final report

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Phase 2: Validating the antimethanogenic properties of red macro algae for provisional patent

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Executive Summary

In Australia, agriculture is regarded as one of the major contributors to greenhouse gas emissions, accounting for 16% of total emissions from all sectors. Enteric fermentation from ruminant livestock contributes a large proportion of these emissions as methane.

Diet manipulation is generally regarded as an effective approach in which the ruminant livestock sector could reduce enteric contributions to Australia's GHG emissions. A number of feed additives have previously been described with potential to reduce enteric methane production, but few are persistent over time and most have a negative impact on intake and/or digestibility when fed in quantities that result in a mitigation response. The use of macroalgae as a feed additive to support animal productivity and thereby reduce emission intensity has been investigated. The inclusion of algae biomass *in vitro* has been described and forms the basis of an international patent for the use of a red macroalga to reduce enteric methanogenesis in ruminants with International Publication Number WO 2015/109362 A2.

This project has now determined the effect of supplementing a pelleted grain diet with a marine red macroalga on enteric methane production and rumen function. The overall objective was to provide a sufficient data set to support claims based on previous *in vitro* trials and a short term *in vivo* trial using Brahman steers. The effectiveness of accumulated bioactives in *Asparagopsis* biomass on reducing rumen methanogenesis was assessed across five inclusion rates compared to a control (no alga) using sheep as the experimental animal. The potential of including *Asparagopsis taxiformis* in the diet to mitigate methane production has now been described and clearly demonstrates the antimethanogenic potential of naturally occurring algal bioactives *in vivo*.

Twenty four merino cross wethers were allocated to one of five groups based on the daily inclusion rate of *Asparagopsis* [0 (control), 0.5, 1.0, 2.0, 3.0 % on an organic matter basis]; equivalent to 0, 13, 26, 58 and 80 g/d algae as fed, respectively. Individual feed intake was measured over 75 d, rumen fluid and blood samples were collected to determine the effect of *Asparagopsis* on rumen fermentation and animal health, respectively. Individual animal methane production was measured on three occasions using open-circuit respiration chambers.

The *Asparagopsis* biomass used as the supplement for the sheep in this study was found to contain approximately 0.4 mg/g DM of bromoform and 0.004 mg/g of dibromochloromethane. The biomass processing was completed using the gold standard of freeze drying, however the product supplemented to the sheep was kiln dried and some downward variation in content is likely. Inclusion rates in the daily diet were lower than those previously reported for the antimethanogenic compound bromochloromethane (BCM), but a similar trend in methane reduction was reported. The manufacture and use of substances such as BCM have been banned in Australia since 2004, however *Asparagopsis* is rich in halogenated analogues and the mode of action described for BCM is considered to be the same for the accumulated bioactives found in *Asparagopsis*.

Sheep fed the lower inclusion rates of *Asparagopsis* (< 2.0 %) consumed all the algae on a daily basis when mixed with a palatable carrier (crushed lupins). Higher doses generally resulted in maximum intakes of the milled algae material of approximately 30 g/d, and suggest a maximum inclusion rate equivalent to 0.45 g/kg LW or ~0.18 mg Bromoform/kg LW per day for the class of sheep and diet used in this project. The inclusion of *Asparagopsis* in the diet had a significant effect on methane production compared with the control. Mean methane production from control (0% *Asparagopsis*) sheep was 14.7 g/kg DM intake, compared with 12.8, 6.8, 5.7 and 2.9 g/kg DM intake for sheep supplemented with

Asparagopsis at inclusion rates of 0.5, 1.0, 2.0 and 3.0 % (OM basis), respectively. Consistent reductions in methane emissions over time, up to 80% compared with the control, were observed when *Asparagopsis* was included the daily diet of sheep fed a high grain pelleted diet. Adding *Asparagopsis* biomass, a source of halogenated metabolites, to a pelleted diet was an effective inhibitor of enteric methanogenesis with emissions decreased in a dose response manner for up to 72 d.
Residues of bromoform and dibromochloromethane could not be found in muscle tissue and fat of sheep supplemented with *Asparagopsis* for 72 d. This agrees with studies using a similar trihalomethane (THM) in experiments with beef steers and thus indicates that these THM's don't accumulate in tissues and are decomposed or excreted. At the doses fed and the limits of reporting of NMI this study indicates safe meat products from sheep supplemented with *Asparagopsis*.

