Communication

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Bonnemaisonia hamifera, a Temperate Macroalga to Reduce Methane Emissions from Ruminants

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Simple Summary: In efforts to reduce the environmental impact of livestock farming, we explored a red macroalga called *Bonnemaisonia hamifera*. This macroalga was collected from the shores of Sweden and used in an *in vitro* digestion experiment to evaluate its effects in reducing methane emissions from dairy cows. Methane is a gas that ruminants naturally release during digestion, and it's a significant contributor to global warming. Increasing amounts of the macroalga were mixed with grass silage. We noticed an increase in the proportion of propionate in rumen fluid and a reduction in methane production with increasing levels of the macroalga. This is important because if we can find ways to lower methane emissions from ruminants, we could help the environment. *B. hamifera* exhibited antioxidant properties, which could be beneficial for the animals. In conclusion, this study shows that *B. hamifera* from Sweden has the potential to make livestock farming more eco-friendly by decreasing methane gas emissions.

Abstract: Researchers have been exploring seaweed to reduce methane (CH_4) emissions from livestock. This study aimed to investigate the potential of a red alga, *Bonnemaisonia hamifera*, as an alternative to mitigate CH_4 emissions. *B. hamifera*, harvested from the West coast of Sweden, was used in an *in vitro* experiment using a fully automated gas production system. The experiment was a randomized complete block design consisting of a 48-h incubation that included a control (grass silage) and *B. hamifera* inclusions at 2.5%, 5.0% and 7.5% of grass silage OM mixed with buffered rumen fluid. Predicted *in vivo* CH_4 production and total gas production were estimated by applying a set of models to the gas production data and *in vitro* fermentation characteristics were evaluated. The results demonstrated that the inclusion of *B. hamifera* reduced ($P = 0.01$) predicted *in vivo* CH_4 and total gas production, and total gas production linearly decreased ($P = 0.03$) with higher inclusion of *B. hamifera*. The molar proportion of propionate increased ($P = 0.03$) while isovalerate decreased ($P = 0.04$) with inclusion of *B. hamifera*. There was a tendency for increased ($0.06 \leq P \leq 0.10$) total volatile fatty acid production, as well as lower proportions of butyrate, isobutyrate, and 2-methylbutyrate. Chemical analyses revealed that *B. hamifera* had moderate concentrations of polyphenols. The iodine content was low and there was no detectable bromoform, suggesting quality advantages over *Asparagopsis taxiformis*. Additionally, *B. hamifera* exhibited antioxidant activity comparable to the positive control Resveratrol. The findings of this study indicated that *B. hamifera* harvested from temperate waters in Sweden possesses capacity to mitigate CH_4 *in vitro*.

Keywords: dairy cow; greenhouse gas; macroalga; methane

1. Introduction

Over the past decade, the discussion on the negative impact of meat and dairy production on the environment has gained a considerable momentum due to methane (CH_4) emissions and global warming. Globally, as much as 44% of the total CH_4 emissions can be attributed to agriculture [1].

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Approximately 40% of these emissions can be attributed to the fermentation of feed by cattle [2]. Research has demonstrated that the macroalga *Asparagopsis taxiformis* is among the most effective feed additives for mitigating enteric CH_4 emissions from ruminants [3,4]. The mechanism of reduction is largely attributed to halogenated secondary metabolites, particularly bromoform [3], which acts by directly inhibiting methanogenesis [5]. Researchers concluded that commercial production of *A. taxiformis* could create new economies due to the fact that small quantities of this seaweed in the diet of ruminant animals reduced CH_4 emissions by up to 98% when included at 0.05% of organic matter (OM) intake [5]. However, bromoform is a known carcinogen, and there has been elevated concentrations bromide and iodine in the milk of dairy cows fed with *A. taxiformis* [6,7]. *A. taxiformis* is native to South Australia and it is currently not cultivated in large quantities in the Northern hemisphere.

Bonnemaisonia hamifera is also a type of red alga of the same order *Bonnemaisoniales* and family *Bonnemaisoniaceae* as *A. taxiformis*. In New Zealand *B. hamifera* was shown to have a strong CH_4 inhibitory effect *in vitro* of 95.4%, and 98.8% relative to the basal feed substrate at inclusion levels of 6% and 10% on OM basis [8]. Furthermore, Mihaila et al. [8] showed that the primary bioactive compound bromoform in *A. taxiformis* was not detected in *B. hamifera*. We hypothesized that native *B. hamifera* wild-harvested from the West coast of Sweden was going to display a CH_4 inhibitory effect *in vitro* and be a temperate seaweed alternative to cultivate, and less susceptible to the loss of harmful volatile bioactives during processing and handling. The objectives of this study was to measure the CH_4 inhibitory effect *in vitro* of *B. hamifera* harvested in temperate water in Sweden.

