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Enteric methane mitigation through *Asparagopsis taxiformis* supplementation and potential algal alternatives

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Research is increasingly directed towards decreasing the greenhouse gases contribution, specifically methane, from the livestock agriculture sector. Macroalgae supplementation has emerged as a promising tool to mitigate enteric methane emission in ruminants. The mode of action responsible for the mitigation effect centers around the content of volatile halogenated compounds, primarily bromoform. The sub-tropical red seaweed, *Asparagopsis taxiformis*, is the most well researched bromoform containing species. While several studies, both *in vitro* and *in vivo*, have demonstrated the effectiveness of *A. taxiformis* at reducing enteric methane emission (> 80% reduction), questions surrounding sustainability, animal productivity, animal product quality, and commercial practicality remain. These questions by no means disqualify the practice of feeding macroalgae to cattle to reduce methane emission, but they must be answered before implementing macroalgae as a feed additive commercially. Also, limiting scientific inquiry to a few species reduces the potential of discovering other compounds and modes of action that could produce the desired mitigation effect without the inherent drawbacks of the current options. Work conducted in both ruminant nutrition and human health fields have identified numerous bioactive compounds within plants that exhibit anti-microbial functions that could modify the rumen microbiome for beneficial outcomes. These compounds are also found in macroalgae. Phlorotannins, saponins, sulfonated glycans, other halocarbons and bacteriocins found within macroalgae have demonstrated antimicrobial activity *in vitro*. However, it is unclear what effect these compounds may have when used *in vivo*. Once identified, extracting these compounds for supplementation in lieu of feeding the entire plant may be a more practical solution. Dietary inclusion levels of macroalgae in ruminant diets can be limited by variation in active ingredient concentration, palatability to cattle, and excessive dietary mineral content. There are multiple *in vitro* studies that have demonstrated a methane reduction potential of non-bromoform containing species, but inclusion levels are often well above the effective levels of *A. taxiformis* (< 0.5% of dietary dry matter). In some animal studies, *A. taxiformis* supplementation has led to decreased dry matter intake and productivity and elevated mineral accumulation, such as iodine, in animal

products. Therefore, methane mitigation by macroalgae will likely have to occur at low dietary concentrations to be practical. This review aims to highlight potential benefits and challenges that feeding macroalgae as a tool for methane reduction may have on animal production, the environment, animal and consumer health.

KEYWORDS

seaweed, enteric methane, cattle, sustainability, climate

Introduction

Efforts to decrease the carbon footprint of human activities to mitigate climate change have increased globally across multiple economic sectors. In the animal agriculture sector, enteric methane emission from ruminant livestock has been identified as a key contributor to greenhouse gas (GHG) emissions. Methane (CH₄) emissions from animal production as a whole need to be reduced by 24 to 47% by 2050 compared to 2010, in order to meet projected goals from the Paris Agreement (Masson-Delmotte et al., 2019; Arndt et al., 2022). It is estimated that one third of anthropogenic CH₄ emissions is produced by ruminant livestock (Jackson et al., 2020). Methane emission from anaerobic manure storage and enteric fermentation in ruminants is a byproduct of the metabolism of archaea, which reduces carbon dioxide (CO₂) with hydrogen (H₂) as a source of energy (Thauer, 2012). To further complicate matters, production and consumption of animal protein is projected to increase 68% by 2050 (Searchinger et al., 2018). Therefore, research has been even more directed towards finding methods which reduce enteric CH₄ emission from dairy, beef and small ruminant operations. Animal and feed management, diet formulation, and rumen manipulation have been identified as feasible strategies to reduce absolute and/or product-based enteric CH₄ emissions in a recent review by Arndt et al. (2022); this review highlighted rumen manipulation through feed additives, specifically macroalgae, as a potentially viable mitigation practice.

Macroalgae, commonly known as seaweed, represent a large domain of aquatic plants separated into three main taxa: *Chlorophyta* (green), *Phaeophyceae* (brown) and *Rhodophyta* (red). There are also three smaller taxa of seaweed which are not pertinent to this review and therefore will not be discussed. Green, brown, and red seaweeds are key marine habitats, and are harvested commercially for food and the abundance of bioactive compounds contained within them. One such compound, bromoform (CHBr₃), has been proven to be highly effective at inhibiting methanogenesis (Machado et al., 2016a). It is thought

that CHBr₃, along with other halogenated volatile organic compounds (VOC), competitively bind to the enzymes and reductases that facilitate the final steps of reducing CO₂ and H₂ by archaea into CH₄ (Wood et al., 1968). Bromoform is found within many seaweed species in low concentrations but has been discovered to accumulate in higher levels in the red seaweed *Asparagopsis taxiformis* (Carpenter and Liss, 2000; Machado et al., 2016a). Within the last ten years, researchers have investigated the effects of *A. taxiformis* on CH₄ production in both *in vitro* and *in vivo* ruminant models and have concluded that it is highly effective as a CH₄ mitigant for livestock. The aim of this paper is two-fold: (1) to describe the practicality and utilization of *A. taxiformis* as a mitigating feed additive for ruminants and (2) to explore the potential of other bioactive algal compounds to mitigate CH₄ or improve animal performance.