This project has demonstrated that *Asparagopsis* can be fed to ruminants to reduce enteric methanogenesis. Adding low levels (<3.0%) of *Asparagopsis* biomass to a lupin/grain based pelleted diet has been shown to result in considerable reductions in enteric methane emissions; however this strategy may only become available to the livestock sector when commercial scale harvesting or production of this marine macro algae is demonstrated.

The natural accumulation of halogenated analogues in algal biomass provides the livestock industry with a unique opportunity to adopt a novel abatement strategy where *Asparagopsis* production can be integrated with allied sectors such as aquaculture. The use of *Asparagopsis* in the feedlot sector has almost immediate adoption potential once commercial production of the alga can be realised. The potential reduction in emissions intensity for red meat production is therefore substantial if inclusion in the diet of intensively managed cattle is adopted across the industry. Economic and sustainable benefits may be realised under an Emissions Reductions Fund which approves the use of algae rich in natural antimethanogenic bioactives for beef production systems.

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1 Background

1.1 Halogenated metabolites in food products and the environment

Guidelines for food quality associated with bromoform and dibromochloromethane are centred on drinking water and no guidelines for acceptable limits in meat were found in the literature. The world Health Organization (WHO) publishes guidelines for drinking water, and bromoform and dibromochloromethane are both recommended at maximum 0.10 mg/L (WHO 2011). The guideline is cumulative in that the total of trihalomethanes (THM) has a maximum of 0.10 mg/L. However, bromoform is typically the highest concentration of halogenated analogues in *Asparagopsis* (Paul et al. 2006).

It is known that halocarbons in the environment, and specifically bromoform, are mainly of biogenic natural origin (Ballschmiter 2003). High concentrations of bromoform and dibromochloromethane found in the marine environment correlate with abundance of macroalgae. These compounds are detected in both water and atmosphere and levels are typically 5-10 fold higher in coastal tropical regions compared to cold water and the open ocean. Coral reef levels of up to10 ng/L compare to 1.5 ng/L in the North Atlantic and open Indian Ocean, and coastal regions have higher levels than pelagic zones (Ballschmiter 2003). The authors describe that the major source of biogenic organohalogens in the terrestrial environment is fungi living in soils. One of the largest anthropogenic sources of THM's is water disinfection where reaction of halogens with oxidizers, frequently ozone, generates THM (Cotruvo 1982). The presence of THM's due to water disinfection may be reasoning for developing guidelines for THM's in drinking water and lack of guidelines in meat may due to low or no sources of THM's in traditional meat production.

1.2 Macroalgae

Species-specific bioactive compounds in marine macroalgae and their characteristics and functions are well described by Holdt and Kraan (2011). The exploitation of algae as a natural ingredient for methane abatement and livestock production enhancement has far reaching benefits for Australia's livestock sector. In these environments algal based supplements can be added directly to diets at known rates to achieve a production and/or abatement outcome. To date, there have been no natural or persistent dietary additives identified for a significant reduction of enteric methanogenesis for ruminants. The antimethanogenic compound bromochloromethane (BCM) prepared in an α-cyclodextrin matrix has been shown to decrease methane production by up to 93% for steers fed a feedlot ration (Tomkins *et al*., 2009). Sawyer *et al.,* (1974) have previously fed BCM in corn oil to mature wether sheep and reported similar decreases in methane production, expressed as % of gross energy intake. Despite its antimethanogenic effect BCM is not commercially available for use in Australia as manufacture, import and export was prohibited by the Australian Government in 2004 under the Ozone Protection and Synthetic Greenhouse Gas Management Act 1989.

Methane reducing compounds are known to naturally exist in macroalgae in varying concentrations. Methane reduction *in vitro* has been clearly demonstrated in a dose response trial where the marine red macroalgae *Asparagopsis taxiformis* was incubated with a tropical forage substrate (Kinley et al. 2016; Machado *et al.,* 2014). *Asparagopsis* produces more than 100 low molecular weight metabolites containing bromine, iodine and chlorine that have antimicrobial activity. Burreson *et al*., (1976) identified 42 volatile compounds in *Asparagopsis taxiformis*, including haloforms, dihalomethanes, halogenated acetones and butenones. Methane analogues such as BCM inhibit methane production by reacting with reduced vitamin B_{12} which inhibits cobamide-dependent methanogenesis. The mechanism of inhibiting methanogenesis is presumed in some algae. Conveniently the

antimethanogenic properties of *Asparagopsis* can be described by the mode of action for BCM. Bromoform is a secondary metabolite produced by *Asparagopsis* and inhibits methanogenesis by reacting with a vitamin B_{12} cofactor.

