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REVIEW

Seaweed as a feed additive to mitigate enteric methane emissions in ruminants: Opportunities and challenges

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Highlights

- *Asparagopsis taxiformis* has demonstrated significant potential in reducing enteric methane emissions in ruminants, achieving reductions of up to 99% *in vitro* and *in vivo*, primarily due to its bromoform content.
- The application of seaweed as a feed additive faces challenges, including potential heavy metal contamination, environmental risks associated with bromoform, and the need for a sustainable cultivation and processing supply chain.
- Further research is needed to identify low-bromoform seaweed species and investigate additional bioactive compounds to optimize methane mitigation strategies.

Abstract

Cutting farming-related methane emissions from ruminants is critical in the battle against climate change. Since scientists initially investigated the potential of marine macroalgae to reduce methane emissions, using seaweeds as an anti-methanogenic feed additive has become prevailing in recent years. *Asparagopsis taxiformis* is the preferred species because it contains a relatively higher concentration of bromoform. As a type of halogenated methane analogue, bromoform contained in *A. taxiformis* can specifically inhibit the activity of coenzyme M methyltransferase, thereby blocking the ruminal methanogenesis. However, bromoform is a potential toxin and ozone-depleting substance. In response, current research focuses on the effects of bromoform-enriched seaweed supplementation on ruminant productivity and safety, as well as the impact of large-scale cultivation of seaweeds on the atmospheric environment. The current research on seaweed still needs to be improved, especially in developing more species with low bromoform content, such as *Bonnemaisonia hamifera*, *Dictyota bartayresii*, and *Cystoseira trinodis*. Otherwise, seaweed is rich in bioactive substances and exhibits antibacterial, anti-inflammatory, and other physiological properties, but research on the role of these bioactive compounds in methane emissions is lacking. It is worthy of deeper investigation to identify more potential bioactive compounds. As a new focus of attention, seaweed has attracted the interest of many scientists. Nevertheless, seaweed still faces some challenges as a feed additive to ruminants, such as the residues of heavy metals (iodine and bromine) and bromoform in milk or meat, as well as

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the establishment of a supply chain for seaweed cultivation, preservation, and processing. We have concluded that the methane-reducing efficacy of seaweed is indisputable. However, its application as a commercial feed additive is still influenced by factors such as safety, costs, policy incentives, and regulations.

Keywords: seaweed, *Asparagopsis taxiformis*, bromoform, methane emission, ruminant

1. Introduction

Methane has a global warming potential (GWP) of 86 or 28 times greater than CO_2 on a 20- or 100-year time scale, respectively (IPCC 2021). Agriculture contributes to nearly half of the global anthropogenic methane production, followed by fossil fuels and waste (Opio *et al.* 2013). The livestock sector is among the most significant contributors to methane emissions, especially ruminant production. Numerous approaches have been proposed, with dietary manipulation being one of the most effective ways to mitigate enteric methane emissions from ruminants (Arndt *et al.* 2022). Seaweeds (macroalgae) are marine plant resources extensively used in human foods, natural cosmetics, pharmaceuticals, and nutraceuticals (Holdt and Kraan 2011; Rengasamy *et al.* 2020). Interest in the application of macroalgae to mitigate enteric methane emissions from ruminants has increased dramatically in recent years (Machado *et al.* 2016a; Antaya *et al.* 2019; Roque *et al.* 2019; Kinley *et al.* 2020; Vijn *et al.* 2020). Although bromoform is believed to be the primary active ingredient responsible for methane mitigation (Machado *et al.* 2016b), other bioactive compounds such as phlorotannins, alkaloids, and peptides may also play a role in reducing enteric methane emissions (Abbott *et al.* 2020). This review summarizes recently published studies using seaweed as an anti-methanogenic feed additive, discusses the challenges and limitations, and proposes perspectives for future research.

2. Rumen methanogens and mitigation of methanogenesis

2.1. Pathway of methanogenesis in the rumen

Ruminants rely on rumen microbes to ferment dietary components into volatile fatty acids (VFAs), the major energy source for the host (Moss *et al.* 2000). During fermentation, the hydrogenases in bacteria or protozoa transfer electrons from reduced cofactors to H^+ forming H_2, which is further transferred to methanogens and used for methanogenesis (Pitta D *et al.* 2022). Primary substrates can be used by rumen methanogens to produce methane including CO_2, methyls (methanol, methylamines), and acetate (Fig. 1). Based on substrate utilization, rumen methanogenesis is divided into 3 pathways, including hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis (Berghuis *et al.* 2019). In hydrogenotrophic methanogenesis, rumen methanogens reduce CO_2 to methane using H_2 as an electron donor through the Wolf cycle (Thauer 2012). Briefly, CO_2 binds initially to methanofuran (MFR) and is subsequently reduced to formylmethanofuran (CHO-MFR) by formylmethanofuran dehydrogenase (Fmd) and Fd_{red} (Wagner *et al.* 2016). The formyl group is then transferred to tetrahydromethanopterin (H_4MPT) to form CHO-H_4MPT, which is subsequently dehydrated to CH_3-H_4MPT with reduced F_{420} ($F_{420}H_2$) as the electron donor (Thauer 1998). The methyl group of CH_3-H_4MPT is transferred into HS-CoM to form CH_3-S-CoM, a typical central intermediate shared by all 3 methanogenic pathways. Finally, CH_3-S-CoM is converted to methane using HS-CoB as the electron donor (Wongnate *et al.* 2016). In acetoclastic methanogenesis, methanogens convert acetate to acetyl-coenzyme A (CH_3-CO-S-CoA) using adenosine triphosphate (ATP) and HS-CoA. Acetyl-coenzyme A is split to form enzyme-bound methyl and carbonyl groups (CO-S-CoA). The methyl group is then transferred to H_4MPT, which follows the last 2 steps of the hydrogenotrophic pathway. Following the methyl-coenzyme M reductase-catalyzed reaction, the carbonyl group is oxidized to CO_2 with Fd_{ox} as the electron acceptor. Methylotrophic methanogens utilize methylated compounds including trimethylamine, dimethyl sulfate, and methanol (Enzmann *et al.* 2018). The methyl group from methyl-containing compounds (CH_3-R) is transferred to a cognate corrinoid protein and then to CoM-SH in the methylotrophic pathway (Lyu *et al.* 2018).

2.2. Compounds that potentially mitigate rumen methanogenesis

The conversion of methyl-CoM to methane is the final step in all 3 methanogenic pathways. This step is catalyzed by methyl-coenzyme M reductase (MCR), an enzyme found in all methanogens in which nickel is bound to cofactor F_{430},