Asparagopsis taxiformis

As a member of the Bonnemaisoniaceae family, *Asparagopsis taxiformis* is a sub-tropical seaweed distributed in warm temperate waters that grows as a creeping rhizomatous structure on rocks and algal beds. It is considered 'native' in Indo-Pacific waters and parts of the Atlantic (Canary and Azores Islands), but has also been 'introduced' in waters off the coast of California and the eastern Mediterranean (Andreakis et al., 2004). This family of seaweeds, compared to other seaweeds, is known for producing and accumulating high concentrations of halogenated compounds (Paul et al., 2006). Bromoform and other brominated compounds are stored in gland cells residing within pericentral (parent) cells of Bonnemaisoniaceae, and are considered to be released into the environment upon mechanical exposure to combat predation and antagonistic bacteria (Paul et al., 2006). The unique structure of these red seaweeds allows accumulation of VOC to reach 2 to 6% of dry weight biomass (Mata et al., 2012). This high VOC content is what makes *A. taxiformis*, and to a lesser extent *A. armata*, appealing candidates for enteric CH₄ emission reduction through rumen manipulation.

Body of work

A growing body of work has established that low level inclusion ($\leq 1\%$) of dietary dry matter (DM) of *A. taxiformis* can reduce, if not eliminate, enteric CH₄ production (Table 1). Machado et al. (2014) demonstrated that inclusion of *A. taxiformis* at 20% organic matter (OM) with Flinders grass hay reduced CH₄ production by 99% *in vitro*. Subsequent *in vitro* studies confirmed this antimethanogenic effect at doses below 2% substrate DM and identified CHBr₃ as the primary compound responsible for this effect (Kinley et al., 2016;

Machado et al., 2016b; Machado et al., 2016a; Machado et al., 2018). Chagas et al. (2019) further demonstrated that *A. taxiformis* was effective at reducing *in vitro* CH₄ production at even lower inclusion rates (0.5 mg/g of DM).

Following on the *in vitro* work, *in vivo* supplementation of *A. taxiformis* (0.5 to 3% OM basis) in sheep reduced CH₄ emissions 50 to 80% over 72 d (Li et al., 2018). Work conducted in steers decreased enteric CH₄ yield (g/kg of DM) between 80 and 98% when diets were supplemented with *A. taxiformis* (0.2 to 0.5% OM basis), dependent on the dietary fiber content (Kinley et al., 2020; Roque et al., 2021). Interestingly, Roque et al. (2021)

TABLE 1 Summary of *in vivo* seaweed studies, in which enteric CH₄ emission was measured.