The potential of including *Asparagopsis* in the diet of ruminant livestock to mitigate methane production has now been described and formed the basis of a provisional patent for the use of red macroalgae to reduce enteric methanogenesis in ruminants and is published with International Publication Number WO 2015/109362 A2.

2 Project objectives

2.1 Intent

To provide evidence to support the hypothesis that the red macroalga *Asparagopsis* can significantly reduce ruminant enteric methane emissions without trace of the bioactive metabolites in the products of the animal. The project was conducted to determine the effect of the macroalga supplementation, when fed to sheep, on product quality. The overall objective was to provide data to support claims that *Asparagopsis* is safe for livestock and their product quality in association with enteric methane mitigation.

2.2 Research objectives of B.CCH.2095 Phase 2

- Collect and analyse tissue and fat samples from the *Asparagopsis* supplemented sheep for residues of bromoform and dibromochloromethane for the inclusion rates described in the original project B.CCH.2095.

- Integrate data, statistical analysis and report

3 Methodology

3.1 Experimental site

The sheep *Asparagopsis* supplementation experiment was conducted at the CSIRO Centre for Environment and Life Sciences Floreat, WA between September and December 2014. The tissue and fat samples were collected at the termination of the feeding period and the sheep were sacrificed. The experimental protocol was approved by the local animal ethics committee (AEC1404) and was operated under the guidelines described by the Australian code for the care and use of animals for scientific purposes (8th Edition, 2013).

3.2 Experimental design and management

3.2.1 Animals

Twenty four merino cross wethers [mean ± sem live weight (LW); 65.8 ± 1.03 kg] were allocated to one of five groups based on the daily inclusion rate [organic matter, (OM) basis] of the red macroalgae *Asparagopsis taxiformis* [0 (control), 0.5, 1.0, 2.0, 3.0 %]. Inclusion rates (% OM intake) were equivalent to 0, 13, 26, 58 and 80 g/d algae as fed, respectively.

Animals were adapted to the pelleted basal diet over an initial 21 d period, including a staggered and gradual addition of the algae, presented with 200 g crushed lupins over 14 d to achieve the four inclusion rates of Asparagopsis as described. This staggered adaptation period facilitated the use of only eight open circuit respiration chambers for the proceeding

72 d experimental period. Throughout this experimental period the algae/lupin mix was added to the pelleted ration on a daily basis. Individual animal live weight (LW) and body condition score (BCS; 1-5) was measured at 14 d intervals throughout the trial.

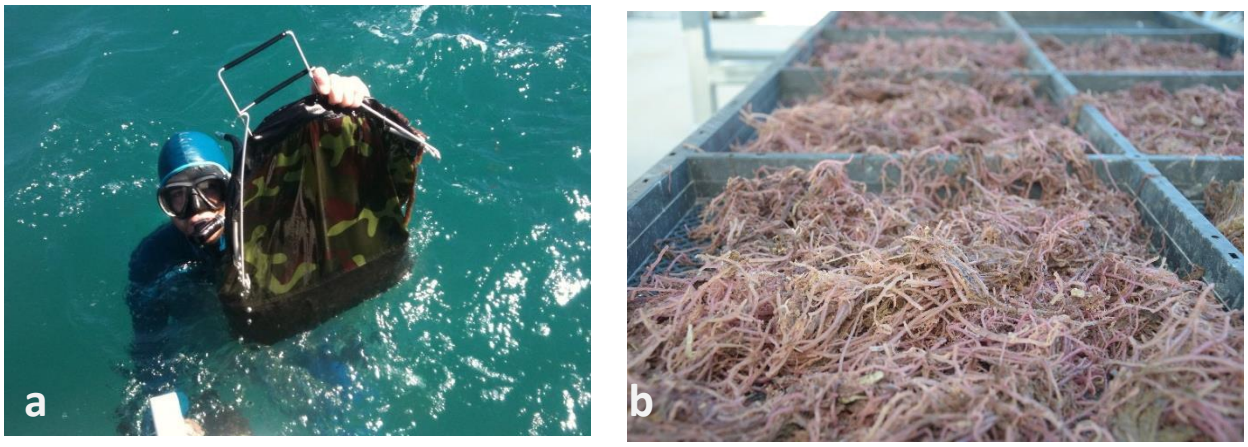

Fig 1: *Asparagopsis* biomass was harvested by hand (a) from Keppel Bay by JCU staff (College of Marine & Environmental Sciences) and initially dried on open racks (b) prior to being dried to constant weight in a solar kiln.