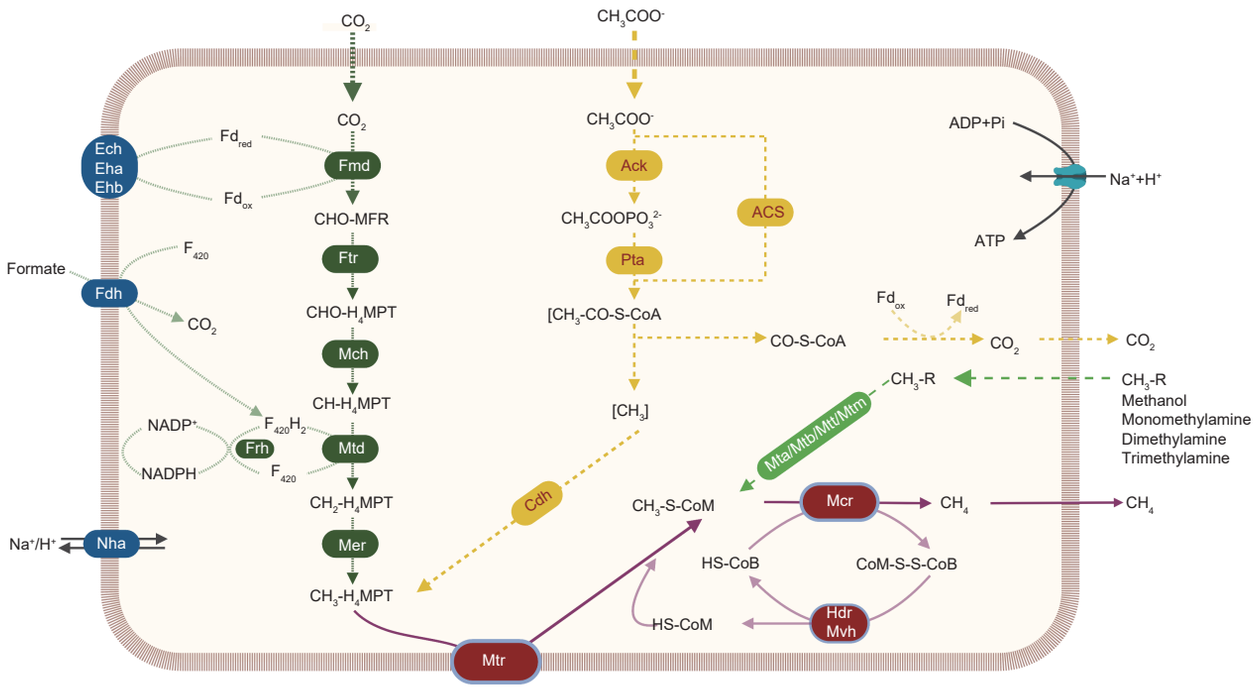

Fig. 1 Three pathways of methanogenesis. Hydrogenotrophic methanogenesis is the most widespread pathway which uses CO_2 and H_2 to produce methane. Acetoclastic methanogenesis splits acetate into methane and CO_2. Methylotrophic methanogenesis is a group of methanogens that can use reduced one-carbon compounds, such as methanol, monomethylamine, dimethylamine, and trimethylamine. Frh, F_{420}-reducing hydrogenase; Nha, Na^+/H^+ antiporter; Fmd, formylmethanofuran dehydrogenase; Ftr, formylmethanofuran-tetrahydromethanopterin N-formyltransferase; Mch, methenyltetrahydromethanopterin cyclohydrolase; Mtd, 5,10-methylenetetrahydromethanopterin dehydrogenase; Mer, 5,10-methylenetetrahydromethanopterin reductase; Ack, acetokinase; Pta, phosphotransacetylase; ACS, acetyl coenzyme A synthetase; Cdh, CO dehydrogenase/acetyl-CoA synthase; Mta, methyltransferase; Mtb, methanol cobalamin methyltransferase; Mtt, trimethylamine methyltransferase; Mtm, monomethylamine methyltransferase; Ech/Eha/Ehb, energy-converting hydrogenase; Fdh, formate dehydrogenase; Mtr, coenzyme M methyltransferase; Mcr, methyl coenzyme M reductase; Hdr, heterodisulfide reductase; Mvh, F_{420}-non-reducing hydrogenase.

a tetrapyrrole derivative (Ermler *et al.* 1997; Wongnate *et al.* 2016). The structural analog of methyl-coenzyme M that binds to the active site of methyl-coenzyme M is one of the most promising enteric methane inhibitors (Fig. 2). Various halogenated, sulfonated compounds, such as 2-bromoethanesulfonate (BES) (Robert *et al.* 1978; Gräwert *et al.* 2014), 2-chloroethanesulfonate (CES) (Ungerfeld *et al.* 2004), and 3-bromopropanesulfonate (BPS) (Ungerfeld *et al.* 2004), are structural analogues of CoM, and they can competitively and specifically inhibit MCR activity, decreasing methane production at relatively low concentrations (Ellermann *et al.* 1989; Gräwert *et al.* 2014; Patra *et al.* 2017). In addition, 3-nitrooxypropanol (3-NOP) has a similar molecular structure to methyl-coenzyme M, which targets the nickel enzyme of MCR (Duin *et al.* 2016; Pitta D W *et al.* 2022). It has been reported that 3-NOP reduces methane emissions in ruminants by over 30% on average (Melgar *et al.* 2021).

Coenzyme M methyltransferase (MTR) is another essential enzyme in methanogenesis and halogenated methane analogues (HMAs) could potentially inhibit its activity by interfering with the cobamide-dependent methyl transferase step of methanogenesis (Fig. 2) (Bauchop 1967; Wood *et al.* 1968; Tomkins *et al.* 2009). The HMAs including bromoform and dibromochloromethane could inhibit methane production even at low concentrations (Machado *et al.* 2016b). Bromoform, a naturally occurring halogenated volatile organic compound derived from chemical and biological sources such as marine phytoplankton and macroalgae, is believed to be the primary active ingredient in seaweeds responsible for methane mitigation (Machado *et al.* 2016b).

Besides bromoform, seaweeds contain abundant bioactive compounds, such as carbohydrates and phlorotannins, alkaloids and peptides. These bioactive compounds may also play a role in reducing enteric methane (Abbott *et al.* 2020). For example, phlorotannins can act as H_2 sinks and reduce the H_2 concentration in the rumen (Kim *et al.* 2022). It is hypothesized that the presence of these metabolites in the rumen reduces the availability of H_2, thereby reducing the substrate available for methanogenesis.

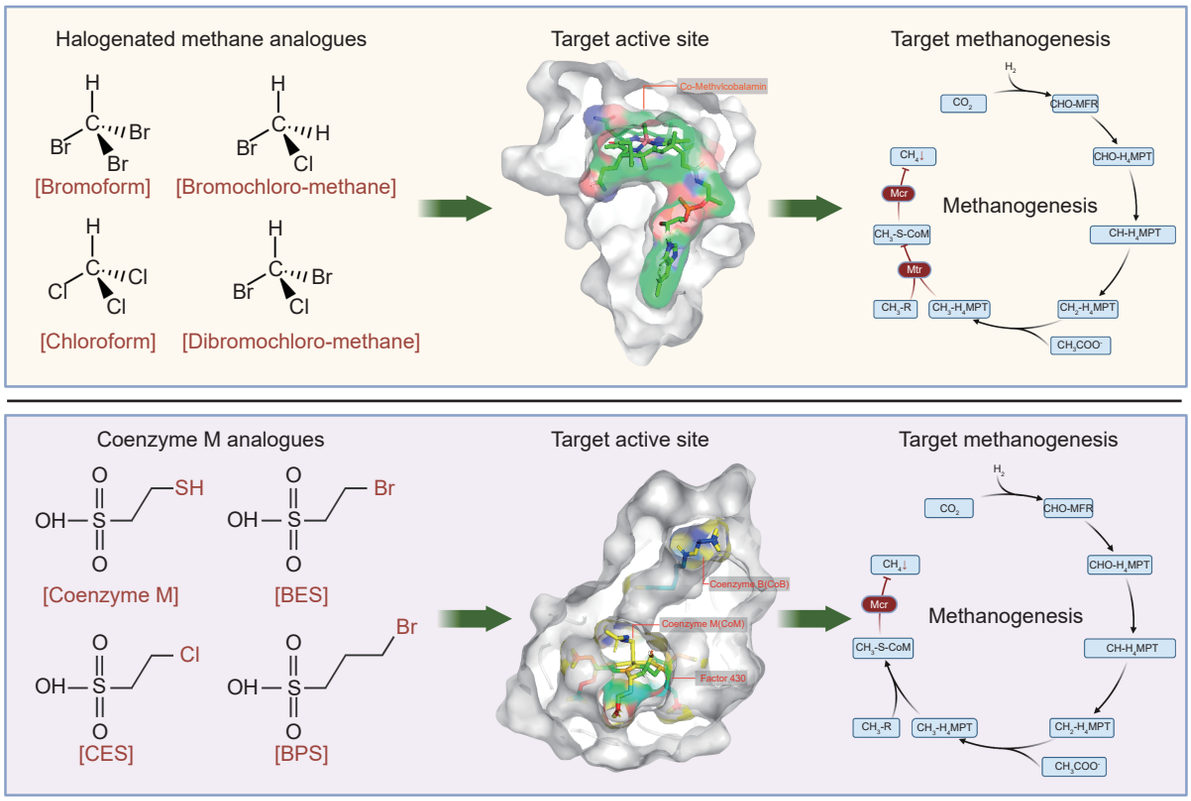

Fig. 2 The chemicals of the halogenated methane analogue and coenzyme M analogue target key enzymes in the methanogenesis pathway. BES, 2-bromoethanesulfonate; CES, 2-chloroethanesulfonate; BPS, 3-bromopropanesulfonate.

3. Seaweeds affect the methane mitigation of ruminant animals

There has been growing interest in feeding seaweeds to ruminants in recent years because of their significant effects on methane mitigation. The seaweeds used in previous studies mainly belong to red (Rhodophyta), brown (Phaeophyceae), and green algae (Chlorophyta).

3.1. Methane mitigation potential of red algae

Red algae, with more than 6,100 species, are essential coastal ecosystem components and economically significant as natural foods and a source of gelling agents (Guiry 2012). Their color results from the presence of pigments, phycoerythrin, and phycocyanin. Red algae are the most studied seaweeds in ruminants, particularly *Asparagopsis taxiformis*, and exhibit strong methane mitigation effect both *in vitro* (66–99%) and *in vivo* (30–99%) (Table 1 and Fig. 3-A). Numerous studies demonstrated that even low levels (2–10% dietary organic matter, OM) of *A. taxiformis* supplementation could exhibit strong methane mitigation capabilities (Table 1). Mihaila *et al.* (2022) compared the effect of multiple red algae on methane mitigation *in vitro*, and found that adding *Asparagopsis armata* at 2% OM completely inhibited methane production and significantly increased hydrogen production. In addition, *Bonnemaisonia hamifera* demonstrated the most significant reduction in methane (98.9%) and an increase in the production of hydrogen with the addition of 10% OM, while *Euptilota formisissima*, *Gracilaria vermiculophylla*, *Laurencia filiformis*, *Hypnea pannosa*, and *Plocamium cirrhosum* reduced methane production by 40 to 50% (Mihaila *et al.* 2022). Several *in vivo* studies also showed that *A. taxiformis* significantly mitigates methane emissions from ruminants (Table 1). For example, Li *et al.* (2018) observed an 80% reduction in methane production when *A. taxiformis* was added to the diets of sheep. Dietary supplementation with *A. taxiformis* (0.2 to 0.5 OM basis) decreased enteric methane production in beef steers and heifers by 67 to 98% (Kinley and Fredeen 2015; Roque *et al.* 2021; Almeida *et al.* 2022). Methane emissions from dairy cows decreased by 30–35% when *A. taxiformis* was supplemented (Stefenoni *et al.* 2021; Angellotti *et al.* 2022; Wasson *et al.* 2022a). In general, the results of *in vivo* experiments were consistent with those obtained *in vitro*, although factors such as dosage, experimental