2. Materials and Methods

The macroalga *B. hamifera* was harvested from Kristineberg Center for Marine Research and Innovation in Fiskebäckskil (58°14′N 11°27′E) on the West coast of Sweden. The seaweed was harvested from the shore in accordance with the Nagoya protocol guidelines (https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf), packed in cool boxes, and transported via overnight courier to Swedish University of Agricultural Sciences in Umeå on dry ice. Samples were washed to remove sand and epiphytes and stored at -18°C. All samples were freeze-dried using a laboratory-scale Labconco FreeZone freeze dryer equipped with tray dryers (Labconco, Kansas city, Missouri, USA) operating at -84°C.

The donor animals of rumen inoculum, equipment used and procedures of the *in vitro* followed the recent work reported by Krizsan et al. [9]. In brief, rumen fluid was directly transported to the laboratory after collection and filtered through cheesecloth into Thermos flasks. The samples were in total repeated across two water baths to get one bottle with blank (i.e. bottles with 60 mL of buffered rumen fluid with no sample or treatment in), duplicate bottles with control sample consisting of grass silage, and three replicates of treatment samples containing grass silage and *B. hamifera* in each bath. The *B. hamifera* was added at inclusion levels of 2.5%, 5% and 7.5% on OM basis. All samples were randomly distributed among the *in vitro* bottles in each bath. Gas production was measured with a fully automated system (Gas Production Recorder, GPR-2, Version 1.0 2015, Wageningen UR). Measurement of CH_4 was performed by withdrawing gas samples (0.2 mL) at 2, 4, 8, 24, 32, and 48 h from all *in vitro* bottles. The concentration of CH_4 was determined immediately after collection by injecting the gas sample in a Trace 1300 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA). The CH_4 concentration (mL/g sample) of all samples were used in model simulations to achieve *in vivo* predicted CH_4 according to Ramin and Huhtanen [10].

For the alga, the N percentage in the sample was determined using the LECO FP628 (LECO Corp., MI, USA) protein analyser applying the Dumas AOAC method 992.15 (1990) [11] and protein content was obtained using a conversion factor of 5.0 [12]. The NDF concentration was determined free of residual ash following the protocol outlined by Van Soest et al. [13], using a 1020 hot and 1021 cold extractor (Tecator Fibertec System; Foss Analytical) with addition of heat-stable α-amylase and sodium sulphite. The percentage lipid in each sample was assessed using the Oracle NMR Smart Trac rapid Fat analyser (CEM Corporation, USA) using AOAC official methods 985.14. The ash and moisture contents were determined according to [11].

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As detailed in Krizsan et al. [9], the total polyphenol concentration (TPC) of the macroalga was estimated using the Folin Ciocalteau reagent; the iodine content was determined using the Iodine Colorimetric Assay Kit (BioVision, Milpitas, California, USA), and the antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazine (DPPH) Antioxidant Assay Kit (AbCam, The Netherlands -ab289847, K2078). Bromoform concentration in Macroalga extract was carried out as described in Krizsan et al. [9].

Individual volatile fatty acid (VFA) concentrations in *in vitro* rumen fluid samples were determined using a Waters Alliance 2795 UPLC system as described by Puhakka et al. [14].

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.). Data were subjected to ANOVA using the MIXED procedure in SAS with treatment, water bath, and their interaction as fixed effects and bottle position in water bath as a random effect.

Treatments were compared using orthogonal contrasts; contrasts were constructed to evaluate the effects of inclusion of *B. hamifera* and the linear and quadratic effects of inclusion levels.

3. Results

The macroalga had DM, OM, CP, NDF and crude fat concentrations of 152±1.3 g/kg of fresh weight, and 505±6.7, 97±3.1 and 4.3±0.26 g/kg of DM.

The inclusion of *B. hamifera* decreased ($P = 0.01$) predicted *in vivo* CH_4 (Figure 1a) and total gas production (Figure 1b). There was a quadratic effect ($P = 0.01$) of increased levels of *B. hamifera* on predicted *in vivo* CH_4 production. The predicted *in vivo* total gas production linearly decreased ($P = 0.03$).