Table 1: Composition of main dietary components [g/kg dry matter (DM) unless stated otherwise] fed to merino cross wethers over 72 d (DM basis)

	Asparagopsis[1]	Lupins	Pellets[2]
DM g/kg	966	918	890
Organic matter	431	968	928
Ash	569	32	72
Crude protein[3]	92	343	108
Neutral detergent fibre[4]	_	286	519
Acid detergent fibre[4]	_	251	338

[1]*Asparagopsis taxiformis*, dried and milled, mixed with crushed lupins as a palatable carrier when fed; [2]Macco Feeds WA as 9mm proprietary shipper pellets free of Co, Se or ionophores; [3]6.25 X N for lupins and pellets, 4.59 X N for Asparagopsis (Lourenço *et al*., 2002); [4]Neutral detergent fibre (aNDFom), with the addition of 4.0 mL heat stable α-amylase and 20 g sodium sulphite, and acid detergent fibre (ADFom) exclusive of residual ash was determined using an ANKOM220 Fibre Analyser, (Ankom Technology, Fairport, NY) as determined by Symbio Alliance (Eight Mile Plains, Queensland).

Table 2: Elemental analysis of *Asparagopsis taxiformis*, included in the daily ration at one of four different dose rates, and a high grain pelleted ration fed to merino cross wethers over 72 d (DM basis)

Element		*Asparagopsis*	Pellets
B	mg/kg	114.78	5.90
Ca	%	2.73	1.00
Cl	%	9.72	0.82
Co	mg/kg	4.32	
Cu	mg/kg	7.38	3.75
Fe	mg/kg	10993.53	783.18
Mg	%	0.95	0.11
Mn	mg/kg	174.22	68.31
Mo	µg/kg	1027.10	537.30
NO_3	mg/kg	249.87	<40.00
P	%	0.38	0.20
K	%	1.36	0.72
Se	mg/kg	35.61	
Na	%	7.05	0.51
S	%	2.26	0.15
TN[#]	%	2.01	1.37
Zn	mg/kg	34.55	39.96

Fig 2: Sheep were confined in open circuit respiration chambers for 24 h to determine individual methane emissions (g/kg DM intake) when fed at 1.0 X maintenance.

3.2.2 Necropsy and tissue residue analysis

Two and seven days after the last series of methane measurements two groups of six sheep were selected across the treatments and transported by road to the College of Veterinary Medicine, Murdoch University in Perth, WA for necropsy and collection of tissues for

histopathological examination. On each occasion sheep were euthanized using Sodium Pentobarbitone (160 mg/kg LW IV) and presented as a blind study to University staff to remove biases in tissue evaluation and reporting. In addition, from each animal, samples of depot fat (kidney), and muscle (*M. Longissimus lumborum*) were dissected from the carcass, sealed in individual poly bags and immediately placed on dry ice. Retained samples of depot fat and muscle were stored at -80°C until transported to the National Measurement Institute (NMI) Port Melbourne, VIC for quantification of the halogenated compounds Bromoform and dibromochloromethane.

The method is based on USEPA 8260 VOC. Whole samples were homogenised and then sub-samples were analysed by NMI method VL 234 "Volatile Organic Compounds in biota, soils and water by Head Space with Gas Chromatography with Tandem Mass Spectrometer detection (HS-GC-MS)" (Griffith 2004; USEPA 1996).

Whole samples (100 g) were homogenised and then sub-samples of 5g (+/- 0.1g) were analysed by headspace vapor generation using HS-GC-MS. Samples were incubated at 100 degrees for 15 minutes in the headspace generation sample vials with internal standard added into each sample. A subsample (1 mL) of generated vapour containing volatilized bromoform, dibromochloromethane, and internal standard was subsequently analysed by HS-GC-MS. Identification of analytes is carried out using retention time, primary characteristic and qualifier ions. Quantitation of analytes is completed using the primary characteristic ion using the internal standard method. A multi-point external (target) analyte matrix matched calibration of the instrument is employed. Quality control protocols included a control blank, duplicates and recovery for every 10^{th} or last sample (if batch is less than 10). The NMI limit of reporting for this method is < 0.05 mg/kg.