Seaweed	Animal	n (design)	Study duration (d)	Seaweed inclusion level	CHBr ₃ intake (mg/d)	CH ₄ g/kg of DMI ($\Delta\%$)	DMI ($\Delta\%$)	Productivity ¹ ($\Delta\%$)	CHBr ₃ residue ²	Reference
<i>A. taxiformis</i>	Sheep	5 (Ctn)	72	0.5% OM	0.07	n.s.	n.s.	-	n.s.	Li et al., 2018
<i>A. taxiformis</i>	Sheep	5 (Ctn)	72	1.0% OM	0.14	-52	n.s.	-	n.s.	Li et al., 2018
<i>A. taxiformis</i>	Sheep	5 (Ctn)	72	2.0% OM	0.16	-62	n.s.	-	n.s.	Li et al., 2018
<i>A. taxiformis</i>	Sheep	5 (Ctn)	72	3.0% OM	0.18	-81	n.s.	-	n.s.	Li et al., 2018
<i>Ascophyllum nodosum</i>	Dairy cows	7 (Ctn)	112	113 g/h/d	-	n.s.	n.s. ^a	n.s.	-	Antaya et al., 2019
<i>A. armata</i>	Dairy cows	12 (3 × 3 LS)	21	0.50% OM	0.44	-26	-10	n.s.	n.s.	Roque et al., 2019
<i>A. armata</i>	Dairy cows	12 (3 × 3 LS)	21	1.0% OM	0.62	-67	-38	-11.6 ^b	n.s.	Roque et al., 2019
<i>A. taxiformis</i>	Beef cattle	5 (Ctn)	60	0.05% OM	0.1	-9	n.s.	n.s.	n.s.	Kinley et al., 2020
<i>A. taxiformis</i>	Beef cattle	5 (Ctn)	60	0.10% OM	0.25	-38	n.s.	51 ^c	n.s.	Kinley et al., 2020
<i>A. taxiformis</i>	Beef cattle	5 (Ctn)	60	0.20% OM	0.45	-98	n.s.	42 ^c	n.s.	Kinley et al., 2020
<i>A. taxiformis</i>	Beef cattle	6 (Ctn)	147	0.25% OM	0.87	-51	-8	n.s.	n.s.	Roque et al., 2021
<i>A. taxiformis</i>	Beef cattle	6 (Ctn)	147	0.50% OM	1.58	-75	-14	n.s.	n.s.	Roque et al., 2021
<i>A. taxiformis</i>	Dairy cows	20 (4 × 4 LS)	28	0.25% DM	-	n.s.	n.s.	n.s.	n.s.	Stefenoni et al., 2021
<i>A. taxiformis</i>	Dairy cows	20 (4 × 4 LS)	28	0.5% DM	-	-34	-7	-6.5 ^b	n.s.	Stefenoni et al., 2021
<i>A. taxiformis</i>	Dairy cows	8 (Ctn)	19	67 g/h/d	0.08	-	-5	15 ^b	9.1 $\mu\text{g/L}^{\text{d}}$	Muizelaar et al., 2021
<i>A. taxiformis</i>	Dairy cows	2 (Ctn)	19	133 g/h/d	0.17	-	-9	-5 ^b	11 $\mu\text{g/L}^{\text{d}}$	Muizelaar et al., 2021
<i>A. taxiformis</i>	Dairy cows	2 (Ctn)	19	333 g/h/d	0.42	-	-14	-4 ^b	35 $\mu\text{g/L}^{\text{d}}$	Muizelaar et al., 2021

CH₄, methane; CHBr₃, Bromoform; Ctn, Continuous design; DM, dry matter basis; OM, organic matter basis; DMI, dry matter intake; $\Delta\%$, treatment response over control; (-), not quantified; n.s., not statistically significant; LS, Latin square.

¹Reported as average daily gain or milk yield.

²Reported CHBr₃ residue in milk or meat products.

^aTotal DMI = estimated pasture DMI + partial total mixed ration DMI.

^bMilk.

^cAverage daily gain.

^dHighest single day CHBr₃ concentration in milk reported during the study.

reported a decrease of dry matter intake (DMI) by 14% with an equivalent increase in feed conversion efficiency in steers fed *A. taxiformis* (0.5% OM). A similar VOC containing species, *A. armata*, reduced CH₄ intensity (g/kg milk yield) by 60% and CH₄ yield by 43% when fed at 1% OM to lactating dairy cows (Roque et al., 2019). However, DMI was also decreased by 38% in that study. Stefenoni et al. (2021) fed *A. taxiformis* to lactating Holsteins at 0.5% DM and observed a 55% reduction in CH₄ yield. This study also reported decreased DMI and milk yield in cows fed *A. taxiformis*. Additionally, Stefenoni et al. (2021) reported a loss of antimethanogenic effect of the seaweed during the second half of the experiment. As this experiment was a 4 × 4 Latin square design, rumen adaptation was eliminated as a possible explanation for this inefficiency. The authors concluded the decreased effectiveness was a result of decreased CHBr₃ concentration within the seaweed over time, from ~10 mg/kg to 3 mg/kg (day 0 and 120 of storage, respectively). Other previously mentioned studies did not report any decrease in CH₄ mitigation effectiveness over time. Furthermore, a study conducted to establish a methodology for CHBr₃ quantification found no decrease in CHBr₃ concentration from *A. taxiformis* that had been freeze dried and stored at -20°C for 20 weeks (Romanazzi et al., 2021). Muizelaar et al. (2021) fed *A. taxiformis* at three levels (67, 133 and 333 g/d) and reported decreased DMI across all treatment levels. These authors also reported elevated CHBr₃ excreted in milk during days 1 through 9, but CHBr₃ was not consistently found in days 10 to 22 of feeding *A. taxiformis*. This was attributed to the inconsistency of seaweed intake. Upon completion of the study, two cows were sacrificed and their rumen walls were evaluated for abnormalities. The authors reported loss of rumen papillae and histological conformation of inflammation. These findings were comparable to rumen wall evaluation of sheep sacrificed in Li et al. (2018). However, both studies were unable to conclude that damage to the rumen epithelial were a direct result of *A. taxiformis* supplementation. Li et al. (2018) suggested that these changes could have been caused by mild ruminal acidosis associated with carbohydrate ingestion via the pelleted diet used to deliver the treatments. Muizelaar et al. (2021) did not report any clinical signs of acidosis. Additionally, both studies only examined rumen tissue from *A. taxiformis* animals, preventing comparison with control animals.

