carbon dioxide intensity between control and 1% *A. armata* inclusion remained relatively similar; however, the low level inclusion showed a reduction in carbon dioxide intensity of about 3.5% ($P = 0.02$).

Bromoform concentrations in milk were numerically greater in cows fed diets with *A. armata* at both 0.5 and 1% levels. However, the differences were not significant compared to control (Table 2).

3.2. Animal and production parameters

Animal characteristics, milk yield, and milk composition responses to *A. armata* inclusion in the TMR are given in Table 2. Dry matter intake decreased significantly by 2.98 kg/d ($P < 0.001$) at low level of inclusion and further went down by 10.6 kg/d at the highest level compared to control, representing a 10.8 and 38.0% decrease, respectively. There was no significant body weight change between cows receiving *A. armata* at low inclusion compared to control; however, cows receiving the 1% level gained 9.72 kg less than control cows. Adjusted feed conversion efficiency was numerically greater in cows consuming *A. armata* at 0.5% level compared to control. However, it was significantly greater (by 0.95 kg milk/kg intake) in cows fed diet with *A. armata* at 1% level compared to control. Milk yield did not differ significantly between cows in the control group and those at low level of *A. armata* inclusion. However, cows fed at the higher level of *A. armata* inclusion produced 11.6% less milk compared to control ($P < 0.001$). Milk protein content decreased as *A. armata* inclusion increased; however, significant differences were only observed in cows fed diets with 1% *A. armata* ($P < 0.0001$). No significant differences were found in milk fat, lactose, solids non-fat, milk urea nitrogen, or somatic cell count with both levels of macroalgae inclusion.

4. Discussion

Livestock systems, particularly ruminants, contribute to greenhouse gas emissions, and particularly in the form of enteric methane. A review of mitigation options for enteric methane from ruminants showed that some of the effective strategies include increasing forage digestibility, replacing grass silage with corn silage, feeding legumes, adding dietary lipids and concentrates (Hristov et al., 2013). Although effective, these types of system management options may not offer the scale of reduction required to dramatically change the agriculture contribution to the global GHG inventory and subsequent negative effects on climate change. However, the results of the present study and others suggest that feed additives may provide potent emissions reduction methodology.

Feed additives have been tested to reduce methane emissions with mixed results (Table 3). For example, Appuhamy et al. (2013) showed about a 10% reduction using ionophores, specifically monensin in dairy and beef diets. Nitrates have also shown a potential to reduce emissions by 16% (Van Zijderveld et al., 2011a). Dijkstra et al. (2018) conducted a meta-analysis on the effect of 3-nitrooxypropanol to reduce methane emissions and reported that it is effective in reducing enteric methane by 39% in dairy and 22% in beef. The seaweed tested in this study was reported to have antimethanogenic effect that reduces methane yield during *in vitro* fermentation (Kinley et al., 2016), which was confirmed in *in vivo* using sheep (Li et al., 2018).

4.1. Enteric methane emissions

The methane production reductions demonstrated in this study are among the highest ever reported. Adjusting for differences in intake, methane yield in cows fed diets with *A. armata* showed a sharp decline for low and high levels of inclusion compared to the control group (Fig. 1). Using a closely-related species of *Asparagopsis* (*A. taxiformis*) Li et al. (2018) reported a reduction of 50–80% in enteric methane emissions over a 72-day period at inclusion levels between 1 and 3% of OM in sheep. The difference in effectiveness reported in the *in vivo* trials may be related to the concentration of the active compounds responsible for anti-methanogenic activity. The bioactive compounds are halogenated