3.3 Statistical analysis

The statistical analysis for the variables measured in project B.CCH.2095 was conducted by fitting linear mixed models to each response variable. These models were able to account for the design of the experiment (the allocation of animals to particular groups and chambers), the structure of the data (repeated measures) and the missing values which occurred. The "fixed effects" in the mixed model consisted of the treatment effect (five inclusion rates of *Asparagopsis*), the time effect (three sampling dates), the treatment by time interaction, and any covariates. Initial live weight was included as a covariate when analysing live weight. It was also tested as a potential covariate for other response variables, but was not significant, and so was not included in the final model.

The analysis produced means for all combinations of treatment and time, adjusted for all other terms in the model. P-values were calculated for testing the overall effect of time, treatment, and their interaction. Least significant differences (P = 0.05) were calculated for comparing pairs of means.

4 Results

4.1 Asparagopsis

Biomass of wild *Asparagopsis* in the benthic gametophyte phase collected from a site near Humpy Island, Keppel Bay contained approximately 0.41 mg/g DM halogenated metabolites consisting of > 96% bromoform; < 2% dibromochloromethane; and trace amounts of bromochloro acetic acid; and dibromoacetic acid. The biomass processing was completed using the gold standard of freeze drying, however the product supplemented to the sheep was kiln dried and some downward variation in content is likely.

The proximate and elemental analysis of the dried *Asparagopsis* biomass fed to sheep at rates equivalent to 0, 0.5, 1.0, 2.0 and 3.0 % (OM intake basis) is shown in Tables 1 and 2, respectively.

4.2 Methane emissions

Individual methane emissions (g/kg DM intake) were measured after an initial 30 d period of algae inclusion in the diet and then at 21 d intervals over the remaining experimental period (Fig 3). The inclusion of *Asparagopsis* in the diet had a significant effect ($P < 0.001$) on methane production compared with the control (Fig 3). Mean methane production from control (0% *Asparagopsis*) sheep was 14.7 g/kg DM intake, compared with 12.8, 6.8, 5.7 and 2.9 g/kg DM intake for sheep supplemented with *Asparagopsis* at inclusion rates of 0.5, 1.0, 2.0 and 3.0 % (OM basis), respectively. There was no significant difference ($P > 0.05$) in methane emissions for control and *Asparagopsis* inclusion at 0.5 % (OM basis). There was no significant difference ($P > 0.05$) in methane emissions for *Asparagopsis* inclusion at 1.0% and 2.0 % (OM basis).