Table 1 Summary of studies using red algae (Rhodophyta) for methane reduction, including methods, inclusion level, results, and references[1)]

Seaweed	*In vivo*/ *In vitro*	Animal	Dose	CH_4 (Δ%)	TVFA (Δ%)	C2 (Δ%)	C3 (Δ%)	C2/C3 (Δ%)	DMI (Δ%)	References
Asparagopsis taxiform	*In vitro*	–	2% OM	99 ↓	11.5 ↓	37 ↓	68 ↑	63 ↓	–	Machado *et al.* (2016a)
A. taxiform	*In vitro*	–	5% DM	74 ↓	–	–	–	–	–	Brooke *et al.* (2020)
A. taxiform	*In vitro*	–	2% DM	66 ↓	7 ↓	8 ↓	17 ↓	10 ↑	–	Terry *et al.* (2022)
A. taxiform	*In vitro*	–	9 mg	99 ↓	–	–	–	–	–	Alvarez *et al.* (2022)
A. taxiform	*In vitro*	–	2% DM	81 ↓	–	–	–	–	–	Gunter *et al.* (2022)
A. armata	*In vitro*	–	2% OM	100 ↓	22 ↓	23 ↓	47 ↑	–	–	Mihaila *et al.* (2022)
Bonnemaisonia hamifera	*In vitro*	–	10% OM	98.9 ↓	25 ↓	21 ↓	53 ↑	–	–	Mihaila *et al.* (2022)
Devaleraea mollis	*In vitro*	–	2% DM	23 ↓	–	–	–	–	–	Gunter *et al.* (2022)
Euptilota formisissima	*In vitro*	–	10% OM	51 ↓	13 ↓	10 ↓	27 ↑	–	–	Mihaila *et al.* (2022)
Gracilaria vermiculophylla	*In vitro*	–	25% DM	38 ↓	5 ↓	3 ↑	5 ↑	11 ↑	–	Maia *et al.* (2016)
Laurencia filiformis	*In vitro*	–	0.2 g OM	40 ↓	12 ↓	3 ↑	0.6 ↓	1.6 ↑	–	Machado *et al.* (2014)
Hypnea pannosa	*In vitro*	–	0.2 g OM	43 ↓	ns	4 ↑	6 ↓	9 ↑	–	Machado *et al.* (2014)
Plocamium cirrhosum	*In vitro*	–	0.2 g OM	40 ↓	12 ↓	8 ↓	21 ↑	–	–	Mihaila *et al.* (2022)
A. taxiform	*In vivo*	Sheep	3% OM	80 ↓	29 ↓	16 ↓	54 ↑	45 ↓	–	Li *et al.* (2018)
A. taxiform	*In vivo*	Beef steer	0.5% OM	80 ↓	–	–	–	–	14.2 ↓	Roque *et al.* (2021)
A. taxiform	*In vivo*	Beef steer	0.2% OM	98 ↓	–	–	–	–	10.8 ↓	Kinley *et al.* (2020)
A. taxiform	*In vivo*	Beef cattle	0.4% OM	67 ↓	–	–	–	–	ns	Almeida *et al.* (2022)
A. taxiform	*In vivo*	Dairy cow	0.5% DM	34 ↓	11 ↓	6.6 ↓	8.5 ↑	12 ↓	7 ↓	Stefenoni *et al.* (2021)
A. taxiform	*In vivo*	Dairy cow	0.3% OM	35 ↓	–	–	–	—	7.6 ↓	Angellotti *et al.* (2022)
A. taxiform	*In vivo*	Red cow	0.5% OM	60 ↓	–	11 ↓	23 ↑	–	13.8 ↓	Krizsan *et al.* (2023)
A. taxiform	*In vivo*	Goats	0.5% DM	30–40 ↓	–	–	–	–	–	Romero *et al.* (2022)
A. taxiform	*In vivo*	Dairy cows	0.75% OM	30 ↓	–	–	–	–	22 ↓	Wasson *et al.* (2022a)
A. taxiform	*In vivo*	Dairy cows	1% OM	67.2 ↓	–	–	–	–	38 ↓	Roque *et al.* (2019)
Chondrus crispus	*In vivo*	Dairy cows	126–136 g d^{-1}	ns	–	–	–	–	ns	Muizelaar *et al.* (2023)
C. crispus	*In vivo*	Dairy cows	6% DM	13.9 ↓	–	–	–	–	ns	Reyes *et al.* (2023)

[1)] CH_4, methane; TVFA, total volatile fatty acids; C2, acetate; C3, propionate; C2/C3, the ratio of acetate to propionate; DMI, dry matter intake; OM, organic matter; DM, dry matter. Δ%, treatment response over control; –, not quantified; ↑ and ↓ represent the percentage increase and decrease, respectively, of the value in the treatment group supplemented with seaweed compared with the control group. ns, not statistically significant.

period, and animal species may affect its efficacy.

3.2. Methane mitigation potential of brown and green algae

Brown algae have high concentrations of carotenoids, such as yellow-brown fucoxanthin. There are approximately 1,800 species of brown algae worldwide, and most are known to exist in marine water (Makkar *et al.* 2016). Most of the studies using brown algae were conducted *in vitro* (Table 2 and Fig. 3). Overall, the inhibitory effect of most of the brown algae on methane emission is weaker than that of red algae, which decreased methane production by less than 50%, except for *Cystoseira trinodis* (Dubois *et al.* 2013) and *Dictyota bartayresii* (Machado *et al.* 2014) that decreased methane production by 80 and 92%, respectively.

As for green algae, there are 2,200 currently known species, and their color results from the presence of chlorophyll (Makkar *et al.* 2016). Similar to brown algae, the methane inhibitory effect of green algae was basically evaluated *in vitro* (Table 3). Specifically, *Cladophora patentiramea* was the most effective species that could reduce methane production by 66%. *Caulera taxifolia*, *Cladophora coelothrix*, *Chaetomorpha linum*, *Ulva* sp. and *Ulva ohnoi* could reduce methane production by 27 to 50% after 72 h of incubation (Machado *et al.* 2014).

The DNA-barcoding using the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene has proven its usefulness in studying seaweed phylogenetic diversity (Alshehri *et al.* 2019). The NCBI nucleotide submitted sequences of the *rbcL* gene of 23 seaweed species have been used to generate a phylogenetic tree (Fig. 3-B), which contains 3 clades, Chlorophyta, Ochrophyta, and Rhodophyta. We found that the close evolutionary relationships of seaweeds exhibited similar methane mitigation potential in only a few clades but not in other clades. These results indicated that seaweeds of similar species do not all exhibit the same methane inhibition potential, which may be related to the types and contents of methane-inhibiting compounds contained in the seaweeds. The bromoform content of seaweed was

Table 2 Summary of studies using brown algae (Ochrophyta) for methane reduction, including methods, inclusion level, results, and references[1)]

Seaweed	*In vivo*/ *In vitro*	Animal	Dose	CH_4 (Δ%)	TVFA (Δ%)	C2 (Δ%)	C3 (Δ%)	C2/C3 (Δ%)	References
Ascophyllum nodosum	*In vitro*	–	5% DM	8.9 ↓	16 ↓	17 ↓	6 ↓	14 ↓	Künzel *et al.* (2022)
Fucus vesiculosus	*In vitro*	–	5% DM	3.6 ↓	19 ↓	22 ↓	3 ↓	21 ↓	Künzel *et al.* (2022)
Colpomenia sinuosa	*In vitro*	–	0.2 g OM	49 ↓	17 ↓	2 ↓	14 ↑	15 ↓	Machado *et al.* (2014)
Cystoseira trinodis	*In vitro*	–	160 mg OM	80 ↓	–	–	–	–	Dubois *et al.* (2013)
C. trinodis	*In vitro*	–	0.2 g OM	45 ↓	29 ↓	6.7 ↓	25 ↑	21 ↓	Machado *et al.* (2014)
Dictyota bartayresii	*In vitro*	–	0.2 g OM	92 ↓	39 ↓	4.8 ↓	41 ↑	32 ↓	Machado *et al.* (2014)
Ecklonia radiata	*In vitro*	–	10% OM	21 ↓	7 ↓	2.4 ↓	15.7 ↑	–	Mihaila *et al.* (2022)
Hormophysa triquetra	*In vitro*	–	0.2 g OM	44 ↓	24 ↓	1.5 ↑	10 ↑	7 ↓	Machado *et al.* (2014)
Laminaria ochroleuca	*In vitro*	–	25% DM	14 ↑	10 ↓	2.4 ↑	5.8 ↓	8.4 ↑	Maia *et al.* (2016)
Saccharina latissima	*In vitro*	–	-	80 ↓	–	–	–	–	Olijhoek *et al.* (2022)
Sargassum flavicans	*In vitro*	–	0.2 g OM	34 ↓	ns	3.8 ↑	4.4 ↓	8.6 ↑	Machado *et al.* (2014)
Padina australis	*In vitro*	–	0.2 g OM	50 ↓	12 ↓	2 ↑	1.8 ↑	0.8 ↓	Machado *et al.* (2014)
Ascophyllum nodosum	*In vivo*	Jersey cows	113 g d^{-1}	11.5 ↓	–	–	–	–	Antaya *et al.* (2019)
Saccharina latissima	*In vivo*	Dairy cows	126–136 g d^{-1}	ns	–	–	–	–	Muizelaar *et al.* (2023)
Fucus serratus	*In vivo*	Dairy cows	126–136 g d^{-1}	ns	–	–	–	–	Muizelaar *et al.* (2023)

[1)] CH_4, methane; TVFA, total volatile fatty acids; C2, acetate; C3, propionate; C2/C3, the ratio of acetate to propionate; DMI, dry matter intake; Δ%: treatment response over control; ↑ and ↓ represent the percentage increase and decrease, respectively, of the value in the treatment group supplemented with seaweed compared with the control group. –, not quantified; ns, not statistically significant.

determined to be the key inhibitory component for enteric methanogenesis. Seaweeds with differing levels of bromoform will have distinct methane-inhibiting properties. A paper published by Thapa *et al.* (2020), found the genetic mechanism of biosynthesis of bromoform production in various seaweed. The key genes were *mbb1* and *mbb4* (vanadium-dependant haloperoxidases, VHPOs), with the highest bromoform production levels. The gene encoding reactive oxygen species-producing enzyme (*mbb2*) will be clustered with genes encoding bromoform-producing (Zhu *et al.* 2021). These results suggest that differences in bromoform-encoding genes contribute to disparities in the bromoform content of algae. However, knowledge of the bromoform biosynthesis routes, gene regulation, and catalytic activities remains to be resolved. More research is needed to determine the reasons for this discrepancy.