Opportunity and concerns

In addition to the contribution of enteric CH₄ to climate change, it also accounts for gross feed energy intake losses between 2 and 12% (Johnson and Johnson, 1995). The extent of energy loss is tightly linked to carbohydrate fermentation and the resulting supply of available H₂, which in turn influences the acetate:propionate ratio in ruminal contents (Wolin et al., 1997).

In ruminal fermentation, acetate is regarded as a net H₂ source, and propionate a H₂ sink. Diets and conditions that produce a rumen environment with high H₂ concentrations will drive fermentation away from pathways that produce H₂ (acetate), resulting in higher propionate and lower CH₄ production per unit of feed substrate (Janssen, 2010). The redirection and capture of H₂ from CH₄ to propionate is where opportunity exists to increase feed efficiency in livestock (Kinley et al., 2020).

When methanogenesis is inhibited using an additive like *A. taxiformis*, archaea are unable to consume the excess H₂ resulting from fermentation, leading to an increase in propionate production and H₂ accumulation within the rumen (Goel et al., 2009). In studies feeding *A. taxiformis* where eructated H₂ was measured, H₂ emission increased from 234 to 1700% compared to control treatments, depending on the inclusion level (Kinley et al., 2020; Roque et al., 2021; Stefenoni et al., 2021). *In vivo* studies which measured rumen VFA profile while feeding *A. taxiformis* observed decreases in acetate:propionate ratio between 12 and 45% (Li et al., 2018; Kinley et al., 2020; Stefenoni et al., 2021). With the exception of Li et al. (2018), where *A. taxiformis* was fed to sheep at 0.5 to 3.0% (OM basis), these studies did not report a decrease in total VFA concentration. Kinley et al. (2020) observed increased average daily weight gain of 26 and 22% for steers fed 0.1 and 0.2% (OM basis) *A. taxiformis* over 90 days, respectively. However, Stefenoni et al. (2021) reported a 6.5% decrease in milk yield, following a decrease in DMI, in lactating Holstein cows fed 0.5% (DM basis) *A. taxiformis*, with no difference in feed efficiency between treatments. Li et al. (2018) did not observe any change in liveweight gain in sheep fed 0.5 to 3.0% (OM basis) *A. taxiformis*.

It is difficult to reconcile the discrepancy in the above results, although species, diet composition and type of animal production (weight gain vs. milk yield) likely play some role, as animals will partition energy differently during lactation or growth. Moreover, 100% recovery and repartitioning of energy from available H₂ into VFA production does not seem possible without the expansion of H₂ sinks to the rumen. An *in vitro* study, in which methanogenesis was inhibited with 5 or 10 μM bromochloromethane reported 38 and 64% recovery of H₂ in the form of short chain fatty acids, respectively (Goel et al., 2009). A study with lactating cows fed a different type of inhibitor, 3-nitroxypropanol (3-NOP), estimated 89 and 84% H₂ recovery for control and 3-NOP treatments, respectively (Melgar et al., 2020). The authors were unable to determine the fate of the remaining H₂ produced and highlighted the need for further research into rumen H₂ dynamics. A recent study feeding dairy goats *A. taxiformis* combined with phloroglucinol, a H₂ sink yielding acetate as a final product, observed a 74% decrease in eructated H₂ compared with *A. taxiformis* alone (Romero et al., 2022a); total VFA and acetate concentrations in the rumen were also increased by *A. taxiformis* and phloroglucinol in that study. However, animal productivity metrics from this study have yet

to be reported. Further research into combined inhibitor and H₂ sink supplementation could potentially increase animal performance while simultaneously decreasing CH₄ emissions and CH₄ intensity (g/kg of milk or meat).

Results from the aforementioned studies indicate the large CH₄ mitigation potential of feeding *A. taxiformis* but raise questions about animal safety and production effects of supplementation. Of primary concern for a livestock producer is the inconsistencies in DMI and performance. In studies where intake loss did not affect animal performance or was unobserved, a gradual increase in *A. taxiformis* inclusion was conducted over the animal's adaptation period (Li et al., 2018; Kinley et al., 2020; Roque et al., 2021). This gradual adaptation was not applied in the studies that had substantial decreases in DMI and animal performance (Muizelaar et al., 2021; Stefenoni et al., 2021). If this product is to be utilized in commercial animal production, it is critical that a protocol of adaptation be established in order to mitigate performance loss in the form of meat or milk. The animal's stage of production will also be of importance. For example, it has been established that DMI intake during the postpartum period in lactating dairy cattle is the key to maximize performance over the lactation and prevent inflammation and subsequent metabolic diseases (Kvidera et al., 2017). If a prepartum cow has not been acclimated to seaweed in their diet, a drop in intake during adaptation could trigger a decrease in animal health or performance across the rest of their lactation. The authors are unaware of any studies feeding *A. taxiformis* to prepartum animals or during the transition period.