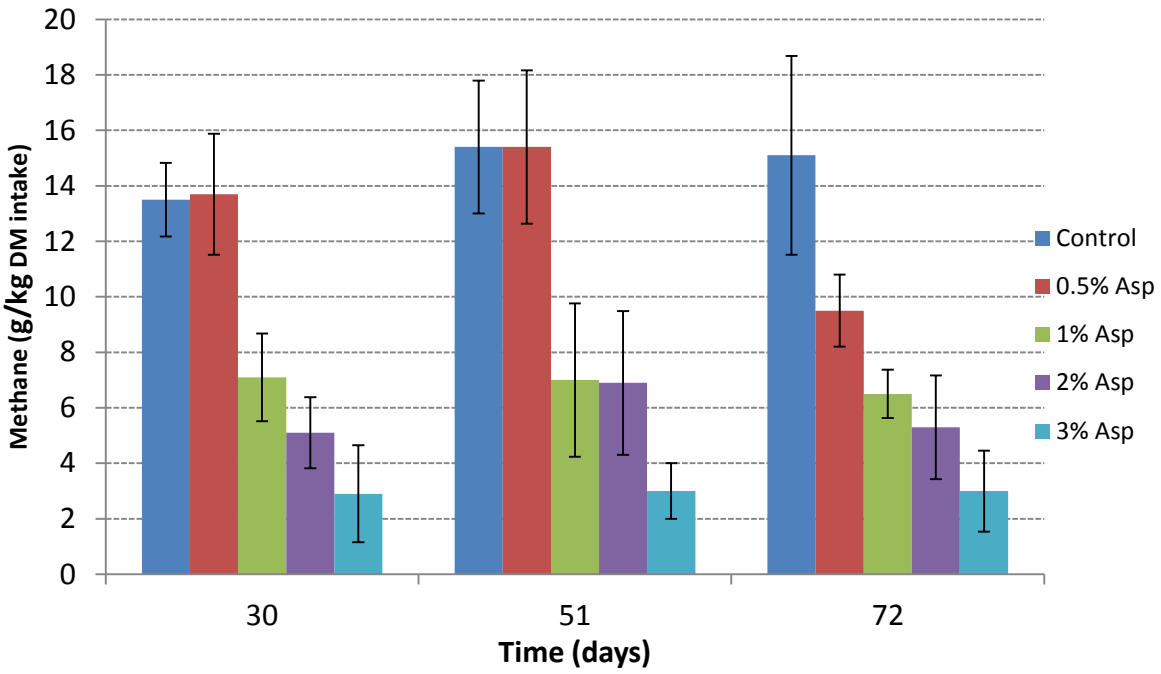

Fig 3: Mean (± sem) methane emissions (g/kg DM intake) measured at three intervals throughout the experimental period for sheep fed a pelleted diet and supplemented (% OM basis) with and without *Asparagopsis* (Asp.) on a daily basis.

The inclusion rates of *Asparagopsis* at 1.0%, 2.0% and 3.0% (OM basis) demonstrated consistent reductions in methane emissions over time compared with the control, equivalent to 53%, 62% and 80%, respectively. There was a strong relationship between inclusion rate of *Asparagopsis* biomass in the diet and decrease in enteric methane emissions (Fig 4).

The mean methane emission for control sheep (0%) was 14.7 ± 1.46 g/kg DM intake. After 72 d of *Asparagopsis* inclusion at 0.5% to the basal diet mean emissions decreased numerically by 35% compared to 30 d and 51 d. However, at 0.5% there was no significant effect of *Asparagopsis* inclusion over time on mean methane production.

4.3 Trihalomethane residues in tissue and fat

After the last series of methane measurements 12 sheep were presented for necropsy and collection of tissues for histopathological examination. Post mortem interval was < 1 h. Carcasses were reported to be in good body condition with minimal autolysis.

The post-mortem analysis of tissue and fat performed by NMI for THM's was conclusive in that bromoform and dibromochloromethane residues were not detectable in any of the sheep regardless of the dose level of *Asparagopsis* fed in this study. Table 3 shows that all samples from all sheep had the same non-detect values. The detection limits offered by NMI of < 0.05 mg/kg tissue and fat for bromoform, and < 0.025 mg/kg for dibromochloromethane, is indicative of zero or extremely low accumulation of their residues when *Asparagopsis* is consumed by sheep at the dose levels used in this study. All values in Table 3 are < 0.05 mg/kg of sample and although the analytical detection limit is < 0.025 mg/kg for dibromochlormethane NMI has a reporting limit of < 0.05 mg/kg due to factors of uncertainty in the methodology. The estimated measurement uncertainty at the Limit of Reporting is ± 40%.

Table 3: Residual bromoform and dibromochloromethane in muscle and fat of the *Asparagopsis* supplemented sheep

Sheep ID Tag #	Supplement Rate	Sheep Muscle		Sheep Fat	
		Bromoform	Dibromochloromethane	Bromoform	Dibromochloromethane
	(% of Ration OM)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
1125	0	< 0.05	< 0.05	< 0.05	< 0.05
658	1	< 0.05	< 0.05	< 0.05	< 0.05
1149	3	< 0.05	< 0.05	< 0.05	< 0.05
1141	0	< 0.05	< 0.05	< 0.05	< 0.05
634	1	< 0.05	< 0.05	< 0.05	< 0.05
1142	3	< 0.05	< 0.05	< 0.05	< 0.05

5 Discussion

5.1 *Asparagopsis* and rumen enteric methane production

This project has determined the effect of a marine red macroalga on enteric methane production and rumen function when fed to sheep daily for 72 d. Additional data has now been generated to support claims based on previous *in vitro* trials and a short term *in vivo* trial in beef. Reductions in ruminant methane emissions are central to national inventories because total methane per animal or methane relative to GE intake are used in IPCC reporting.

The effectiveness of algae bioactive compounds on reducing enteric methanogenesis has now been assessed across five inclusion rates compared with a control (no algae) using sheep as the experimental animal. Adding up to 3% *Asparagopsis* (OM basis), as a source of THM's to a high grain pelleted diet was an effective abatement strategy and enteric methanogenesis was decreased by up to 80% throughout the 72 d of algal inclusion in the daily ration.

