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The red macroalgae *Asparagopsis taxiformis* is a potent natural antimethanogenic that reduces methane production during *in vitro* fermentation with rumen fluid

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Abstract. Livestock feed modification is a viable method for reducing methane emissions from ruminant livestock. Ruminant enteric methane is responsible approximately to 10% of greenhouse gas emissions in Australia. Some species of macroalgae have antimethanogenic activity on *in vitro* fermentation. This study used *in vitro* fermentation with rumen inoculum to characterise increasing inclusion rates of the red macroalga *Asparagopsis taxiformis* on enteric methane production and digestive efficiency throughout 72-h fermentations. At dose levels ≤1% of substrate organic matter there was minimal effect on gas and methane production. However, inclusion ≥2% reduced gas and eliminated methane production in the fermentations indicating a minimum inhibitory dose level. There was no negative impact on substrate digestibility for macroalgae inclusion ≤5%, however, a significant reduction was observed with 10% inclusion. Total volatile fatty acids were not significantly affected with 2% inclusion and the acetate levels were reduced in favour of increased propionate and, to a lesser extent, butyrate which increased linearly with increasing dose levels. A barrier to commercialisation of *Asparagopsis* is the mass production of this specific macroalgal biomass at a scale to provide supplementation to livestock. Another area requiring characterisation is the most appropriate method for processing (dehydration) and feeding to livestock in systems with variable feed quality and content. The *in vitro* assessment method used here clearly demonstrated that *Asparagopsis* can inhibit methanogenesis at very low inclusion levels whereas the effect *in vivo* has yet to be confirmed.

Additional keyword: greenhouse gas, ruminant, seaweed.

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Introduction

Methane (CH_4) in the atmosphere is a potent greenhouse gas (GHG) with an IPCC Fifth Assessment Report (AR5) global warming potential 28 times that of carbon dioxide (CO_2, IPCC 2014). Between 2000 and 2009, agriculture and waste management accounted for 62% of global anthropogenic CH_4 emissions (Kirschke *et al*. 2013) with ruminant enteric fermentation responsible for 58% of agricultural contributions (Olivier *et al*. 2005). In Australia, the contribution of CH_4 from ruminant livestock is approaching 10% of total GHG emissions (Henry *et al*. 2012). These levels have resulted in a universal effort to reduce enteric CH_4 emissions. Enteric CH_4 is a consequence of anaerobic fermentation of feed organic matter (OM) by a microbial consortium that produces substrate CO_2 and hydrogen in a reduction pathway used by methanogens (Morgavi *et al*. 2010). Feed additives have been used to interfere with this pathway or otherwise reduce the numbers of functional methanogens. Patra (2012) reviewed dietary supplementation options for rumen enteric CH_4 management that included ionophores, chemical compounds, legumes, essential oils, fats, saponins, tannins, probiotics, and plant secondary metabolites. Unfortunately, an antimethanogenic effect may be concomitant with some detrimental impacts. Most commonly there is a decrease in fermentation efficiency leading to a decrease in feed intake and a measurable decline in animal productivity.

Macroalgae also have potential for use as a supplement for livestock feeds (Machado *et al*. 2015*a*). The antimethanogenic properties of macroalgae-based functional products in rumen *in vitro* cultures has been demonstrated (Wang *et al*. 2008; Dubois *et al*. 2013; Kinley and Fredeen 2015; Machado *et al*. 2014), however there is much variability in the antimethanogenic potency between types and species of macroalgae. Algae are generally classified by size (micro or macro) whereas macroalgae are broadly classified based on pigmentation (green, red or brown) and habitat (freshwater or

marine). Both freshwater and marine macroalgae are used in human nutrition, cosmetics, and pharmaceutical products (Paul and Tseng 2012). The opportunity to use macroalgae as a feed additive for livestock is growing due to the increasing exploitation of algae for other purposes such as bioremediation. Macroalgae are unique in their rich and diverse lipid and tannin content and secondary metabolites, which in some cases have demonstrated antimethanogenic properties (Wang *et al*. 2008; Kinley and Fredeen 2015).