4. Challenges of using seaweeds in ruminant diet

4.1. Detrimental effect on intake, lactation performance, and rumen fermentation

In vivo studies showed decreased DMI by 10.8 to 14.2% in beef cattle (Kinley *et al.* 2020; Roque *et al.* 2021; Almeida *et al.* 2022) and 7 to 38% in dairy cows (Roque *et al.* 2019; Stefenoni *et al.* 2021; Angellotti *et al.* 2022; Wasson *et al.* 2022a) fed *A. taxiformis* and *A.armata*, respectively (Table 1). Energy-corrected milk (ECM) was decreased by 6 to 14% when *A. taxiformis* was fed to lactating dairy cows (Stefenoni *et al.* 2021; Wasson *et al.* 2022a; Krizsan *et al.* 2023). However, Angellotti *et al.* (2022) found no difference in milk yield when *A. taxiformis* was supplemented at 0.3% OM of the diet, suggesting that dosage may not impact on milk production. In addition to milk production, Stefenoni *et al.* (2021) reported that contents in milk fat, milk true protein, and solid not fat percentages were not affected by the high inclusion level of *A. taxiformis*. However, when *A. taxiformis* was fed to dairy or beef cattle, increased concentrations of iodine and bromide in milk (Stefenoni *et al.* 2021) or meat (Roque *et al.* 2021) were observed. Conversely, the concentration of bromoform did not show a significant difference, which was consistent with the findings of Krizsan *et al.* (2023). Seaweeds are known to be accumulators of halogens, and previous studies have identified high levels of iodine in *A. taxiformis* (Mairh *et al.* 1989; Nunes *et al.* 2018). Consequently, high-dose feeding of this seaweed may pose a risk of elevated iodine residues in animal products. Although iodine consumption is necessary for humans, overconsumption is a health concern (Leung and Braverman 2014).

Methanogenesis prevents the accumulation of hydrogen in the rumen, which inhibits the activity of dehydrogenase (Martin *et al.* 2010). However, when methanogenesis was inhibited by seaweeds, methanogens were unable to consume the H_2, leading to a redirection of metabolic hydrogen toward propionate (H_2 sink) (Ungerfeld 2015). By summarizing previous studies in Table 1, we found a negative relationship between the methane reduction rate and the total VFA

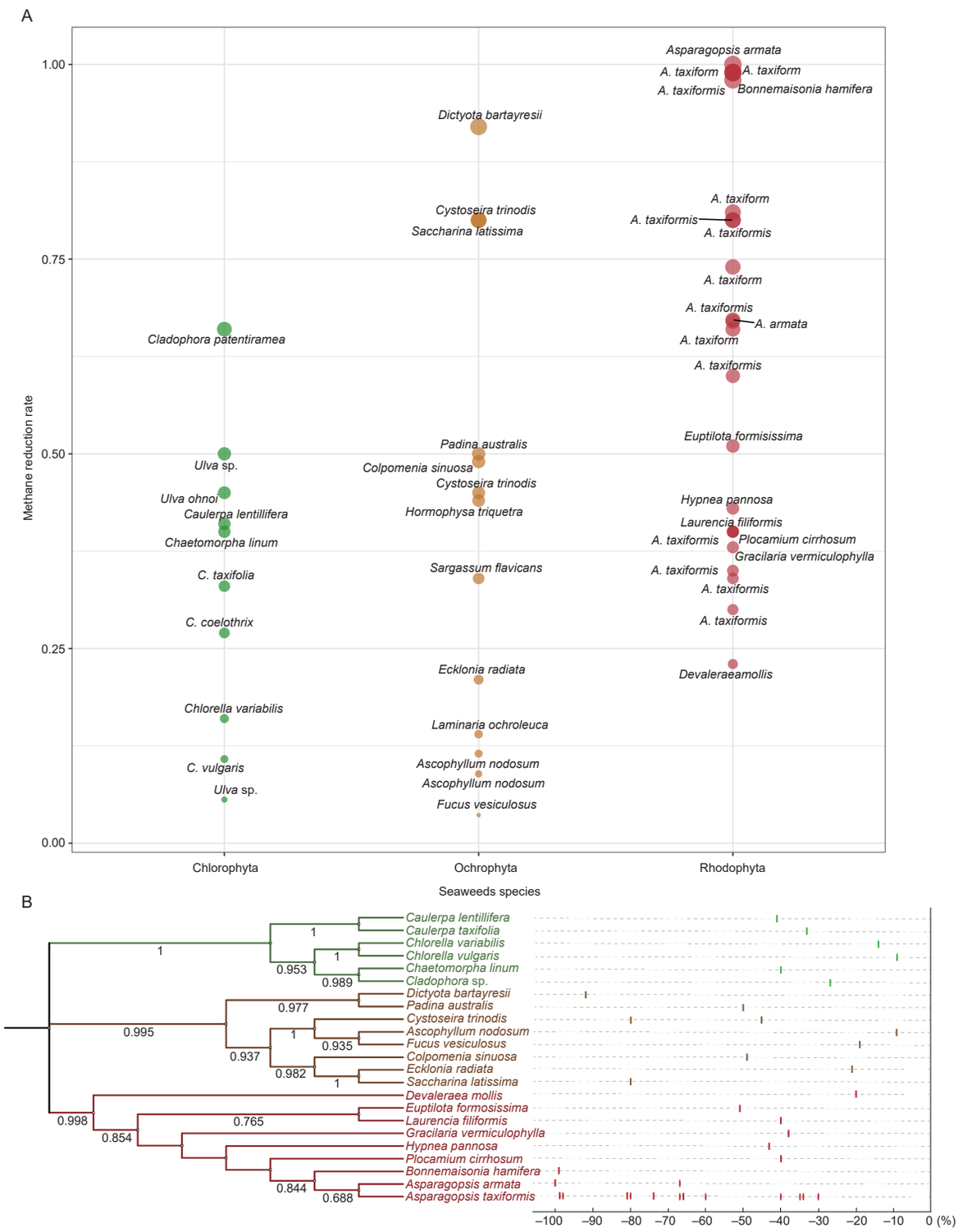

Fig. 3 The methane mitigation potential and evolutionary relationships of different seaweeds. A, summary of methane reduction rates of different seaweed species. According to seaweed species, this figure was divided into three sections (Chlorophyta, Ochrophyta and Rhodophyta). The size of the bubble represents the methane mitigation ability of different seaweeds. B, the phylogenetic tree was inferred by using the maximum likelihood method as can the General Time Reversible model. The tree with the highest log likelihood (–15,156.92) is shown. This analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 2,181 positions in the final dataset. Evolutionary analysis was conducted in MEGA11. On the right side of the figure, the methane reduction abilities of various seaweed species are presented to evaluate the relationship between the phylogenetic proximity of the seaweeds and their potential for methane mitigation.

reduction rate (Fig. 4-A), and acetate reduction rate (Fig. 4-B). Conversely, the methane reduction rate was positively correlated with the propionate increase rate (Fig. 4-C), suggesting that reducing methane emissions by seaweeds may redirect metabolic hydrogen in the rumen to propionate production.