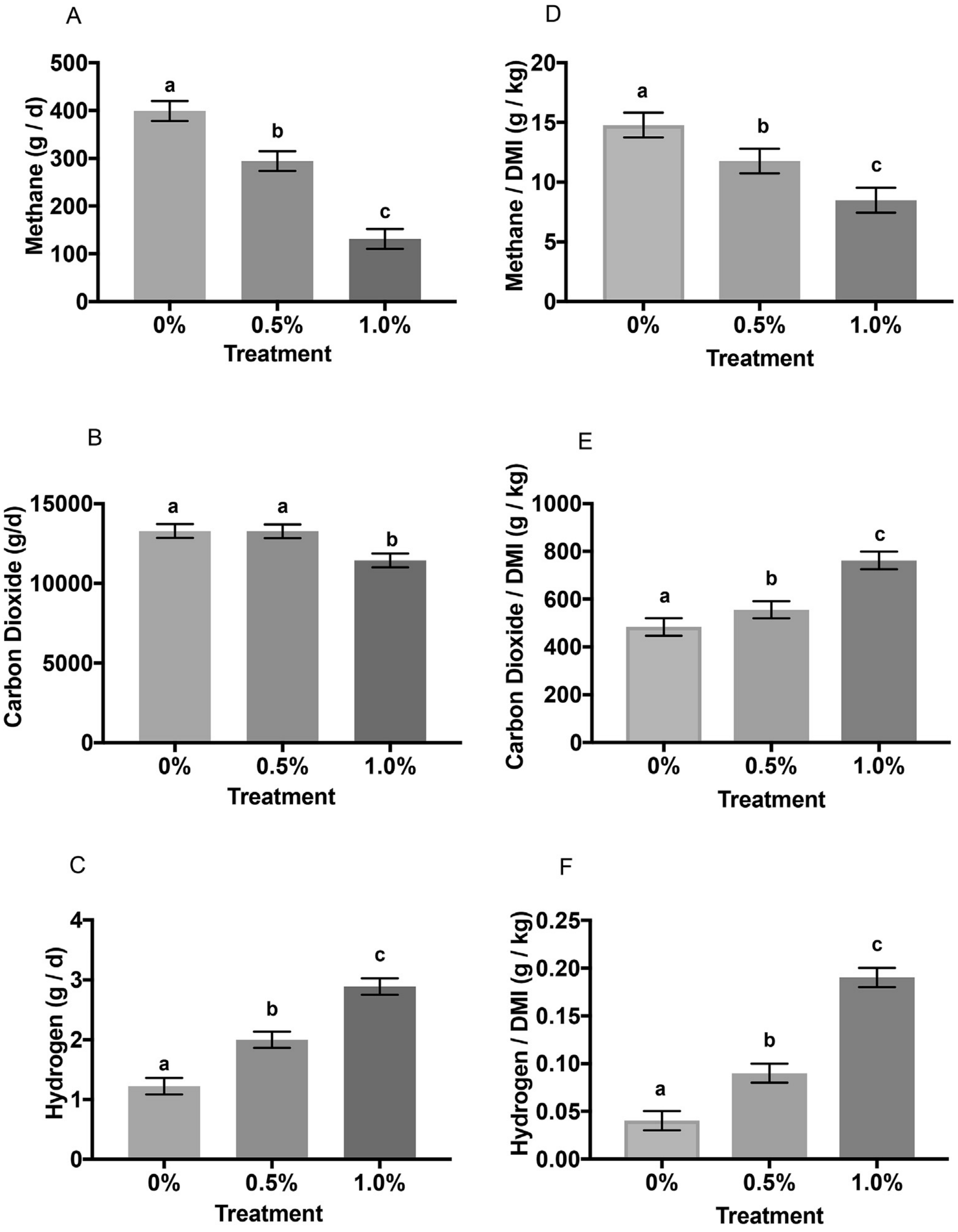

Fig. 1. Means, standard deviations, and statistical differences of methane, hydrogen, and carbon dioxide production, g/d, **(A,B,C)** and yield, g/kg dry matter intake (DMI), **(D,E,F)** for 0%, 0.5%, and 1% *A. armata* inclusion. Means within a graph with different alphabets differ ($P < 0.05$).