One novel antimethanogenic strategy seeks to harness the effect of secondary metabolites found in some macroalgae. These have been demonstrated to be variable in effect on *in vitro* fermentation in a dose-dependent manner (Dubois *et al*. 2013; Machado *et al*. 2015*b*). Machado *et al*. (2014) reported the antimethanogenic effect of 20 different macroalgae species *in vitro* when fermented with a low quality dry rangeland Rhodes grass (*Chloris qayana*). This work demonstrated that at high inclusion rates (17% OM basis) highly variable effects on methanogenesis were possible. *Asparagopsis* is a marine genus of red macroalgae characterised by secondary metabolites with antibacterial properties (Paul *et al*. 2006) that demonstrates a potent antimethanogenic effect *in vitro* (Machado *et al*. 2015*b*).

It was hypothesised that the red macroalga *Asparagopsis taxiformis* at low inclusion rates can dramatically reduce CH_4 emissions from *in vitro* fermentations with rumen fluid (RF) without detrimental effects on fermentation while using a grass feed substrate. The objective of this study was to demonstrate the *in vitro* antimethanogenic potency of the red macroalga *Asparagopsis taxiformis* at low inclusion rates over 72 h using an irrigated Rhodes grass as the feed substrate. The effects on parameters of rumen fermentation were examined using standardised *in vitro* culture methods.

Materials and methods

Preparation of macroalgae and Rhodes grass substrate

The *Asparagopsis taxiformis* (hereafter *Asparagopsis*) was harvested from Nelly Bay, Magnetic Island (19°16′S, 146°85′E) near Townsville, Qld, Australia. The macroalga biomass was rinsed in seawater for 2 min then dipped in freshwater to remove residual salt to maximise alga OM content. The clean biomass was placed in 100-µm mesh and centrifuged at 1000*g* for 6 min at ambient temperature in a commercial washing machine to remove excess water and then stored at −10°C. The biomass was then freeze-dried (SP Industries VirTis K, Warminster, PA, USA) and ground to 1 mm and stored at −10°C. The high quality Rhodes grass (HQR) was grown under irrigation and harvested locally. Subsamples of HQR were air-dried and ground to 1 mm. Table 1 describes the composition of the *Asparagopsis* and HQR biomass used as the fermentation substrates. Dry matter was determined by achievement of constant weight at 105°C, and OM was measured as loss on combustion at 550°C for 8 h (Horwitz 2000). Neutral and acid detergent fibre were determined using an Ankom (Macedon, NY, USA) model 200 fibre analyser. Crude protein content was determined using a LECO (St Joseph, MI, USA) model CHN628 series nitrogen analyser.

Table 1. Nutritional composition of the Rhodes grass substrate and *Asparagopsis* biomass (g/kg DM unless stated otherwise)

Composition	Rhodes grass	*Asparagopsis*
Dry matter (g/kg as used)	916	945
Organic matter	878	811
Crude protein	167	252
Neutral detergent fibre[A]	645	–
Acid detergent fibre	315	–

[A]Without α amylase.

Donor animals and preparation of RF inoculum

Rumen fluid inoculum was collected from four fistulated Brahman steers (*Bos indicus*; LW 490 ± 45 kg) fitted with 10-cm Bar Diamond (Parma, OH, USA) rumen cannulas. The steers were maintained at the College of Public Health, Medical and Veterinary Sciences at James Cook University (Townsville) according to current guidelines (NHMRC 2013) and approved by the local animal ethics committee (A5/2011). The steers were maintained on Rhodes grass *ad libitum* for 6 months before the collection of RF, which was extracted 2 h after morning feeding by sampling from four quadrants of the rumen and hand-squeezing to completely fill pre-warmed 1-L stainless steel thermal flasks.

Inoculation and in vitro fermentation

The RF was pooled and immediately processed by filtration through a 0.5-mm sieve and combined with Goering and van Soest (1970) buffer (GVB) at a ratio of 1 : 4 (RF : GVB). Maintenance of 39°C and mixing of the RF buffer fermentation media (RFB) was continuous to ensure homogeneity (Major Science SWB 20 L-3; Saratoga, CA, USA). The full system was N_2 purged and a Dose-It pump (Integra Biosciences, Hudson, NH, USA) was used to aspirate 125 mL of RFB into incubation bottles containing the *Asparagopsis* and HQR. The bottles were sealed with an Ankom RF1 gas production module (Macedon, NY, USA) and placed in an incubator (Ratek OM11; Boronia, Vic., Australia) maintained at 39°C and oscillating at 85 RPM.