4.2. Potential bromoform residue in products

According to research by the Environmental Protection Agency (EPA) (EP 2015), nervous system injury due to high levels of bromoform inhalation or ingestion in humans and animals leads to brain malfunction and liver and

Table 3 Summary of studies using green algae (Chlorophyta) for methane reduction, including methods, inclusion level, results, and references[1)]

Seaweed	*In vivo*/ *In vitro*	Dose	CH_4 (Δ%)	TVFA (Δ%)	C2 (Δ%)	C3 (Δ%)	C2/C3 (Δ%)	References
Caulerpa lentillifera	*In vitro*	160 mg OM	41 ↓	–	–	–	–	Dubois *et al.* (2013)
Caulerpa taxifolia	*In vitro*	0.2 g OM	33 ↓	17 ↑	4.8 ↑	8.9 ↓	17 ↑	Machado *et al.* (2014)
Cladophora coelothrix	*In vitro*	0.2 g OM	27 ↓	1 ↓	0.3 ↓	4.9 ↑	6.3 ↓	Machado *et al.* (2014)
Cladophora patentiramea	*In vitro*	0.2 g OM	66 ↓	9 ↓	0.2 ↓	4.9 ↑	6.3 ↓	Machado *et al.* (2014)
Chaetomorpha linum	*In vitro*	0.2 g OM	40 ↓	ns	2.7 ↓	13 ↑	14 ↓	Machado *et al.* (2014)
Chlorella vulgaris	*In vitro*	25% DM	10.8 ↓	7 ↓	21 ↓	30 ↑	–	Sucu (2020)
Chlorella variabilis	*In vitro*	25% DM	16 ↓	13 ↓	25 ↓	17 ↑	–	Sucu (2020)
Uva sp.	*In vitro*	0.2 g OM	50 ↓	ns	0.8 ↓	4.5 ↑	5.5 ↓	Machado *et al.* (2014)
Ulva sp.	*In vitro*	10% OM	5.6 ↓	6 ↓	0.1 ↑	0.5 ↓	–	Mihaila *et al.* (2022)
Ulva ohnoi	*In vitro*	0.2 g OM	45 ↓	6 ↓	2.9 ↑	4.2 ↓	6.3 ↑	Machado *et al.* (2014)

[1)] CH_4, methane; TVFA, total volatile fatty acids; C2, acetate; C3, propionate; C2/C3, the ratio of acetate to propionate; DMI, dry matter intake; Δ%, treatment response over control; ↑ and ↓ represent the percentage increase and decrease, respectively, of the value in the treatment group supplemented with seaweed compared with the control group. –, not quantified; ns, not statistically significant.

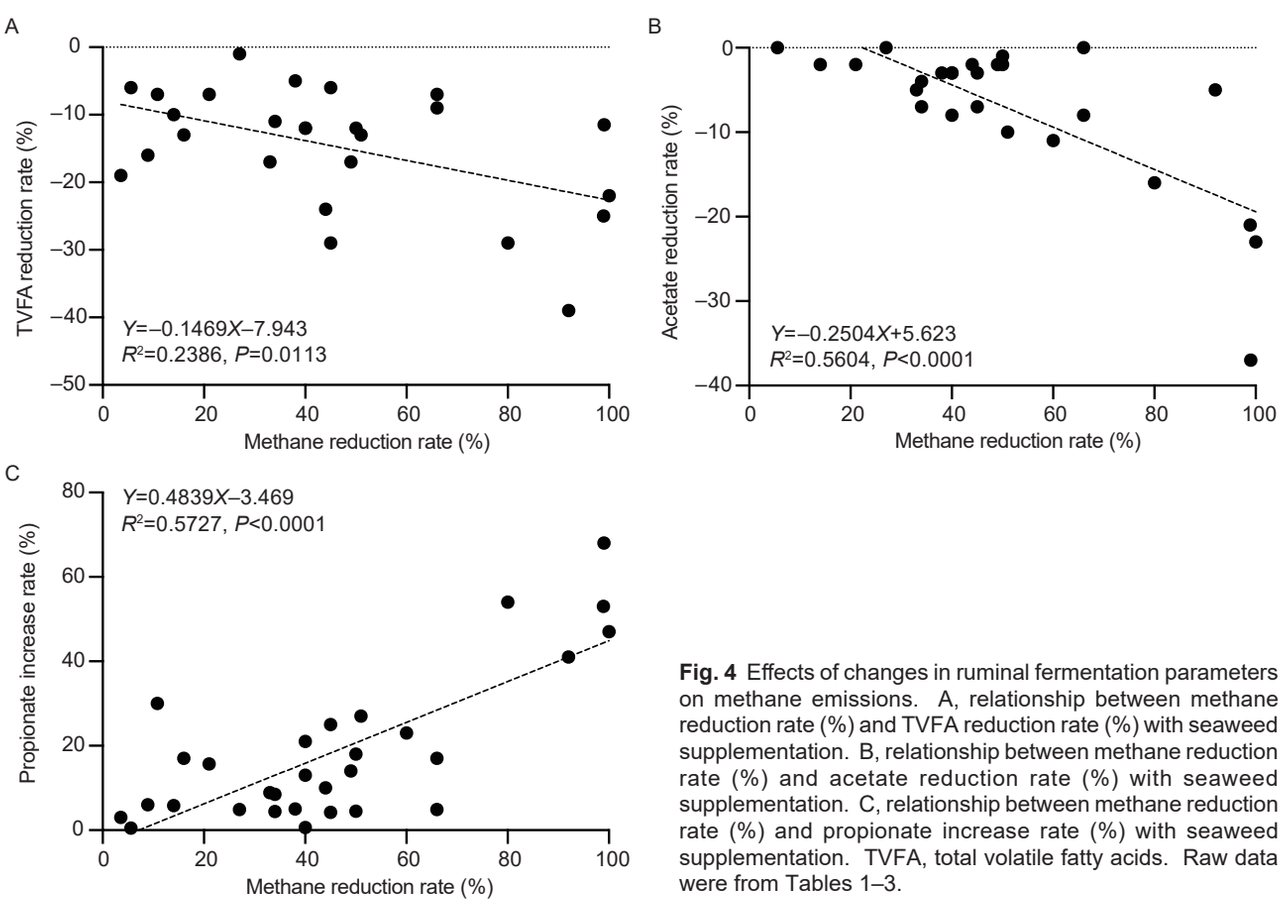

Fig. 4 Effects of changes in ruminal fermentation parameters on methane emissions. A, relationship between methane reduction rate (%) and TVFA reduction rate (%) with seaweed supplementation. B, relationship between methane reduction rate (%) and acetate reduction rate (%) with seaweed supplementation. C, relationship between methane reduction rate (%) and propionate increase rate (%) with seaweed supplementation. TVFA, total volatile fatty acids. Raw data were from Tables 1–3.

kidney damage. Therefore, the EPA has set a limit of 80 μg L^{-1} for bromoform consumption in drinking water. The bromoform concentrations in milk produced by dairy cows fed low (0.5%) and high (1%) *A. taxiformis* levels were 0.11 to 0.15 μg L^{-1}, which is over 500 times lower than the maximum standard, according to Roque *et al.* (2019). Similarly, Kinley *et al.* (2020) found no changes in the meat quality of beef cattle supplemented with *A. taxiformis* at a concentration of 0.00, 0.05, 0.10, and 0.20% of feed OM. Romero *et al.* (2022) showed that bromoform in *A. taxiformis* was degraded quickly during incubation, with 70% degradation in the first 30 min and nearly 90% after 3 h. After 12 h of incubation, the bromoform was not detectable. However, Muizelaar *et al.* (2021) found that although bromoform did not accumulate in animal tissue, it was excreted in urine and milk and caused abnormalities of rumen wall papillae in lactating dairy cows fed *A. taxiformis*. According to Li *et al.* (2018), no detectable levels (<0.05 mg kg^{-1}) of bromoform or dibromochloromethane were identified in any muscle or fat sample of sheep. However, five of ten sheep offered *A. taxiformis* had the mucosal lining of the rumen floor examined grossly after death, showing a variable-sized area of nodular proliferation, white-tan discoloration, and blunted ruminal papillae. The effects of seaweeds on the safety of ruminant products need further evaluation. Most countries or regions are currently cautious about using seaweeds as feed additives to reduce enteric methane emissions from ruminants. So far, only the California Department of Food and Agriculture has approved one of the most effective seaweeds for cattle as an inhibitor. Moreover, a recent report from Sweden's EPA proposes immediate government action to encourage the adoption of these feed additives through incentives.

4.3. Storage of bromoform in seaweeds

The efficacy of seaweeds in mitigating enteric methane heavily depends on the processing and storage of bioactive compounds within their gland cell walls (Paul *et al.* 2006). Vucko *et al.* (2017) utilized a factorial design to determine the effects of rinsing (unrinsed, dip rinsed, and submerged), freezing (frozen and not frozen), and drying (freeze-dried, kiln-dried, and dehydrated) of *A. taxiformis* on methane production *in vitro* and concentration of bromoform in the biomass. The results indicated that freeze-drying was the most effective processing method for maintaining bromoform concentration and anti-methanogenic activity. Stefenoni *et al.* (2021) evaluated the effect of storage time, temperature, and light exposure on the concentration of bromoform in *A. taxiformis*, and found that after 4 mon of storage, the concentration of bromoform decreased by 75% when stored in the dark and by 84% when stored under luminescent light, while storage temperature did not affect the concentration of bromoform. Magnusson *et al.* (2020) demonstrated that immersion of *Asparagopsis* genus biomass in oil could maintain high concentrations of bromoform for at least 12 wk, even when stored at room temperature (25°C), while water immersion resulted in 40% less bromoform extraction and at least 47% loss after 12 wk of storage. Tan *et al.* (2022) investigated the relatively long-term storage of Freeze-Dried-Asp and Asp-Oil under different storage conditions (different temperatures, presence of fluorescent light, and exposure to open air). Asp-Oil was a more desirable method of preserving bromoform compared to Freeze-Dried-Asp. Freeze-Dried-Asp was only stable at –20°C, whereas Asp-Oil had a higher or no change in bromoform concentrations after 24-wk storage at 40, 25, 4 and –20°C in dark conditions (Tan *et al.* 2022). We have concluded that the storage and stability of bromoform in seaweed are influenced by factors such as cultivation, processing, and transportation. This poses challenges and shortcomings in establishing a commercial supply chain for seaweed utilization. In addition, more studies are needed to explore the influence of factors such as harvest time and regional differences on bromoform concentration in seaweeds.