Bromoform is categorized as a 'potential human carcinogenic' compound by the EPA, and although fed at low levels to livestock, it is currently unclear as to what impact feeding CHBr₃ may have on animal health long term (U.S. Environmental Protection Agency National Center for Environmental Assessment (1987)). This concern is of higher priority in dairy cattle that persist within the herd for several years versus in a beef finishing system where animals are only supplement seaweed for ~90 d (Kinley et al., 2021). Personnel involved in *A. taxiformis* processing and feed manufacturing facilities could also potentially be at risk, if continuously exposed to *A. taxiformis*. This is a purely hypothetical concern, however, since potential exposure time and levels, manufacturing conditions, and *A. taxiformis* handling procedures have not been established or described. Future research should be directed to finding appropriate timing and implementation of seaweed supplementation to avoid these possible complications. Finally, controlled histological evaluations of rumen epithelial tissue should be conducted to assess the effects of VOC on these tissues over extended periods of exposure.

Seaweed cultivation, processing and storage conditions also need to be considered (Vijn et al., 2020). Previous work has utilized freeze dried product kept in some form of cold storage. The capability to store feed additives in a freezer or refrigerator is

not common for dairy or livestock operations and will render the strategy impractical. Freeze dried *A. taxiformis* stored at 25°C had a 38% reduction in CHBr₃ over the course of 12 weeks (Magnusson et al., 2020). In the same study, however, an oil immersion of *A. taxiformis* had no measurable decrease in CHBr₃ concentration. This oil immersion technique could provide a solution to the issue of storage. Variables in seaweed production also need to be addressed. For example, phenotypic and temperature variation have an effect on CHBr₃ content of *Asparagopsis* with content of bioactive compounds reaching tenfold differences (Mata et al., 2017). This suggests that wild harvested or commercial oceanic cultivation may produce vastly different products in terms of CHBr₃ content. Land-based culture techniques could standardize plant variety as well as optimize growing conditions for consistent CHBr₃ concentrations. For instance, providing a higher C/N ratio and increasing the availability of CO₂ within the growing medium has been shown to increase CHBr₃ content within the biomass of *A. taxiformis* (Mata et al., 2012).

Effects of CH₄ inhibiting feed additives on methane production from anaerobic digesters is another relevant concern for livestock producers who utilize manure digesters on their operations. Digesters are being deployed by farmers in the United States as a way to offset carbon emissions and obtain carbon credits through government policy (Key and Sneeringer, 2011). Fortunately, CHBr₃ appears to be quickly metabolized by the rumen microbes (Romero et al., 2022b) and is not excreted in animal manure (Kinley et al., 2020; Muizelaar et al., 2021). In situations where producers add feed refusals to their digester this may still present an issue. A recent *in vitro* study analyzed CH₄ production from incubated manure from cows fed no inhibitor or 0.5% (OM basis) *A. taxiformis*, and the same manures with added *A. taxiformis* (Ramin et al., 2022). Enteric CH₄ production was only numerically lower in *A. taxiformis* fed cows, and the addition of *A. taxiformis* to the manure decreased manure CH₄ production by 50%. These results may indicate that the addition of *A. taxiformis* to manure digesters in the form of feed refusals may have a negative impact on their energy production. Nkemka et al. (2019) reported no effect on CH₄ yield from anaerobic leach bed digesters supplied manure from 3-NOP steers compared with control. Similarly, manure stored for 202 d from animals fed the CH₄ inhibitor, 3-NOP, did not differ in CH₄ emission when compared with animals fed no inhibitor (Owens et al., 2020). Both studies may indicate resilience to effects of dietary CH₄ inhibitors in anaerobic digester systems.