Experimental design

To characterise the effect of *Asparagopsis* on *in vitro* rumen fermentation a series of 20 incubation periods were conducted using five *Asparagopsis* dose rates ranging from 0.5% to 10% (OM basis) of the HQR substrate OM and compared with a control (no macroalgae) and RFB blanks. Each dose level was characterised in duplicated incubation periods containing quadruplicate repetitions at each sampling time point (12, 24, 36, 48, and 72 h). Controls and blanks were included in all periods and time points in duplicate. Each 72-h fermentation series was split into two periods with the first monitoring at 12, 24 and 36 h [plus a 6-h sample for *in vitro* apparent digestibility of substrate OM (IVD-OM only)] and the second monitoring 36, 48, and 72 h. The data was then combined to provide time series curves covering the full 72 h. Each fermentation contained 1.0 g of the HQR (OM basis) and appropriate quantities of

Asparagopsis to achieve dose rates of 0.5%, 1%, 2%, 5% and 10% according to the biomass composition described in Table 1.

Fermentation monitoring and sample analyses

Total gas production

The methods used in this study were similar to those described by Cone *et al*. (1996), Pellikaan *et al*. (2011) and Machado *et al*. (2014). Total gas production (TGP) was measured continuously for a maximum of 72 h. The Ankom parameter settings were kept constant with maximum pressure in the fermentation bottle of 3 psi, which when exceeded, would vent for 250 ms and the pressure change accounted in the cumulative pressure recording. Gas pressure was measured every 60 s and cumulative pressure was recorded at 20-min intervals. The cumulative TGP expressed in mL/g of substrate OM was determined by application of the natural gas law to the accumulation of the recorded gas pressure while accounting for individual bottle volume.

Methane production

In vitro CH_4 production was determined and time series production curves prepared by collection of samples at multiple time points. Production in mL CH_4/g of substrate OM was estimated by application of CH_4 concentration in time series samples using the relative TGP while assuming constant homogeneity of bottle headspace. Concentration of CH_4 in headspace collected in 10-mL Labco Exetainer vacuum vials (Lampeter, Great Britain) were measured by gas chromatography (GC) on a Shimadzu GC-2014 (Kyoto, Japan) equipped with a Restek (Bellefonte, PA, USA) ShinCarbon ST 100/120 column (2 m × 1 mm × micropacked) with a flame ionisation detector (FID). Column temperature was 150°C, injector was 240°C, and 380°C in the FID. Ultra high purity N_2 was the carrier gas at 25 mL/min and injection volume was 250 µL.

In vitro *apparent digestibility of substrate OM*

The IVD-OM was quantified to coincide with CH_4 determinations (plus a 6-h sample for IVD-OM only). The fermentation was chilled to terminate bacterial activity then *in vitro* fluid (IVF) was vacuum filtered through a Duran No. 1 porosity glass fritted crucible with a 0.5-cm layer of sand filtration aid. The crucible and fermentation residue was oven-dried to constant weight at 105°C for DM determination. Residue OM was determined as loss on ignition in a muffle furnace at 550°C for 8 h (Carbolite AAF 11/18; Derbyshire, Great Britain).

Volatile fatty acid production

Volatile fatty acids (VFA) in the IVF were quantified after 72 h of fermentation. The preparation of IVF for VFA analysis was at a ratio of 4 mL of IVF to 1 mL of 20% metaphosphoric acid spiked to 11 mM with 4-methylvaleric acid (Sigma-Aldrich; Castle Hill, NSW, Australia) as internal standard and stored at −20°C. A 1.5-mL subsample was centrifuged for 15 min at 13 500*g* and 4°C (Labnet Prism R; Edison, NJ, USA). The supernatant was filtered through 0.2-µm PTFE syringe tip filters (Agilent; Santa Clara, CA, USA) and analysed using a Shimadzu GC17A equipped with a Restek Stabilwax (30 m × 0.25 mm × 0.25 µm) fused silica column and FID. The column was ramped from 90°C to 155°C at 3°C/min and held for 8.3 min. The temperature was 220°C in the injector and 250°C in the FID. Ultra high purity N_2 was the carrier gas at 1.5 mL/min and the injection was 1.0 µL.