Antimethanogenic compounds, such as BCM, directly inhibit a terminal step in the enzymatic pathway of methanogenesis, rather than act as a toxin to methanogens (Johnson *et al*.

1972). Real-time quantitative PCR has been used to monitor methanogenic numbers in cattle treated with a complexed BCM (Denman *et al*. 2007). A decrease of 42% in total rumen methanogenic archaeal population for steers (relative to total bacteria) was observed but only after minimum of 8 h treatment with the complexed BCM. This supports that THM's directly interfere with methanogenesis through inhibition of a key enzymatic pathway. However, the reduction in methane release only has detrimental effect on methanogenic archaea after a lag of many hours. Tomkins *et al*., (2009) indicated that methane production could be partially suppressed for up to 90 d indicating some adaption of the rumen biome to BCM, however with the use of *Asparagopsis*, which can contain bioactive metabolites consisting of up to 87% THM's (Burreson *et al.,* 1976), no rumen adaption could be observed based on the persistent antimethanogenic effect over 72 d in sheep. Additional ruminal diversity and abundance analysis will be required to fully understand the effect of *Asparagopsis* on the rumen archaea population where cobamide-dependent methanogenesis is inhibited.

Ironically, Tomkins *et al*., (2009) speculated toward the future use of alternative antimethanogenic compounds other than BCM, with a similar mechanism of action. Such alternatives, now possible with *Asparagopsis*, would have practical commercial relevance given that cobamide-dependent methane production could be inhibited for up to 90 days, which is a period typically used for feedlot finishing of cattle.

5.2 Trihalomethane residues

If BCM is to be used as a model for the mode of action associated with feeding *Asparagopsis* to ruminants, then dissociated algal bioactives could similarly complex with reduced vitamin B_{12} in the rumen (Wood *et al*. 1968). Vitamin B complexes are not stored in animal tissue in appreciable amounts, with the exception of cyanocobalamin. It is likely that dissociation of algae bioactives in the rumen results in products that are either readily lost by eructation, or are further metabolised in the gastro-intestinal tract.

Burreson *et al.,* (1976) identified 42 volatile compounds in the extracted oil of *Asparagopsis taxiformis*, of which 87% by weight were haloforms of bromine and/or iodine. Inorganic bromide has been shown to accumulate in the blood of monogastrics when subjected to inhalation of BCM over extended periods (Svirbely et al. 1947). The serum anion gap is used in the detection of metabolic acidosis and bromide intoxication (Kraut and Madias, 2007). Although the normal value for serum AG can vary widely, the absence of a significant difference for this parameter between the treatment groups suggest sheep in this study were not subjected to either bromide toxicity or metabolic acidosis induced by feeding *Asparagopsis* on a daily basis. The absence of detectable levels of BCM in bovine tissues reported by Tomkins *et al.,* (2009) suggests that THM may not accumulate in ruminants fed the compound over extended periods. The bioactives identified in *Asparagopsis* are equally volatile and attempting to determine residue concentrations in tissue near the temporary maximum residue limit (tMRL) of 0.02 mg/kg for BCM (Tomkins et al. 2009) may be inconclusive. Nevertheless, samples of fat and muscle have been collected as part of the current project and were analysed independently by NMI for residues of the major bioactive metabolites produced by *Asparagopsis*. The results are in Table 3 and none of the samples of either fat or tissue contained detectable levels bromoform or dibromochloromethane (< 0.05 mg/kg). This agrees with previous studies feeding brominated THM to cattle (Tomkins et al. 2009) which suggest lack of accumulation of the compounds in tissue and fat.

6 Conclusions/recommendations

The characteristics and function of species-specific bioactive compounds in macroalgae have been well described. Similarly the potential of macroalgae as a feed for ruminant

livestock in northern Australia has been highlighted by Machado *et al.,* (2015). Green freshwater and marine macroalgae have the greatest potential to be integrated into livestock production systems due to their biomass production and crude protein content. *Asparagopsis* sp. however, is not unique for its biomass production by comparison, or by crude protein content (~ 9% DM basis), but has been shown to accumulate more than 100 low molecular weight metabolites containing bromine, iodine and chlorine in specialised gland cells or vesicles.