compounds including primarily bromoform, but also to lesser degree dibromochloromethane, bromochloroacetic acid, and dibromoacetic acid (Machado et al., 2016b). *Asparagopsis* accumulate bromoform (Paul et al., 2006), and in all studies to date, the seaweed has been harvested in the wild where there is large variation between collections. The mode of action resulting in methane reduction by rumen methanogens due to dietary *Asparagopsis* containing the halocarbon bromoform has been previously described using bromochloromethane (BCM) as a model for bromoform (Denman et al., 2007; Kinley et al., 2016). This mode of action is a result of inhibition of the methanogenic pathway by inhibition of the cobamide-dependent methyl transferase at the terminal step of the pathway. Prior to this project this theory has been the accepted mode of action however further investigation into any distinctions or deviations is ongoing similar to Duin et al. (2016) who characterized the mode of action for 3-nitrooxyproponal which also has the enzyme inhibition mode of action. Some haloalkanes are structural analogs of methyl coenzyme-M reductase, the enzyme that catalyzes the last step of methanogenesis and inhibit the methyl transfer reactions that are necessary in methane biosynthesis (Ermler et al., 1997; Liu et al., 2011).

The differences in methane reduction when accounting for milk production for cows fed diets with the low and high level of *A. armata* inclusion were similar to the reductions in overall methane production but greater compared to methane yield (Figs. 1 and 2). This is mainly due to milk production being not as greatly affected as would be expected with reductions in DMI for the high

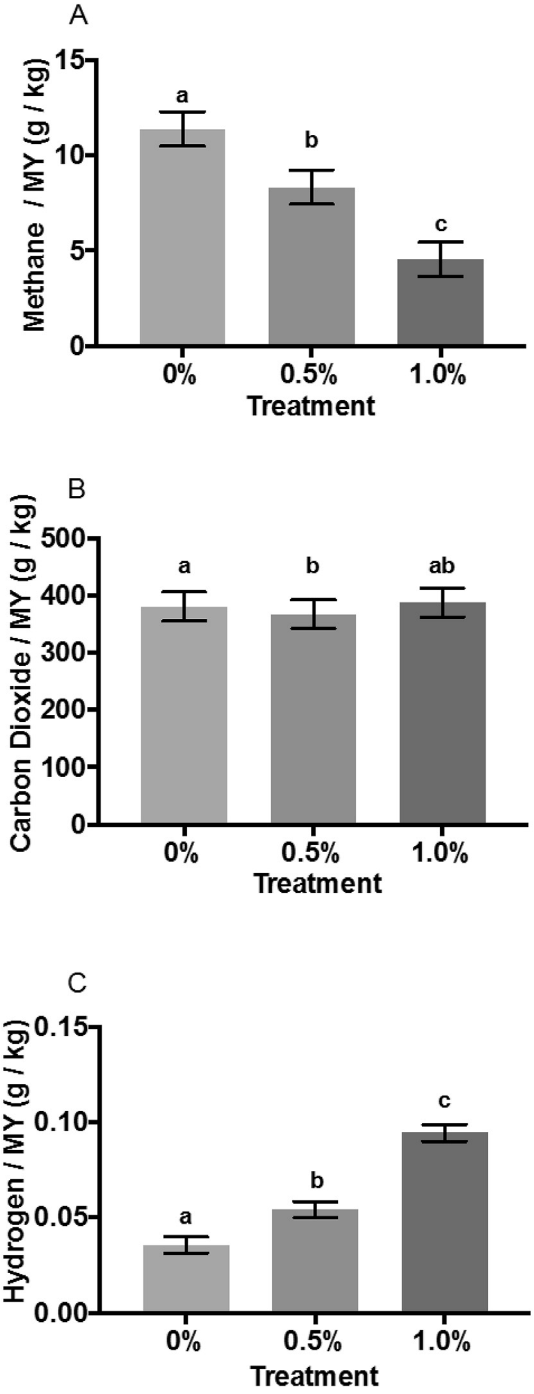

Fig. 2. Means and standard deviations of methane **(A)**, hydrogen **(B)**, and carbon dioxide **(C)** intensity (g/kg milk yield (MY)), for cows supplemented with 0, 0.5, and 1% *A. armata*. Means within a graph with different alphabets differ ($P < 0.05$).

Table 2
Effect of *A. armata* inclusion at 0.5% (Low) and 1% (High) on dry matter intake, body weight, milk production, and milk composition of dairy cows.