Statistical analyses

Two-factor repeated-measures permutational analysis of variance (PERMANOVA) was used to test for significant differences in the TGP, CH_4 production, and, IVD-OM over time and a one-factor PERMANOVA was used to test for significant differences in the production of VFA between the treatments (fixed factor) using Primer 6 (version 6.1.13) statistical software and PERMANOVA+ (version 1.0.3; Clarke and Gorley 2006). Data were also fitted with generalised additive models to predict the relationship and examine differences between TGP over time between treatments and differences in the changes in the rates of TGP. The generalised additive models were produced using the mgcv package within the R language (version 3.0.1; R Core Team 2013).

Results

Asparagopsis had consistent effects on *in vitro* fermentations and these effects were dose dependent. This study applied a HQR (Table 1) as substrate and the dose rate of 2% *Asparagopsis* (OM basis) was near the optimum dose as measured by decrease in TGP, CH_4 abatement, stability of IVD-OM, and beneficial changes in VFA concentrations. Using HQR, the 1% dose was no more effective than 0.5% (Fig. 1). However, there was a significant reduction in TGP of ~30% with the inclusion at 2% of *Asparagopsis* ($P < 0.001$). There was good reproducibility of TGP lending to standard error (s.e.) of <1.5 mL/g representing 0.7% of the TGP values at 214 mL/g substrate OM.

The inclusion of *Asparagopsis* had the effect of reducing CH_4 production in a dose and time-dependent manner (Fig. 2). Methane production had a similar trend as TGP. There was minimal CH_4 produced with 1% *Asparagopsis* inclusion in the first 24 h, however after 24 h CH_4 production began to increase rapidly. After 48 h there was no longer measurable difference in CH_4 between the 0.5% and 1% dose rates. The prominent effect occurred at dose levels ≥2% ($P < 0.001$) and no detectable CH_4 was produced. Variability in CH_4 production between periods was greater than that observed for other variables monitored in this study.

Substrate degradability *in vitro* was not affected by the inclusion of *Asparagopsis* ≤5.0% (OM basis). There was no difference in IVD-OM over 72-h fermentations between the control and dose rates up to 5% of substrate OM. However, 10% *Asparagopsis* induced significant reduction in IVD-OM ($P < 0.001$). The comparison of IVD-OM between dose rates and the control was consistent and independent of time. The IVD-OM variability within- and between periods was small as reflected by the small s.e. (Fig. 3).

In Fig. 4 it was shown that *Asparagopsis* at doses ≤2% had little effect on total VFA (TVFA) after 72 h of fermentation and little change was induced by dose rates <5%, however with 5% the TVFA was decreased ($P < 0.05$) and more

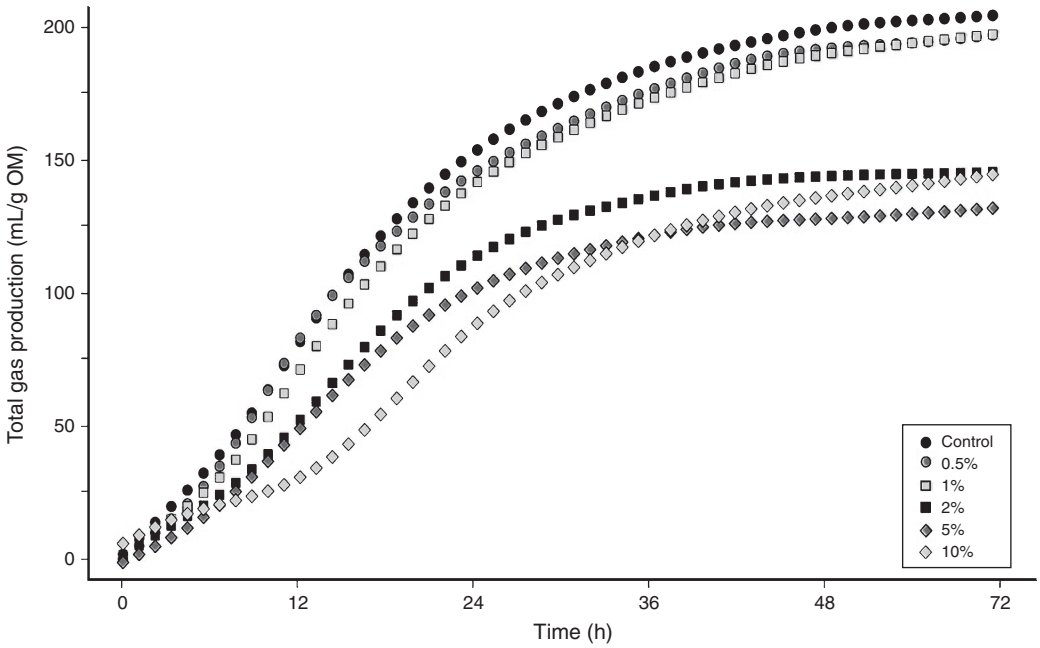

Fig. 1. The time series effect of increasing dose rate of *Asparagopsis* on *in vitro* total gas production (mL/g organic matter). Control was a high quality Rhodes grass hay. Error bars are not shown as they were smaller in size then the symbols used.