The effectiveness of algae bioactive compounds on reducing enteric methanogenesis has now been assessed across five inclusion rates with a pelleted diet fed to sheep. The inclusion rates of *Asparagopsis* at 1.0 %, 2.0 % and 3.0 % (OM basis) demonstrated consistent reductions in methane emissions over time compared with the control and 0.5%, equivalent to 53%, 62% and 80%, respectively. There was a strong relationship between inclusion rate of *Asparagopsis* biomass in the diet and decrease in enteric methane emissions, but also an inclusion rate was identified which restricted algal intake to a maximum of 0.45 g/kg LW (~0.18 mg/kg LW of bromoform) for the class of sheep and diet used in this project. Importantly, adaptation of methanogens to *Asparagopsis* did not occur after 72 d.

A daily dose of 30 g/d, or approximately 2.0% (OM basis) *Asparagopsis* is recommended that would result in significant decreases in enteric methanogenesis for sheep. This inclusion rate is now recognised as dependent on the concentration of key bioactives in the alga biomass and may, in some circumstances, be closer to 1.0% (OM basis). Further work is required to determine the effectiveness of lower doses over time and the effect of bioactive content as a result of commercial *Asparagopsis* production under different temperatures and conditions. In addition the application of the alga in reducing methane emissions for the feedlot and dairy sector require investigation to validate these inclusion rates. Further work may be extended to extensively managed cattle, namely the northern breeder herd, where algal derivatives could be incorporated into existing lick block supplementation strategies.

In this project the *Asparagopsis* biomass contained 0.4 mg/g DM halogenated metabolites, dominated by bromoform, and to a lesser extent dibromochloromethane, and inclusion rates were lower than those previously reported for antimethanogenic agents such as BCM. The manufacture and use of substances such as BCM have been banned in Australia since 2004. The natural accumulation of these substances in algal biomass provides the industry with a unique opportunity to adopt a novel abatement strategy where *Asparagopsis* production can be integrated with allied sectors such as aquaculture. An abatement methodology based on *Asparagopsis* requires development for both feedlot and lactating cattle, although in both applications the impact on animal health and potential food residues requires further investigation. However, in this and previous studies supplementing a similar THM (BCM; Tomkins et al. 2009) no residues could be detected in tissue and fat after extended dietary exposure. Nevertheless, adoption and practice of feeding *Asparagopsis* to decrease enteric methane production has significant implications for the red meat industry in reducing emissions intensity.

We have demonstrated that *Asparagopsis* has a significant antimethanogenic effect when fed to sheep on a daily basis. Further work is recommended to:

- determine the commercial production potential of *Asparagopsis* that maximises bioactive content
- validate the antimethanogenic response in feedlot cattle and confirm that edible tissue entering the human food chain is free of residues
- investigate the production effect: liveweight gains and carcass yield/quality, when *Asparagopsis* is fed over an extended period (feedlot finishing)

- identify the direct effect of *Asparagopsis* bioactives on the rumen microbiome and alternative H_2 sinks associated with the significant reductions in enteric methanogenesis
- populate economic models that identify least cost parameters associated with feeding *Asparagopsis* to ruminant livestock and verify abatement in a life cycle analysis for red meat production
- Confirm that the rumen biome does not adapt to *Asparagopsis* and the antimethanogenic potency remains intact during extended feeding
- demonstrate an innovative application, and antimethanogenic effect of *Asparagopsis* bioactives when incorporated in supplementation practices currently used in northern Australia

7 Key messages

7.1 Methane abatement potential

Few antimethanogenic compounds have been able to demonstrate consistent abatement potential of up to 80% over extended periods. Although BCM is unavailable for commercial use as part of a livestock abatement methodology its natural algal equivalent in *Asparagopsis* has a similar mechanism of action and has practical commercial relevance given that methane production can be inhibited for up to 72 days with no microbial adaptation. The use of *Asparagopsis* in the feedlot sector has almost immediate adoption once commercial production of the alga can be realised. The potential reduction in emissions intensity for red meat production is substantial if inclusion in the diet of intensively managed cattle is adopted across the industry. Economic and sustainable benefits may be realised under an Emissions Reductions Fund which approves the use of natural bioactives in beef production systems.

7.2 Trihalomethane residues

Residues of bromoform and dibromochloromethane could not be found in muscle tissue and fat of sheep supplemented with *Asparagopsis* for 72 d. This agrees with studies using similar THM's in experiments with beef steers and thus indicates that these THM's don't accumulate in tissues and are decomposed or excreted. At the doses fed and the limits of reporting of NMI this study indicates safe food products from ruminants supplemented with *Asparagopsis*.

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