There are also potential concerns for the consumers of products derived from animals fed *A. taxiformis*. Whereas effects on consumer health are beyond the scope of this review, it is important to acknowledge the concerns surrounding the feeding of *A. taxiformis* to animals entering the food chain. As previously stated, Muizelaar et al. (2021) reported elevated levels of CHBr₃ in milk. Levels ranged from not detectable to 9.1 µg/L in milk from cows on the Low

treatment (~67g *A. taxiformis*/cow/d). This relatively small increase, that was transient throughout the study, may have been caused by inconsistent seaweed intake. Maximum reported CHBr₃ levels in Med (133 g/h/d) and High (333 g/h/d) treatments reached 11 and 35 µg/L, respectively. However, these inclusion levels far exceed a minimum effective dose. Future work in this area should continue to quantify CHBr₃ levels in milk over time. In studies involving steers no appreciable level of CHBr₃ was found within the meat. A summary of toxicological risk to animals and humans concluded that at low inclusion levels *A. taxiformis* did not cause problems for ruminant animals or consumers through their products (Glasson et al., 2022). Stefenoni et al. (2021) conducted a milk sensory panel during which consumers were unable to discern between milk from cows fed *A. taxiformis* compared to control, but the ability to detect a difference approached a trend ($P = 0.11$; with 39% of participants correctly identified milk from *A. taxiformis* cows as different from milk from control cows). Additionally, milk from cows fed seaweed had 5- and 8-times higher concentrations of iodine and bromide, respectively. Bromide levels in this study from cows fed *A. taxiformis* at 0.5% (DM basis) averaged 40.4 mg/kg. For context, the World Health Organization recommends ≤ 6 mg/kg in water for adults and ≤ 2 mg/kg for children (WHO, 2009). While acute toxicity to bromide is seldom seen in humans, chronic toxicity in animals has negative effects on endocrine and reproductive systems (VanBriesen, 2013). The increase of iodine in milk could have effects on consumer health; for example, in persons with abnormal thyroid function. Most seaweeds are known to have high mineral content due to the environment they are grown in and are used as a form of mineral supplementation in human and animal diets (Norton et al., 1981). Since minerals are sequestered from the growing environment, future land-based production facilities should be able to reduce mineral levels within seaweeds through water remediation. Monitoring of mineral levels in the diet and in animal products, manure and urine should be conducted to prevent any downstream effects on human health and the environment.

One final area of concern is the environmental impact of seaweed cultivation. It is unlikely that wild harvesting of seaweed will be adequate to meet the demand of the United States cattle market of ~3 million metric tons (MMT) dry product per annum (Vijn et al., 2020). Supplying the global herd of 1.4 billion cattle would not be feasible, not to mention impractical and cost prohibitive for pastoral smallholder farm systems (Vijn et al., 2020). Current supply of *A. taxiformis* is limited and expensive (Hristov et al., 2022). This is untenable for any livestock production system, but the cost should decrease as more production and harvesting entities begin supplying the market. Therefore, large scale cultivation will need to be implemented along the coasts or in commercial facility. This will require energy in order to supply light, heat and salt for

culture systems. In a land based production system it is estimated that *A. taxiformis* cultivation will emit 9.2 kg CO₂e/kg of seaweed produced, with 48% of the CO₂e being emitted from salt (Nilsson and Martin, 2022). It is important to note that this estimate was modeled using wind energy for drying down the seaweed using a refractance window drying technique. Recent studies using *A. taxiformis* have utilized lyophilization to dry the product which provides the highest concentration of CHBr₃ in the final product (Vucko et al., 2017). The authors are unaware of any effects on CHBr₃ content or activity when the seaweed is subjected to refractance window drying. Considering the decrease in mitigation effect observed in Stefenoni et al. (2021), processing of *A. taxiformis* should be optimized to retain CHBr₃ concentration and activity level with the final product.

Bromoform itself poses potential hazards to the environment as an ozone depleting compound. All seaweeds combined are responsible for approximately 70% of the global bromoform flux. It stands to reason that increasing seaweed cultivation will increase the global CHBr₃ emissions, especially when the species is known for higher CHBr₃ content. However, current volume of all commercially harvested seaweeds (3.12 MMT) contribute only ~1% of the global CHBr₃ flux, with the majority being emitted from wild seaweed (Glasson et al., 2022). Therefore, 100% implementation of *A. taxiformis* supplementation (at 1% of feed DM intake) in the U.S. herd of 93 million cattle would increase global CHBr₃ emissions from cultivated seaweed to approximately 1.5%, as it will require half the biomass of current global seaweed biomass production (Vijn et al., 2020). Admittedly, estimates assuming full adoption across the livestock industry is unlikely but illustrates what the maximum contribution of CHBr₃ to the atmosphere could be. As production of *A. taxiformis* ramps up it will be critical to continue monitoring atmospheric CHBr₃ concentrations and quantify contributions from commercial seaweed systems.

It is important to note that seaweed cultivation can provide multiple benefits to the environment through nutrient extraction (Kim et al., 2019). In order to grow, seaweeds consume N, P and C, which can ameliorate harmful algal blooms, prevent eutrophication, and aid in buffering of seawater. If grown in open water, seaweed beds can also provide habitat for aquatic life. However, displacing native seaweed in lieu of growing a commercially viable seaweed may be antagonistic to local biodiversity. Land-based systems could employ this bioremediation to waste waters, reducing nutrient inputs to the system as well as 'downstream'. Future efforts to integrate production systems to efficiently utilize nutrients may provide environmental benefits in addition to CH₄ mitigation.