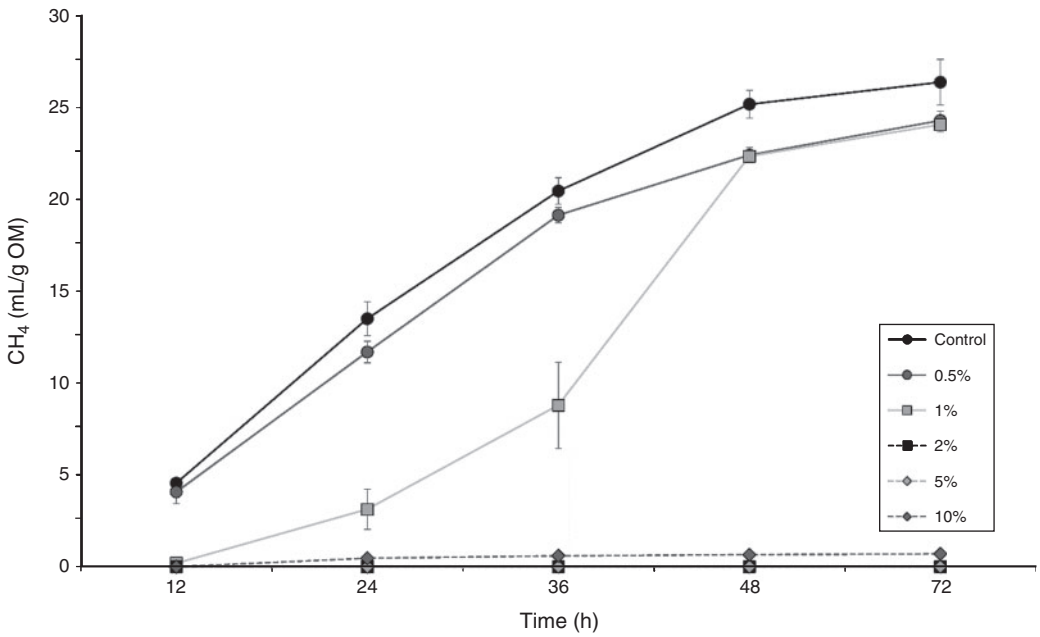

Fig. 2. The time series effect of increasing dose rate of *Asparagopsis* on mean (±s.e.m.) *in vitro* methane production (mL/g organic matter). Control was a high quality Rhodes grass hay.

so at 10% ($P < 0.001$). Although TVFA was not acutely sensitive to low level inclusion of *Asparagopsis* there was a trend towards reduced TVFA with increased dose. For individual VFA a significant change was demonstrated ($P < 0.05$) and the effect was magnified with increasing dose ($P < 0.001$). As the dose rate of *Asparagopsis* increased through the 0.5%, 1%, 2%, 5%, and 10% dose range the acetate concentrations decreased by 4%, 11%, 29%, 41%, and 61%, respectively, compared with the control. For propionate the concentrations increased by 13%, 0%, 56%, 88%, and 106% for the same dose range, respectively. For butyrate the increases were 67% and 116% for the 2% and 10% doses, respectively. These changes in acetate and propionate concentrations reduced the acetate : propionate ratio. With little effect on TVFA and a shift to lower acetate and greater