Item	Treatment groups			
	Control	Low	High	SEM
Animal (kg)				
Dry matter intake	27.9^a	24.9^b	17.3^c	1.29
Initial body weight	720	732	737	24.9
Body weight change	31.0^a	32.7^a	21.3^b	3.23
adj.FCE[a]	1.29^a	1.55^a	2.24^b	0.10
Milk production				
Milk yield (kg)	36.2^a	37.2^a	32.0^b	2.20
Fat (%)	3.98	3.84	3.71	0.13
Protein (%)	3.12^a	3.01^{ab}	2.93^b	0.06
Lactose (%)	4.74	4.75	4.69	0.04
Solids non-fat (%)	8.65	8.55	8.40	0.08
MUN (mg/dl)	16.7	15.1	15.2	1.79
SCC (x 103/ml)	126	100	129	30.9
Bromoform µg/L	0.11	0.15	0.15	0.03

^{a,b,c}Means within row with different superscripts differ ($P < 0.05$).

[a] adj.FCE accounts for average body weight changes = (Milk yield (kg)/DMI (kg)) *(Final body weight (kg)/Initial body weight (kg)).

level of inclusion. During lactation, milk production is given a higher priority and body reserves are used to compensate if the cow is not consuming enough feed intake to support a given level of milk production (NRC, 2001).

Concentrations of volatile fatty acids in ruminal contents were not analyzed in the current experiment because the cows were not rumen cannulated. In vitro studies showed that volatile fatty acid production decreased with inclusion of *Asparagopsis* (Machado et al., 2016a). However, the inclusion level used was twice as much compared to this study. The authors also reported that the proportion of propionate, butyrate, valerate and isovalerate increased in samples with *Asparagopsis* level of 2% OM, which suggests that rumen fermentation favors propionate producing bacteria when methanogenesis is inhibited. *In vivo* experiments of Li et al. (2018) also demonstrated that propionate production (which is a hydrogen sink) increased as the levels of *Asparagopsis* increased. Indeed, virtually all feed additives that reduce methane production demonstrate a concomitant reduction in the acetate to propionate ratio (e.g. Hristov et al., 2015). Hydrogen is a key product of rumen fermentation and has been suggested to thermodynamically control the production of the various volatile fatty acids, which may shift based on hydrogen partial pressure in the rumen (van Lingen et al., 2016).

4.2. Hydrogen and carbon dioxide emissions

The 55- and 79-fold increases in hydrogen emission in low and high macroalgae inclusion groups, respectively, compared to control is remarkable but not surprising as hydrogen would normally be converted to methane and eructated out if methanogenesis were not inhibited. Mitsumori et al. (2012), using bromochlormethane, reported similar effects in hydrogen emissions, where they observed the majority of the metabolic hydrogen to be expelled as hydrogen gas. Hristov et al. (2015) also reported a 64-fold increase in hydrogen emissions in dairy cows supplemented with 3NOP, a compound that inhibits methanogenesis in a similar way as *Asparagopsis*. Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analog showed that methane-inhibited rumen appeared to adapt to the higher hydrogen levels by shifting fermentation to propionate which was mediated by an increase in the population of hydrogen consuming *Prevotella* and *Selenomonas* spp. (Denman et al., 2015).

Carbon dioxide production in livestock fed *Asparagopsis* has not been reported. However, Hristov et al. (2015), using the anti-methanogenic compound 3NOP, reported that there was no significant difference compared to control. In our study the significant increases of carbon dioxide in cows fed both levels of *Asparagopsis* suggest that eructation is one of the ways to expel metabolic carbon dioxide. When adjusted for feed intake or milk yield, carbon dioxide emissions showed small differences.

Table 3
Estimates of methane reduction through the use of feed additives.