4.4. Potential environmental impact of bromoform in seaweeds

In addition to potential damage to animal health, bromoform can catalytically destroy ozone in the troposphere and stratosphere via chemical reactions (WMO 2018). While large-scale *A. taxiformis* farming is one of the most promising options to produce anti-methanogenic feed additives, how *A. taxiformis* farming may affect the atmospheric environment needs to be investigated and elucidated. A recent study by Jia *et al.* (2022) evaluated various cultivation scenarios to determine the potential risks of bromoform released from *A. taxiformis* farming near Australia on the stratospheric ozone layer. The study showed that under typical operating conditions, the planned operation of *Asparagopsis* seaweed cultivation farms at Triabunna, Yamba, Geraldton, and Darwin, with an annual yield of 34,674 mg dry weight, does not have an impact on the ozone layer. In addition, according to Glasson *et al.* (2022), bromoform from cultivated *A. taxiformis* biomass had little impact on the overall natural and anthropogenic atmospheric loading. More studies are needed to verify if large-scale artificial cultivation of

seaweed could potentially cause detrimental effects on ozone.

4.5. Costs of seaweed farming and processing

Asparagopsis taxiformis farming was still in its infancy, with only some startup companies including VOLTA, Greener Grazing, Sea Forest, Symbrosia, and Blu Ocean Barns. Commercial-scale production of seaweeds is still not available, and dried *A. taxiformis* is valued between US$100 to 146 kg^{-1} (Jayvee *et al.* 2021). The cost of wild *A. taxiformis* harvested in Australia was even higher, around US$200 kg^{-1} (Cotter *et al.* 2015). Even if the price was reduced to less than US$5 kg^{-1}, adding 20 kg of dried *A. taxiformis* would cost an additional US$100 per tonne of diet. Therefore, the cost of meat and milk products from cows consuming such a diet would increase roughly 20% (Trevor Whittington 2022). As the main functional component, bromoform could be easily volatilized during preservation and processing. To avoid volatilization, vegetable oil was used (Magnusson *et al.* 2020) which would potentially increase the cost. Another important step, freeze-drying, would also increase the processing cost of seaweeds.

5. Recommendations for future research

5.1. Exploration of more seaweed resources

Although there are more than 12,000 species of seaweeds in the world, only 221 are considered commercially valuable, and even fewer species are cultivated intensively (FAO and WHO 2021). *Asparagopsis taxiformis* species appear to be the best candidate as a feed additive due to their effectiveness at low inclusion rates (Wasson *et al.* 2022b). Other species with low bromoform concentrations, such as *Bonnemaisonia hamifera* (Mihaila *et al.* 2022), *Dictyota bartayresii* (Machado *et al.* 2014), and *Cystoseira trinodis* (Dubois *et al.* 2013) could also reduce methane emissions at a comparable level of inclusion. This research was mainly conducted in coastal countries such as Australia, Canada, Norway, and Denmark. The United States, the United Kingdom, the Netherlands, and New Zealand have also begun exploring the potential of various seaweeds to reduce methane emissions in recent years (Fig. 5). However, almost all of the seaweeds consumed worldwide are produced in Asia, with 57 percent being produced in China alone, followed by Indonesia, the Republic of Korea, and the Philippines (Nin-Pratt *et al.*

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Fig. 5 The country's distribution of seaweed is used as a feed additive to mitigate methane emissions before 2024. Countries highlighted in yellow on the global map have published research on seaweed as a methane inhibitor.

2022). There are still many unexploited seaweed resources in Asian countries. Additional research is still required to evaluate the potential of novel types of seaweeds for methane mitigation in ruminants.

5.2. Identification of bioactive compounds other than bromoform in seaweeds

Halogen compounds, including bromoform, inhibit the ability of methanogen enzymes to produce methane by reacting with vitamin B_{12} (Bauchop 1967). However, other bioactive compounds in seaweeds, including protein hydrolysates, polysaccharides, phlorotannins, and alkaloids (Holdt and Kraan 2011; Hafting *et al.* 2015) may also play a role in reducing methane emissions. Phlorotannins derivatives (phlorofucofuroeckol-A, dieckol, and bieckol), a class of polyphenolic compounds found in brown algae, may reduce fiber digestibility and alter rumen fermentation, resulting in decreased H_2 production (Wang *et al.* 2008). According to Martinez-Fernandez *et al.* (2017), phloroglucinol degradation in the rumen promotes the capture of excess H_2 generated from methanogenesis inhibition. Furthermore, Kim *et al.* (2022) discovered that supplementation with phlorotannins reduced methane emissions without impairing rumen fermentation characteristics. In addition, saponin is known to be a natural detergent with membrane-degrading groups complex with sterols in protozoal cell membranes, which may reduce the population of protozoa in rumen fluid and change ruminal fermentation (Patra and Saxena 2009). Protozoa-associated methanogens are among the most active communities involved in rumen methanogenesis. Reducing protozoa could reduce methanogens, thereby reducing methane emissions (Patra *et al.* 2017). Alkaloids and fucoidans have antibacterial potential (Pérez *et al.* 2016); however, their efficacy on methane mitigation needs further exploration.

In addition to the bioactive components, rumen metabolites potentially inhibit methane emissions when seaweeds are fed to ruminants and metabolized by rumen microorganisms. Olijhoek *et al.* (2022) showed that supplementation of Nordic macroalgae *Saccharina latissimi* could reduce methane production by up to 80% *in vitro* without affecting the degradability of the substrate. Notably, halomethanes were not detected in the *S. latissimi* samples by Gas Chromatography-Mass Spectrometry (Olijhoek *et al.* 2022). In addition, the second principal component (PC2) revealed that several potential compounds, including 'Compound X', were able to reduce methane emissions by 47 and 79%, respectively, with medium dose and high dosage *in vitro* (Thorsteinsson *et al.* 2022). Future research is needed to explore more seaweed compounds that potentially mitigate methane emissions.

5.3. Combination of anti-methanogenic inhibitors

Among the most successful anti-methanogenic compounds tested were coenzyme M analogues (BES, CES, and BPS), and HMAs (chloroform, bromoform, dibromochloromethane, and bromochloromethane), which reduced methane production by up to 50% (Bauchop 1967; Tomkins *et al.* 2009; Liu *et al.* 2011; Abecia *et al.* 2012). In addition, 3-NOP acts on MCR, a key enzyme of the methanogenesis pathway, which is a terminal process used by methanogenesis to produce methane (Kebreab *et al.* 2022). These small molecular compounds can mitigate methane production by inhibiting key enzymes in methanogenesis, when combined the mitigation effect may be stronger. In the rumen, methanogenesis is considered to be the primary pathway for H_2 removal. Several alternative H_2 sinks have been identified when rumen methanogenesis is inhibited, including fumarate, nitrate, sulfate, and homoacetogenesis (Greening *et al.* 2019; Karekar *et al.* 2022). Microbial reduction of nitrates and sulfate to ammonia and sulfide provides a thermodynamically more favorable electron and H_2 sink than methanogenesis (Ungerfeld 2020). Fumarate reduction is also thermodynamically more favorable than the reduction of carbon dioxide to methane (Kohn and Boston 2000). In addition, Homoacetogens have the ability to use gaseous substrates to produce acetic acid (Karekar *et al.* 2022) under normal conditions, the homoacetogenic process can be carried out spontaneously. However, this process only occurs when H_2 partial pressure is high and the homoacetogenic bacteria are in suitable conditions to maintain the ecological environment. In general, homoacetogens are not prevalent when sufficient methanogens are present in the ecosystem. If the methanogenesis process is inhibited, homoacetogens can use excess H_2 to produce acetic acid (Karekar and Ahring 2023). The use of combinations of seaweeds, as well as additional H_2 sinks, which can mitigate methane emission without compromising rumen fermentation functions, should be developed.

6. Conclusion

Seaweeds were proven to have significant methane mitigation potential. However, there are still many limitations, including the potential detrimental effect of high bromoform content on animal health as well as the residuals in products, lack of commercially available resources that may enhance the cost, unexplored novel

species, and bioactive compounds that may also inhibit methanogenesis other than bromoform. Future studies are needed to explore the role of seaweeds play in modulating rumen methanogenesis with the help of integrated omics, which are essential to develop better strategies to maximize the efficacy of seaweeds in methane mitigation.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Ethical statements

This research did not involve animal ethics experiments.

References

Abbott D W, Aasen I M, Beauchemin K A, Grondahl F, Gruninger R, Hayes M, Huws S, Kenny D A, Krizsan S J, Kirwan S F, Lind V, Meyer U, Ramin M, Theodoridou K, von Soosten D, Walsh P J, Waters S, Xing X. 2020. Seaweed and seaweed bioactives for mitigation of enteric methane: Challenges and opportunities. *Animals*, **10**, 1–28.

Abecia L, Toral P G, Martín-García A I, Martínez G, Tomkins N W, Molina-Alcaide E, Newbold C J, Yáñez-Ruiz D R. 2012. Effect of bromochloromethane on methane emission, rumen fermentation pattern, milk yield, and fatty acid profile in lactating dairy goats. *Journal of Dairy Science*, **95**, 2027–2036.