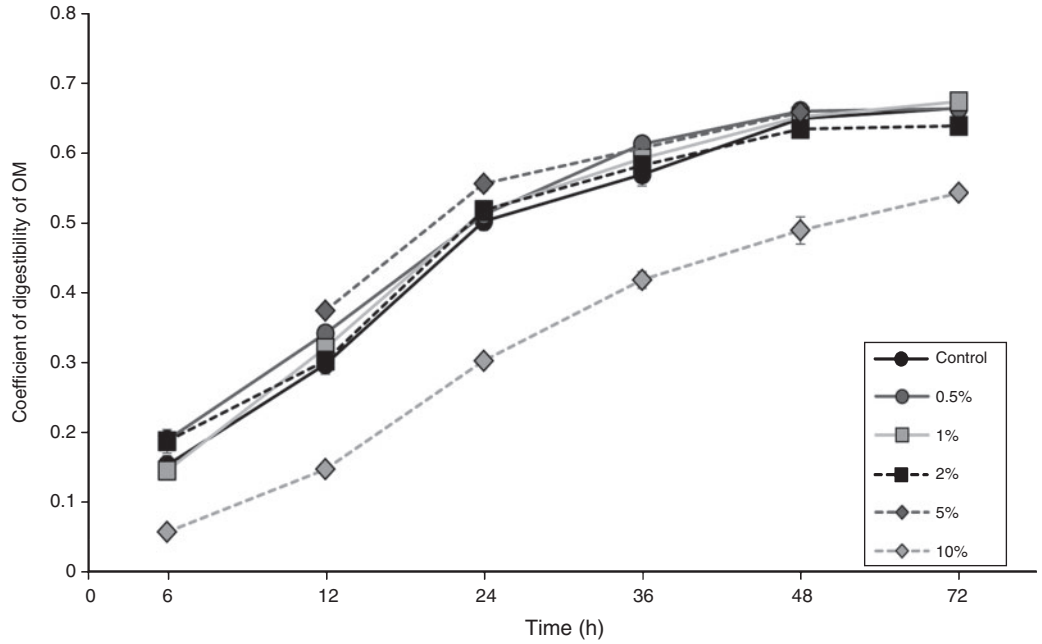

Fig. 3. The time series effect of increasing dose rate of *Asparagopsis* on mean (±s.e.m.) *in vitro* apparent organic matter digestibility. Control was a high quality Rhodes grass hay.

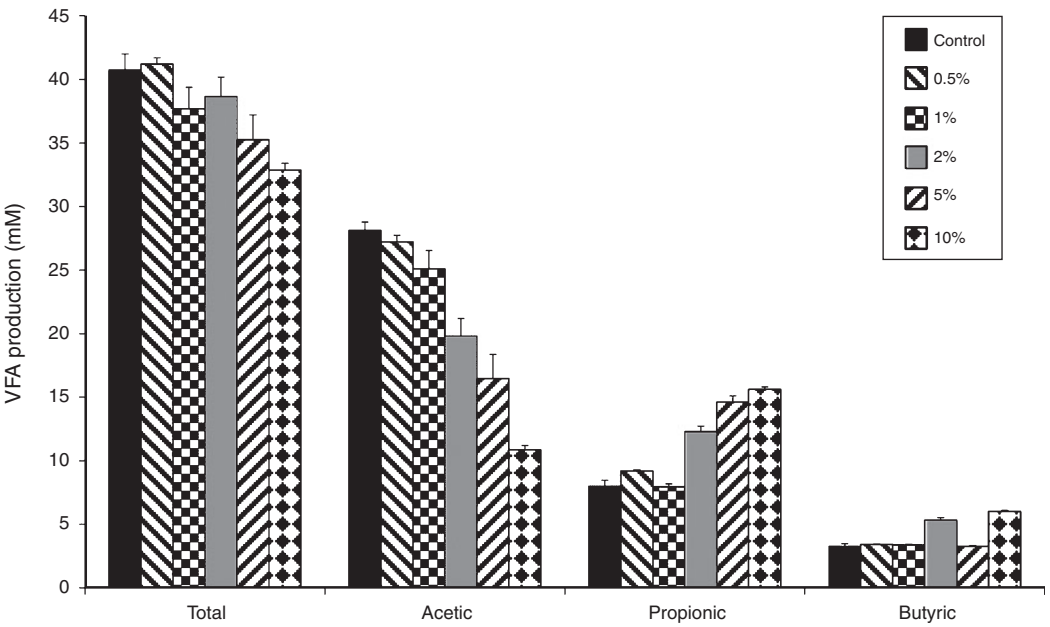

Fig. 4. The effect of increasing dose rate of *Asparagopsis* on mean (±s.e.m.) *in vitro* total volatile fatty acid, acetic, propionic and butyric acid concentrations after 72 h of fermentation. Control was a high quality Rhodes grass.

propionate, there was no negative impact on VFA production due to low dose (<5%) *Asparagopsis* inclusion *in vitro* using HQR substrate.