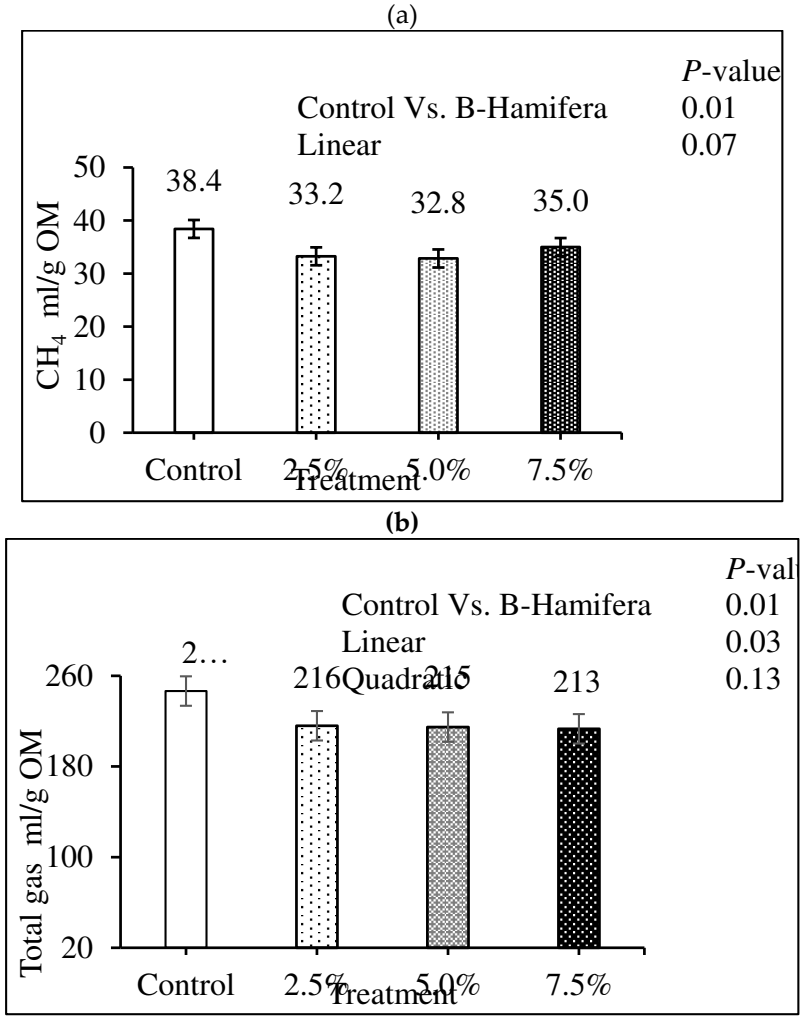

Figure 1. The effect of *B. hamifera* at different inclusion levels on predicted *in vivo* methane (a) and total gas production (b) with SEM of 1.68 and 13.1 ml/g OM, respectively.

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Propionate was greater ($P = 0.03$) and isovalerate, lower ($P = 0.04$) with the inclusion of *B. hamifera* compared to the control (Table 1). A tendency of increased ($0.06 \leq P \leq 0.10$) total VFA production and proportions of butyrate, isobutyrate, and 2-methylbutyrate were observed with the inclusion of *B. hamifera* compared to the control. We also found a quadratic effect ($P \leq 0.05$) on proportions of isobutyrate, 2-methylbutyrate, and isovalerate as well as a quadratic tendency ($0.08 \leq P \leq 0.10$) on total VFA production and the proportion of butyrate with an increase in *B. hamifera* inclusion levels.

The respective average polyphenol and iodine contents of *B. hamifera* sample were 0.165 mg Gallic acid equivalents and 71.1 µg/L iodine. The value obtained for total antioxidant activity of *B. hamifera* was 0.395 µM Trolox equivalents mg/ml sample. This is comparable to the positive control Resveratrol which had a DPPH value of 0.409 µM Trolox equivalents mg/ml (n=3). There was no bromoform detected in the *B. hamifera* used in this study.

Table 1. Effects of *B. hamifera* at different inclusion levels on total volatile fatty acid (VFA) and molar proportions of VFA production at 48 h of incubation *in vitro*.