The associated benefits and risks of *A. taxiformis* need to be investigated and weighed against each other if this mitigation practice is to be adopted across the industry. This review seeks to highlight some of the initial questions that will accompany widescale implementation of the practice, but the decision to implement this technology may ultimately be made by the

industry. A recent study by [Passetti and Hegarty \(2022\)](#) sought to assess industry interest and preparedness to use *A. taxiformis* as a methane inhibitor in livestock. A survey was sent to feed and feed supplement manufacturers and livestock managers in developed (87.5%) and developing (37.5%) countries to gauge their willingness to use additives and the priority to reduce GHG emissions. Methane inhibiting additives were a priority for 14% of feed manufacturers, with 14% of feed manufacturers being aware of using *A. taxiformis* as a CH₄ inhibitor. Greenhouse gas management was not a priority for 81% of livestock managers. Conversely, feed efficiency and animal performance were the main motivation for 92% of livestock managers. This study was, by the admission of the authors, limited. However, the data underline the importance of continued research and development of CH₄ inhibitors and H₂ sinks in order to capitalize on the interest in animal performance and efficiency for the adoption of *A. taxiformis* and related technologies.

Potential alternatives to explore

Exploration of seaweeds as CH₄ mitigating feed additives has not been limited to *Asparagopsis* species. A review by [Abbott et al. \(2020\)](#) highlighted multiple *in vitro* studies with seaweeds using rumen inoculum that measured CH₄ production and quantified CHBr₃ content. While many studies reported decreases in CH₄ production versus a control (no seaweed), supplementation levels often exceeded 2% of substrate DM or OM. Considering observed decreases in DMI with seaweed supplementation, biomass demand on seaweed production systems and the relatively high mineral content of seaweeds; inclusion levels within livestock diets should aim for the lowest effective dose ([Norton et al., 1981](#); [Erickson et al., 2012](#); [Vijn et al., 2020](#); [Muizelaar et al., 2021](#); [Stefenoni et al., 2021](#)). To date, *Asparagopsis* species appear to be the best candidate as a rumen manipulation additive due to effectiveness at low inclusion rates.

Seaweeds that inhibit methanogenesis may not be strictly limited to species with high CHBr₃ content. Preliminary *in vitro* work on a non-CHBr₃ seaweed species has been reported to reduce CH₄ production at similar inclusion rates to *in vitro* work with *A. taxiformis* ([Hristov et al., 2022](#)). However, this undisclosed species and its mode of action requires further investigation and validation through *in vivo* studies. There are more than 200,000 secondary plant metabolites, many of which can be found in seaweeds ([Patra and Saxena, 2010](#)). Polyphenols, peptides, phlorotannins, alkaloids and sulfonated glycans are among the compounds present in seaweed species with known anti-microbial properties ([Pomin, 2009](#); [Abbott et al., 2020](#)). However, seaweed compound specificity against methanogens and interactions with the rumen microbiome remain largely unexplored. Both *in vitro* and *in vivo* studies investigating the effects of secondary plant metabolites on methanogenesis are largely derived from terrestrial sources and have provided mixed

results ([Patra and Saxena, 2010](#); [Patra et al., 2017](#); [Abbott et al., 2020](#)). In a review by [Patra and Saxena \(2010\)](#), saponins, tannins, flavonoids and organosulfur compounds all demonstrated potential to reduce CH₄ production through direct inhibition or indirectly through suppression of protozoa growth.

Generally speaking, tannins have shown efficacy in decreasing CH₄ emission *in vivo* but tend to impact animal performance by decreasing intake, protein digestibility and fiber digestibility ([Arndt et al., 2022](#)). Phlorotannins are structurally one of the simpler tannins and are specific to brown algae. The relatively smaller structural size may limit their effect on CH₄ production, compared to larger compounds which are more effective at reducing CH₄ ([Hatew et al., 2016](#)). Tannins from terrestrial plants are considered one of the few rumen manipulation strategies suitable for grazing systems since tanniferous plants can be grown within the pasture ([Arndt et al., 2022](#)). This advantage would be lost when using a marine source of tannin.