Discussion

The red seaweed *Asparagopsis* has a large CH_4 abatement capacity compared with other natural products when included at low dose in rumen fermentations *in vitro*. The effect of *Asparagopsis* demonstrated in this study agreed with Machado *et al*. (2015*b*), which described end-point results after 72 h of fermentation that demonstrated dose sensitivity to *Asparagopsis* using low quality Rhodes grass (LQR) substrate. Conspicuously however, in their study there was an abrupt reduction in TGP and CH_4 production occurring at 1% *Asparagopsis* rather than the 2% in this study (Figs 1, 2). Consequently, this effective dose

difference observed with variable grass quality may follow a different pattern between grass types or when grain-based substrates are used. Therefore, it is essential to evaluate the effects using the various ruminant feeding systems. Using HQR the 1% dose of *Asparagopsis* was no more effective than 0.5%, and thereafter TGP and CH_4 declined with increasing dose levels. It is an important distinction that the fermentation response to *Asparagopsis* may also be dependent upon the quality of the substrate. Therefore, the requirements for *Asparagopsis* biomass may be only half when feeding LQR compared with HQR. Ruminant production systems utilising low quality forage as the primary feed would require less *Asparagopsis* to achieve equivalent CH_4 abatement.

The production of CH_4 was virtually undetectable at dose rates $\geq$2% OM basis (Fig. 2). For this reason the CH_4 results were not blank corrected, doing so would produce confusing negative values because *Asparagopsis*-treated fermentations produced less CH_4 than the blanks. However, occasionally during other in-house rumen *in vitro* experiments using a 2% dose (data not shown) a small rise in CH_4 was observed after ~36 h. This occasional rise in CH_4 still provided at minimum an abatement of >85% compared with the control. Those fermentations used the HQR substrate with the only difference being the RFB. At the 1% *Asparagopsis* dose rate there was a typical rise in CH_4 from undetectable to ~20 mL/g HQR beginning between 24 and 36 h of fermentation. All experimental periods demonstrated that at an *Asparagopsis* dose rate $\geq$2% of substrate OM typically results in undetectable *in vitro* methanogenesis. At $\geq$5% the CH_4 production was always undetectable. Feed energy is typically lost as CH_4 at a rate of up to 12% of gross energy intake (Johnson and Johnson 1995). Using *Asparagopsis* this energy may be conserved in the rumen for productive use by the ruminant animal at some undefined level which further reduces the cost of abatement. This proportion of retained energy can be quantified with *in vivo* feeding studies that closely monitor feed intake, CH_4 production, and productivity.

It is known that the antibacterial defence mechanism of *Asparagopsis* is predominantly a result of the secondary metabolite bromoform ($CHBr_3$) naturally present in the macroalgal biomass (Paul *et al*. 2006). Bromoform is similar chemically and in antimethanogenic potency to that of bromochloromethane (BCM; CH_2BrCl). In previous *in vivo* experiments investigating enteric CH_4 abatement, BCM induced abatement in Brahman steers of 93% and 50% after separate 28 and 90 days feeding regimes, respectively (Tomkins *et al*. 2009). However, BCM has been banned from manufacture and use in Australia due to its contribution to ozone depletion. The mode of action of BCM was described previously as inhibition of the methanogenic pathway at the final step by inhibition of the cobamide-dependent methyl transferase step in release of CH_4 (Denman *et al*. 2007). In that study inhibition of methanogenesis occurred immediately however the methanogen populations were only found to be reduced after several hours, thus the observed lag in the population decline suggested that the inhibition of methanogenesis directly affected growth of methanogens. They also commented that BCM would be removed from the rumen due to ruminal flow and unless it was replaced CH_4 inhibition would decline, which could not be observed during our *in vitro* batch culture. However, a decline in inhibition was observed with the 1% inclusion (Fig. 2) and was presumably associated with consumption of the antimethanogenic capacity of *Asparagopsis* at very low dose levels.