Almeida de A K, Cowley F, Hegarty R, Kinley R D. 2022. The red seaweed *Asparagopsis taxiformis* inhibits methane emissions in feedlot beef cattle. In: *8th International Greenhouse Gas & Animal Agriculture Conference*, Orlando, USA. pp. 50.

Alshehri M A, Aziz A T, Alzahrani O, Alasmari A, Ibrahim S, Osman G, Bahattab O. 2019. DNA-barcoding and species identification for some Saudi Arabia seaweeds using *rbcL* gene. *Journal of Pure and Applied Microbiology*, **13**, 2035–2044.

Alvarez C, Andersen T O, Eikanger K S, Hamfjord I W, Niu P, Weiby K V, Årvik L, Dörsch P, Hagen L H, Pope P B, Forberg D K, Hustoft H K, Schwarm A, Kidane A. 2022. Insertion depth of oral stomach tubes in cows affects *in vitro* methane inhibition of seaweed. In: *8th International Greenhouse Gas & Animal Agriculture Conference*, Orlando, USA. pp. 51.

Angellotti M, Lindberg M, Ramin M, Krizsan S J, Danielsson R. 2022. Supplementation of *Asparagopsis taxiformis* for mitigation of enteric methane emissions in dairy cows: Feed Intake and milk yield responses. In: *Proceedings of the 11th Nordic Feed Science Conference*. Uppsala, Sweden. pp. 101.

Antaya N T, Ghelichkhan M, Pereira A B D, Soder K J, Brito A F. 2019. Production, milk iodine, and nutrient utilization in Jersey cows supplemented with the brown seaweed *Ascophyllum nodosum* (kelp meal) during the grazing season. *Journal of Dairy Science*, **102**, 8040–8058.

Arndt C, Hristov A N, Price W J, McClelland S C, Pelaez A M, Cueva S F, Oh J, Dijkstra J, Bannink A, Bayat A R, Crompton L A, Eugène M A, Enahoro D, Kebreab E, Kreuzer M, McGee M, Martin C, Newbold C J, Reynolds C K, Schwarm A, *et al.* 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5°C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences of the United States of America*, **119**, 1–10.

Bauchop T. 1967. Inhibition of rumen methanogenesis by methane analogues. *Journal of Bacteriology*, **94**, 171–175.

Berghuis B A, Yu F B, Schulz F, Blainey P C, Woyke T, Quake S R. 2019. Hydrogenotrophic methanogenesis in archaeal phylum Verstraetearchaeota reveals the shared ancestry of all methanogens. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 5037–5044.

Brooke C G, Roque B M, Shaw C, Najafi N, Gonzalez M, Pfefferlen A, De Anda V, Ginsburg D W, Harden M C, Nuzhdin S V., Salwen J K, Kebreab E, Hess M. 2020. Methane reduction potential of two pacific coast macroalgae during *in vitro* ruminant fermentation. *Frontiers in Marine Science*, **7**, 561.

Cotter J, Glass R, Black J, Madden P, Davison T. 2015. A marginal abatement cost analysis of practice options related to the NLMP program (Internet). [2023-7-1]. https://www.mla.com.au/research-and-development/reports/2015/a-marginal-abatement-cost-analysis-of-practice-options-related-to-the-nlmp-program/

Dubois B, Tomkins N W, D. Kinley R, Bai M, Seymour S, A. Paul N, de Nys R. 2013. Effect of tropical algae as additives on rumen *in vitro* gas production and fermentation characteristics. *American Journal of Plant Sciences*, **4**, 34–43.

Duin E C, Wagner T, Shima S, Prakash D, Cronin B, Yáñez-Ruiz D R, Duval S, Rümbeli R, Stemmler R T, Thauer R K, Kindermann M. 2016. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 6172–6177.

Ellermann J, Rospert S, Thauer R K, Bokranz M, Klein A, Voges M, Berkessel A. 1989. Methyl-coenzyme-M reductase from *Methanobacterium thermoautotrophicum* (strain Marburg): Purity, activity and novel inhibitors. *European Journal of Biochemistry*, **184**, 63–68.

Enzmann F, Mayer F, Rother M, Holtmann D. 2018. Methanogens: Biochemical background and biotechnological applications. *AMB Express*, **8**, 1.

Ermler U, Grabarse W, Shima S, Goubeaud M, Thauer R K. 1997. Crystal structure of methyl-coenzyme M reductase: The key enzyme of biological methane formation. *Science*, **278**, 1457–1462.

EP (European Parliament). 2015. Commission Directive 2015/1787 amendment to Council Directive 98/83/EC on the quality of water intended for human consumption.

FAO (Food and Agriculture Organization of the United Nations), WHO (World Health Organization). 2021. *Report of the Expert Meeting on Food Safety for Seaweed*: *Current Status and Future Perspectives*. Rome.

Glasson C R K, Kinley R D, de Nys R, King N, Adams S L, Packer M A, Svenson J, Eason C T, Magnusson M. 2022. Benefits and risks of including the bromoform containing seaweed Asparagopsis in feed for the reduction of methane production from ruminants. *Algal Research*, **64**, 102673.

Gräwert T, Hohmann H P, Kindermann M, Duval S, Bacher A, Fischer M. 2014. Inhibition of methyl-CoM reductase from *methanobrevibacter ruminantium* by 2-bromoethanesulfonate. *Journal of Agricultural and Food Chemistry*, **62**, 12487–12490.

Greening C, Geier R, Wang C, Woods L C, Morales S E, McDonald M J, Rushton-Green R, Morgan X C, Koike S, Leahy S C, Kelly W J, Cann I, Attwood G T, Cook G M, Mackie R I. 2019. Diverse hydrogen production and consumption pathways influence methane production in ruminants. *ISME Journal*, **13**, 2617–2632.

Guiry M D. 2012. How many species of algae are there? *Journal of Phycology*, **48**, 1057–1063.

Gunter S, Kalscheur K, Gossard D, Moffet C, Schuppenhoaur M, Graham M, Hamilton S, Bukowski M, Roemmich J, Thelen T, Gardner L. 2022. Six macroalgaes harvested along the California coast and their ability to mitigate methane emission by a forage diet. In: *8th International Greenhouse Gas & Animal Agriculture Conference*. Orlando, USA. pp. 138.

Hafting J T, Craigie J S, Stengel D B, Loureiro R R, Buschmann A H, Yarish C, Edwards M D, Critchley A T. 2015. Prospects and challenges for industrial production of seaweed bioactives. *Journal of Phycology*, **51**, 821–837.

Holdt S L, Kraan S. 2011. Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology*, **23**, 543–597.

IPCC (Intergovernmental Panel on Climate Change). 2021. Climate change 2021: The physical science basis. In: *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, the United Kingdom and New York.

Jayvee A S, Luigi P V, Kathrina J D D A. 2021. Biological and ecological studies on Asparagopsis taxiformis for culture technology development (BEAT) [Internet]. [2023-7-5]. http://vipcorals.batstate-u.edu.ph/biological-and-ecological-studies-on-asparagopsis-taxiformis-for-culture-technology-development/

Jia Y, Quack B, Kinley R D, Pisso I, Tegtmeier S. 2022. Potential environmental impact of bromoform from Asparagopsis farming in Australia. *Atmospheric Chemistry and Physics*, **22**, 7631–7646.

Karekar S, Stefanini R, Ahring B. 2022. Homo-acetogens: Their metabolism and competitive relationship with hydrogenotrophic methanogens. *Microorganisms*, **10**, 397.

Karekar S C, Ahring B K. 2023. Reducing methane production from rumen cultures by bioaugmentation with homoacetogenic bacteria. *Biocatalysis and Agricultural Biotechnology*, **47**, 102526.

Kebreab E, Bannink A, Pressman E M, Walker N, Karagiannis A, van Gastelen S, Dijkstra J. 2022. A meta-analysis of effects of 3-nitrooxypropanol on methane production, yield, and intensity in dairy cattle. *Journal of Dairy Science*, **106**, 927–936.

Kim Y R, Park K Y, Nejad J G, Yoon W J, Kim S C, Lee J S, Lee H G. 2022. Rumen methane abatement by phlorotannin derivatives (phlorofucofuroeckol-A, dieckol, and 8,8'-bieckol) and its relationship with the hydroxyl group and ether linkage. *Animal Feed Science and Technology*, **293**, 115468.

Kinley R D, Fredeen A H. 2015. *In vitro* evaluation of feeding North Atlantic stormtoss seaweeds on ruminal digestion. *Journal of Applied Phycology*, **27**, 2387–2393.

Kinley R D, Martinez-Fernandez G, Matthews M K, de Nys R, Magnusson M, Tomkins N W. 2020. Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *Journal of Cleaner Production*, **259**, 120836.

Kohn R A, Boston R C. 2000. *The Role of Thermodynamics in Controlling Rumen Metabolism.* Commonwealth Agricultural Bureaux International, Wallingford, UK.

Krizsan S J, Ramin M, Chagas J C C, Halmemies-Beauchet-Filleau A, Singh A, Schnürer A, Danielsson R. 2023. Effects on rumen microbiome and milk quality of dairy cows fed a grass silage-based diet supplemented with the macroalga *Asparagopsis taxiformis*. *Frontiers in Animal Science*, **4**, 1112969.