Results from studies supplementing terrestrial plant flavonoids, saponins and essential oils and their effects on methanogens and methanogenesis are variable. Some of this variation is due to the large diversity of compounds residing in each category. Seaweeds also contain variations of these secondary plant metabolites, although research from compounds specific to seaweeds are sparse. Still, terrestrial plant compound studies may help identify CH₄ mitigating compounds in seaweeds with similar structure. An *in vitro* study reported six flavonoids that reduced CH₄ in a dose dependent manner ([Oskoueian et al., 2013](#)). Reduction in CH₄ yield ranged from 16 to 39% using myricetin, kaempferol, flavone, quercetin, naringin, rutin and catechin (ranked lowest to highest by CH₄ reduction). Flavonoids (purity ≥ 98%) were dissolved in ethanol and included at 4.5% (w/w) of substrate DM, exceeding supplementation rates (%DM basis) of *A. taxiformis* required to produce a CH₄ effect. Based on these concentrations, seaweed flavonoids will likely need to be extracted in order to have an effect on CH₄ production. It is suggested that flavonoids directly inhibit methanogens and, in the case of catechin, act as a H₂ sink ([Oskoueian et al., 2013](#); [Becker et al., 2014](#)). Saponins, such as those found in green tea, seem to inhibit methanogenesis by directly inhibiting protozoa, decreasing protozoa-associated methanogen abundance through lower H₂ production ([Patra and Saxena, 2009](#)). These alkaloids have been quantified within several red and green algae, but so far research has been limited to saponins from terrestrial plants ([Abbott et al., 2020](#)). Future research and classification of seaweed compounds for CH₄ inhibition should focus on direct, qualitative identification of compounds that are structurally similar to terrestrial plant compounds that have proven CH₄ mitigating effect.

Delivery method of anti-methanogenic feed additives should also be considered. In the case of *A. taxiformis* the entire plant is included in the diet. For a compound like CHBr₃ this may well

be the best option as the plant is able to retain the volatile compound within the tissue. Extracted CHBr_3 could be more difficult and dangerous to handle for feed manufacturers and producers. However, other secondary plant metabolites could be extracted. This could produce a more concentrated product; decreasing the mass required in the diet, eliminating seasonal variation in plant concentration of the compound, and avoid dietary enrichment of trace minerals or heavy metals present in ocean water (Mata et al., 2017).

Discussion

Feeding *A. taxiformis* to ruminant livestock may be the most effective rumen manipulation tool in terms of reducing CH_4 emission. When paired with the possible decrease in gross feed energy loss and improved animal performance, *A. taxiformis* could provide a positive outcome for producers and the environment. However, improved animal performance will be contingent on avoiding the decreases in DMI observed in several studies. Potential negative effects on animal performance and health as well as human health should continue to be investigated. Many of these hurdles may have simple solutions. For example, having a defined protocol for diet adaptation to prevent DMI reductions, or reducing trace mineral content through controlled, land-based growing operations with guaranteed analysis on the final product. Supplementation of *A. taxiformis* with the goal of reducing CH_4 only makes sense if there is a net reduction in carbon emissions and no damage to the ozone layer through increased CHBr_3 emissions. Preliminary estimates seem to indicate that the benefits outweigh the risks. If improvements in production efficiency and bio-extraction of N, P from waterways are realized, *A. taxiformis* cultivation may be a beneficial enterprise for livestock producers and the environment. Environmental benefits will also be weighed alongside producers' acceptance as the current cost of *A. taxiformis*, and potential negative effects on performance (i.e., DMI and milk yield) may limit market adoption. Animal productivity and feed efficiency seem to be the main drivers in the adoption of feed additives by livestock producers. Therefore, any concerns of decreased productivity need to be addressed. Possible negative effects from *A. taxiformis* supplementation on the health of animals, manufacturers, producers, and consumers should continue to be investigated. Due to low effective dose required to achieve CH_4 mitigation and the reported lack of CHBr_3 residue in the meat this may not be a cause for concern. However, under conditions of excess supplementation of *A. taxiformis*, CHBr_3 is detected within milk, making it critical to monitor supplementation levels to lactating animals. Transfer of iodine and bromide into milk will still need to be brought to the attention of consumers in order for them to make informed

decisions about their consumption of dairy products. The practicality of handling and storing seaweed products on farm will also need to be addressed. Reluctance to adopt this mitigation tool could be alleviated by increasing production of *A. taxiformis*, establishing dietary adaptation methods, and stabilizing the final product (e.g., oil immersion).

It is possible that other compounds found in macroalgae could have anti-methanogenic effects *in vivo* and should be explored. Secondary metabolites that are not classified as cancerous, stable at ambient temperatures and do not accumulate in milk or meat products would be preferential to both producers and consumers. It is also important that these metabolites do not sacrifice animal performance. Pairing inhibitors with H_2 sinks may provide positive effects on animal performance and efficiency. Until one of these hypothetical compounds are proven to work *in vivo*, *Asparagopsis taxiformis* is the most effective naturally-derived rumen manipulation feed additive livestock producers have as a tool to mitigate enteric CH_4 emissions.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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