The naturally occurring secondary metabolites in *Asparagopsis armata* a temperate species closely related to the tropical *Asparagopsis* used in the present study include di-BCM ($CHBr_2Cl$) at low levels of <0.1% of algal DM, but also bromoform, which at higher levels of ~1.7% (Paul *et al*. 2006) is considered to be the bioactive agent responsible for most of the CH_4 abatement acting in the same way as BCM. The naturally occurring secondary metabolites of *Asparagopsis* have demonstrated activity *in vitro* at dose rates of 1% (Machado *et al*. 2015*b*) and 2% (substrate OM basis) in this study. Thus, the inclusion rate for large abatement of CH_4 and degradation of the bioactive metabolite may be managed. However, intensive study of *Asparagopsis* CH_4 abatement efficacy in cattle and sheep is required.

Reduction effect demonstrated in TGP and CH_4 production at low dose levels of *Asparagopsis* were not reflected equivalently in the IVD-OM results, which remained unchanged until the 10% dose was used. Inclusion of *Asparagopsis* had little effect on IVD-OM at dose levels $\leq$5% of substrate OM, compared with controls (Fig. 3). However, all experiments and studies with *Asparagopsis* demonstrated a significantly reduced IVD-OM ($P < 0.001$) at doses of $\geq$10% (Machado *et al*. 2014, 2015*b*). The IVD-OM represented a demonstration of stability in the fermentation and suggests fibre digesting microbes were not affected by *Asparagopsis* at low dose.

The primary source of energy for ruminant animals is the VFA produced by rumen microbes during digestion of carbohydrates (Bergman 1990) thus negative effects against their production during rumen fermentation is undesirable. The production of TVFA, acetate, and propionate were affected by increasing dose of *Asparagopsis*. At doses between 1% and 2% the decrease in TVFA was not significant with the HQR substrate used in this study; however, this is not in agreement with a study using LQR where there was a significant decrease in TVFA (Machado *et al*. 2015*b*). This may indicate that VFA production may be more sensitive to *Asparagopsis* in LQR possibly due to higher levels of indigestible fibre and lower protein. However, IVD-OM was similar in both studies and not affected at 1% versus 2% doses, thus the mechanism of the effect is unclear. Generally, TVFA was reduced as the dose level increased. The inclusion of *Asparagopsis* at low dose levels induced a beneficial change in VFA in favour of propionate as is common with antimethanogenic inclusions. This is believed to be due to competition for the excess hydrogen and reductive propionate production is more favourable than acetogenesis in these conditions (Mitsumori *et al*. 2012), which may be enhanced by *Asparagopsis*. It was demonstrated for HQR that significant changes ($P < 0.001$) in individual VFA concentrations can be achieved. In the present study acetate concentrations decreased and propionate and butyrate increased with increasing doses of *Asparagopsis*. Changes in acetate and propionate concentrations also reduced the acetate : propionate ratio, which could be partially responsible for corresponding decreases observed

in CH_4 production *in vitro* (Beauchemin *et al*. 2009). The reasoning is that propionate acts as a hydrogen sink; however, production of acetate and butyrate liberates hydrogen thus providing for greater ruminal reduction of CO_2 into CH_4 by methanogens.

Recent studies reporting the antimethanogenic effect of various macroalgae in rumen fermentations has demonstrated variable responses (Dubois *et al*. 2013; Kinley and Fredeen 2015; Machado *et al*. 2014). Some macroalgae have previously indicated potential for enteric CH_4 abatement; however, *Asparagopsis* stands out as the most potent. Other macroalgae, particularly the green species appear to be most suitable as novel protein sources with little value as CH_4 abatement agents for ruminants. The production of *Asparagopsis* at a scale large enough for feeding livestock requires development before commercialisation as a functional feed ingredient. It is unclear how various methods of drying *Asparagopsis* biomass and subsequent storage will affect levels of secondary metabolites and antimethanogenic potency thus characterisation of the most appropriate methods is required.

Conclusions

A dose of *Asparagopsis* at 1% of substrate OM exhibited a significant reduction of CH_4 *in vitro*, and at $\geq$2% demonstrated virtual elimination of CH_4 with minimal effect on fermentation efficiency of HQR. There was no impact on IVD-OM at dose levels $\leq$5% and the effect on VFA was a decrease in acetate with a concomitant increase in propionate and to a lesser degree for butyrate. Other areas requiring characterisation is the most appropriate method for processing (dehydration) and feeding to livestock in systems with variable feed quality and content. Nevertheless, using *in vitro* assessment methods the use of *Asparagopsis* at low inclusion levels in ruminant diets has demonstrated large CH_4 abatement as a natural product.

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