Item	Treatments				SEM	*P*-value		
		B. hamifera inclusion level (% OM)						
	Control	2.5%	5.0%	7.5%		Control vs. *B. hamifera*	Linear	Quadratic
Total VFA, mM	148	162	159	155	6.6	0.10	0.44	0.08
VFA molar proportions, mmol/mol								
Acetate	575	574	575	577	2.4	0.94	0.39	0.42
Propionate	241	246	244	245	2.2	0.03	0.11	0.13
Butyrate	98.4	96.7	96.9	97.5	0.90	0.10	0.39	0.09
Isobutyrate	14.9	14.2	14.2	14.6	0.34	0.06	0.38	0.04
2-Methylbutyrate	11.6	10.9	11.0	11.2	0.31	0.06	0.36	0.05
Isovalerate	13.8	12.8	13.0	13.3	0.39	0.04	0.33	0.03
Valerate	24.5	23.8	23.8	19.9	3.49	0.52	0.24	0.53
Caproate	21.2	21.9	21.3	21.6	0.40	0.29	0.62	0.54

4. Discussion and Conclusion

The potential of feeding red algae to reduce CH_4 emissions from ruminants is a promising solution for a more sustainable production of food from cattle. However, there needs to be a system for use, i.e. cultivating, distributing and storing red algae on the farm without a change in the active substances occurring and assuring safety. Primarily, in order to assure an efficient CH_4 mitigation, but of no less importance to minimize the harmful risk of substances like bromofrom. Poor mixing and an accidently large dose of *A. taxiformis* could cause damage to the rumen wall of single cows [15] and reduced feed intake [6].

It is worth noting that a high concentration of bromoform in red algae have lead to greater CH_4 reduction [5]. In our study, the inclusion of *B. hamifera* resulted in a modest 12.3% reduction in predicted *in vivo* CH_4 production compared to an earlier *in vitro* study conducted in New Zealand that reported CH_4 reductions of most 98.8% at an inclusion of 10% on an OM basis [8]. The inhibitory effect seemed to be mediated by longer-chained halogenated hydrocarbons, likely by the same inhibitory mechanism as *A. taxiformis* [8]. Enge et al. [16] found that *B. hamifera* was chemically defended by producing 1,1,3,3-tetrabromo-2-heptanone (an halogenated secondary metabolites) as the main feeding deterrent compound. This compound could be a prospective candidate for exhibiting anti-methanogenic effect in the rumen.

In terms of ruminal fermentation patterns, most *in vivo* experiments with red algae have demonstrated a shift towards increased propionate production, confirming its role in CH_4 inhibition [5–7,17]. Several CH_4 inhibitory mechanisms could have been the reason for the effect observed in the

present study, but most likely the bioactive substances in Swedish *B. hamifera* affected a broader spectrum of the microbiome since total gas was decreased in supplemented treatments *in vitro*. Depending on where they grow and when they are harvested, algae will contain different levels of bioactive substances [12], which likely can explain the observed differences between *B. hamifera* harvested in Sweden and New Zealand. Ruminal branched-chain VFA (BCVFA; isobutyrate, isovalerate, and 2-methylbutyrate) are derived mainly from the deamination of branched-chain amino acids in the diet. Branched-chain VFA supplementation has been shown to improve digestibility and production in ruminants by providing an additional energy source and promoting the proliferation of cellulolytic bacteria [18]. In our study, the reduction in BCVFA proportions may indicate less microbial activity, contributing to the overall reduction in CH_4 emissions.

In many ways, red algae open up the possibility of producing organic food from dairy cows with reduced CH_4 emissions. *B. hamifera* harvested on the West coast of Sweden does not give a satisfactory reduction of CH_4 compared to other more readily available dietary mitigation strategies that could be suitable also in organic cattle production. On the other hand, the low iodine content and absence of bromoform in *B. hamifera* make it a potentially safer and more environmentally friendly option compared to *A. taxiformis* for CH_4 mitigation in ruminants. These characteristics reduce the risk of negative health effects on animals and minimize potential ecological concerns. However, further research is necessary to fully understand the specific bioactive substances present in *B. hamifera* and their effects on CH_4 production to optimize its utilization as a sustainable solution for reducing greenhouse gas emissions in livestock production. To further understand the differences observed in CH_4 inhibition, it is important to investigate the conditions specific to New Zealand, where more significant reductions in CH_4 emissions were reported in previous studies.

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Institutional Review Board Statement: The animal study protocol was approved by the Swedish Ethics Committee on Animal Research (Dnr A 32-16), represented by the Court of Appeal for Northern Norrland in Umeå, and the experiment was carried out in accordance with laws and regulations governing experiments performed with live animals in Sweden. .

Conflicts of Interest: The authors declare no conflict of interest.

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