Feed additive	N	Methane reduction (%)	P value	Reference
3NOP	11	−39.0	0.002	Dijkstra et al. (2018)
Essential oils	4	−14.8	0.172	Beauchemin and McGinn (2006)
Fibrolytic enzymes	4	+27.0	0.223	Chung et al. (2012)
Tannins	4	−3.7	0.018	Staerfl et al. (2012)
Grape pomace	2	−18.6	0.051	Moate et al. (2014)
Monensin	11	−6.0	0.065	Appuhamy et al. (2013)
Nitrate	43	−39.5	0.001	Van Zijderveld et al. (2011a)
Saponins	3	−16.6	0.018	Mao et al. (2010)
Yeast	9	−9.9	0.015	Chung et al. (2011)
Yucca	12	−2.0	0.172	Van Zijderveld et al. (2011b)
A. armata	1	−46.7 (average)	<0.0001	This study

4.3. Effect of microalgae on dry matter intake

Some differences in DMI were observed and this could be due to the greater mineral concentration in *Asparagopsis*, which may be less palatable, especially at higher level of inclusion in the TMR. As a direct consequence of reduced DMI, high inclusion level cows were found to have a lower milk yield and milk protein percentage when compared to the control group (Table 2). Optimum inclusion levels are likely to change with the concentration of active compounds in the macroalgae used and method of incorporation in the diet. Further research is needed to determine how best *A. armata* could be delivered to the cows in order to maintain DMI more effectively than the molasses mix used in this study. In addition, studies that used methane analogues as feed additives, specifically bromochloromethane, mixed with cottonseed meal found similar results in cattle and lambs (Tomkins et al., 2009; Sawyer et al., 1974). However, decreased DMI when dosing ruminants with bromochloromethane have been reported. For example, McCrabb et al. (1997) reported 8% DMI reduction in growing steers when bromochloromethane was mixed into cottonseed meal. Tomkins et al. (2009) found that 0.60 g/100 kg live weight of bromochloromethane inclusion was sufficient for a 94% reduction in methane with no change in feed intake whereas McCrabb et al. (1997) used 2.4 g/100 kg live weight to obtain a 100% reduction in methane. Chalupa (1977), who observed reductions in DMI while feeding bromochloromethane, hypothesized that these changes may be due to increased hydrogen concentrations in the rumen, impaired B_{12} production, or even taste aversion to the additive. Adjusted feed conversion efficiency increased in cows fed diets with the higher level of *A. armata* compared to control (Table 2) mainly due to the combination of reduced DMI while holding milk production above what is expected for the level of feed intake. Further work needs to be conducted to investigate if increased efficiency will be maintained over a full lactation.

4.4. Bromoform in algae and animal product

The main anti-methanogenic compound in *Asparagopsis*, bromoform, is naturally produced by phytoplankton and seaweeds in the ocean (Wever and van der Horst, 2013). Human consumption of high levels of bromoform could be hazardous, so the US EPA (2008) has set drinking water regulations on bromoform consumption to 80 μg/L. Milk produced by cows fed the low and high *Asparagopsis* additive were in the range of 0.11–0.15 μg/L, which is over 500 times lower than the maximum standard. However, other minerals, iodine in particular, could be present in greater quantities in milk from cows fed macroalgae additive. Processing of the macroalgae to remove minerals may be necessary to support the attainment of the U.S. Food and Nutrition Board's recommended daily allowances without exceeding tolerable upper limits.

5. Conclusions

The potential of the macroalgae *Asparagopsis* to reduce methane emissions shown in *in vitro* studies was investigated *in vivo* using dairy cattle. Adding *Asparagopsis* at 0.5% of diet OM resulted in reductions of 26.4% in methane production, 20.5% in methane yield (adjusted for feed intake) and 26.8% in methane intensity (adjusted for milk production) without compromising milk yield or intake. Increasing the inclusion level to 1% resulted in reductions of 67.2% methane production, 42.6% methane yield, and 60.0% methane intensity. However, feed intake and milk yield were also reduced. Bromoform concentration in milk was not significantly different in cows that consumed macroalgae compared to control. Other mineral concentrations in milk may be increased so some processing may be necessary for *Asparagopsis* to be used as a feed additive effectively. The implication of this study is that enteric methane emissions could potentially be halved by using seaweed as a feed additive to dairy cattle.

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