Künzel S, Yergaliyev T, Wild K J, Philippi H, Petursdottir A H, Gunnlaugsdottir H, Reynolds C K, Humphries D J, Camarinha-Silva A, Rodehutscord M. 2022. Methane education potential of brown seaweeds and their influence on nutrient degradation and microbiota composition in a rumen simulation technique. *Frontiers in Microbiology*, **13**, 889618.

Leung A M, Braverman L E. 2014. Consequences of excess iodine. *Nature Reviews Endocrinology*, **10**, 136–142.

Li X, Norman H C, Kinley R D, Laurence M, Wilmot M, Bender H, de Nys R, Tomkins N. 2018. *Asparagopsis taxiformis* decreases enteric methane production from sheep. *Animal Production Science*, **58**, 681–688.

Liu H, Wang J, Wang A, Chen J. 2011. Chemical inhibitors of methanogenesis and putative applications. *Applied Microbiology and Biotechnology*, **89**, 1333–1340.

Lyu Z, Shao N, Akinyemi T, Whitman W B. 2018. Methanogenesis. *Current Biology*, **28**, 727–732.

Machado L, Magnusson M, Paul N A, Kinley R, de Nys R,

Tomkins N. 2016a. Dose-response effects of *Asparagopsis taxiformis* and *Oedogonium sp*. on *in vitro* fermentation and methane production. *Journal of Applied Phycology*, **28**, 1443–1452.

Machado L, Magnusson M, Paul N A, Kinley R, de Nys R, Tomkins N. 2016b. Identification of bioactives from the red seaweed *Asparagopsis taxiformis* that promote antimethanogenic activity *in vitro*. *Journal of Applied Phycology*, **28**, 3117–3126.

Machado L, Magnusson M, Paul N A, De Nys R, Tomkins N. 2014. Effects of marine and freshwater macroalgae on *in vitro* total gas and methane production. *PLoS ONE*, **9**, 85289.

Magnusson M, Vucko M J, Neoh T L, de Nys R. 2020. Using oil immersion to deliver a naturally-derived, stable bromoform product from the red seaweed *Asparagopsis taxiformis*. *Algal Research*, **51**, 102065.

Maia M R G, Fonseca A J M, Oliveira H M, Mendonça C, Cabrita A R J. 2016. The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. *Scientific Reports*, **6**, 32321.

Mairh O P, Ramavat B K, Tewari A, Oza R M, Joshi H V. 1989. Seasonal variation, bioaccumulation and prevention of loss of iodine in seaweeds. *Phytochemistry*, **28**, 3307–3310.

Makkar H P S, Tran G, Heuzé V, Giger-Reverdin S, Lessire M, Lebas F, Ankers P. 2016. Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, **212**, 1–17.

Martin C, Morgavi D P, Doreau M. 2010. Methane mitigation in ruminants: From microbe to the farm scale. *Animal*, **4**, 351–365.

Martinez-Fernandez G, Denman S E, Cheung J, McSweeney C S. 2017. Phloroglucinol degradation in the rumen promotes the capture of excess hydrogen generated from methanogenesis inhibition. *Frontiers in Microbiology*, **8**, 1871.

Melgar A, Lage C F A, Nedelkov K, Räisänen S E, Stefenoni H, Fetter M E, Chen X, Oh J, Duval S, Kindermann M, Walker N D, Hristov A N. 2021. Enteric methane emission, milk production, and composition of dairy cows fed 3-nitrooxypropanol. *Journal of Dairy Science*, **104**, 357–366.

Mihaila A A, Glasson C R K, Lawton R, Muetzel S, Molano G, Magnusson M. 2022. New temperate seaweed targets for mitigation of ruminant methane emissions: An *in vitro* assessment. *Applied Phycology*, **3**, 274–284.

Moss A R, Jouany J P, Newbold J. 2000. Methane production by ruminants: Its contribution to global warming. *Annales de Zootechnie*, **49**, 231–253.

Muizelaar W, van Duinkerken G, Khan Z, Dijkstra J. 2023. Evaluation of 3 northwest European seaweed species on enteric methane production and lactational performance of Holstein-Friesian dairy cows. *Journal of Dairy Science*, **106**, 4622–4633.

Muizelaar W, Groot M, van Duinkerken G, Peters R, Dijkstra J. 2021. Safety and transfer study: Transfer of bromoform present in *Asparagopsis taxiformis* to milk and urine of lactating dairy cows. *Foods*, **10**, 584.

Nin-Pratt A, Beveridge M C M, Sulser T B, Marwaha N, Stanley M, Grisenthwaite R, Phillips M J, Sulser T. 2022. *Cattle*, *Seaweed*, *and Global Greenhouse Gas Emissions*. International Food Policy Research Institute, Washington, USA. pp. 2111.

Nunes N, Valente S, Ferraz S, Barreto M C, Pinheiro de Carvalho M A A. 2018. Nutraceutical potential of *Asparagopsis taxiformis* (Delile) Trevisan extracts and assessment of a downstream purification strategy. *Heliyon*, **4**, e00957.

Olijhoek D W, Thorsteinsson M, Curtasu M V, Nielsen M O, Nørskov N P. 2022. Identification of potential methane mitigating compounds in ensiled brown seaweed *Saccharina latissimi*. In: *8th International Greenhouse Gas & Animal Agriculture Conference*. Orlando, USA. pp. 218.

Opio C, Gerber P, Mottet A, Falcucci A, Tempio G, MacLeod M, Vellinga T, Henderson B, Steinfeld H. 2013. *Greenhouse Gas Emission from Ruminant Supply Chains*: *A Global Life Cycle Assessment*. Food and Agriculture Organization of the United Nations, Rome.

Patra A, Park T, Kim M, Yu Z. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *Journal of Animal Science and Biotechnology*, **8**, 13.

Patra A K, Saxena J. 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. *Nutrition Research Reviews*, **22**, 204–219.

Paul N A, Cole L, de Nys R, Steinberg P D. 2006. Ultrastructure of the gland cells of the red alga *Asparagopsis armata* (Bonnemaisoniaceae). *Journal of Phycology*, **42**, 637–645.

Pérez M J, Falqué E, Domínguez H. 2016. Antimicrobial action of compounds from marine seaweed. *Marine Drugs*, **14**, 52.

Pitta D, Indugu N, Narayan K, Hennessy M. 2022. Symposium review: Understanding the role of the rumen microbiome in enteric methane mitigation and productivity in dairy cows. *Journal of Dairy Science*, **105**, 8569–8585.

Pitta D W, Melgar A, Hristov A, Challa K, Vecchiarelli B, Hennessy M, Narayan K, Duval S, Walker N. 2022. The effect of 3-nitrooxypropanol, a potent methane inhibitor, on ruminal microbial gene expression proles in dairy cows. *Microbiome*, **10**, 146.

Rengasamy K R, Mahomoodally M F, Aumeeruddy M Z, Zengin G, Xiao J, Kim D H. 2020. Bioactive compounds in seaweeds: An overview of their biological properties and safety. *Food and Chemical Toxicology*, **135**, 111013.

Reyes D C, Meredith J, Puro L, Berry K, Kersbergen R, Soder K J, Quigley C, Donihue M, Cox D, Price N N, Brito A F. 2023. Maine organic dairy producers' receptiveness to seaweed supplementation and effect of Chondrus crispus on enteric methane emissions in lactating cows. *Frontiers in Veterinary Science*, **10**, 1153097.

Robert P G, James A R, Ralph S W. 1978. Preparation of coenzyme M analogs and their activity in the methyl coenzyme M reductase system of *Methanobacterium thermoautotrophicum*. *Biochemistry*, **17**, 2374–2377.

Romero P, Huang R, Jiménez E, Palma-Hidalgo J M, Ignacio Martín-García A, Ungerfeld E M, Yanez-Ruiz D, Popova M, Morgavi D P, Belanche A, Yáñez-Ruiz D R. 2022. *In vivo* study of combining *Asparagopsis taxiformis* and phloroglucinol to reduce methane production and improve

rumen fermentation efficiency in goats. In: *8th International Greenhouse Gas & Animal Agriculture Conference*. Orlando, USA. pp. 239.

Roque B M, Salwen J K, Kinley R, Kebreab E. 2019. Inclusion of *Asparagopsis armata* in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. *Journal of Cleaner Production*, **234**, 132–138.

Roque B M, Venegas M, Kinley R D, de Nys R, Duarte T L, Yang X, Kebreab E. 2021. Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS ONE*, **16**, e0247820.

Stefenoni H A, Räisänen S E, Cueva S F, Wasson D E, Lage C F A, Melgar A, Fetter M E, Smith P, Hennessy M, Vecchiarelli B, Bender J, Pitta D, Cantrell C L, Yarish C, Hristov A N. 2021. Effects of the macroalga *Asparagopsis taxiformis* and oregano leaves on methane emission, rumen fermentation, and lactational performance of dairy cows. *Journal of Dairy Science*, **104**, 4157–4173.

Sucu E. 2020. Effects of microalgae species on *in vitro* rumen fermentation pattern and methane production. *Annals of Animal Science*, **20**, 207–218.

Tan S, Harris J, Roque B M, Askew S, Kinley R D. 2022. Shelf-life stability of *Asparagopsis* bromoform in oil and freeze-dried powder. *Journal of Applied Phycology*, **35**, 291–299.

Terry S, Krüger A, Lima P, Gruninger R, Abbott D, Beauchemin K, Terry S. 2022. Evaluation of rumen fermentation and microbial adaptation to three red seaweeds using the rumen simulation technique. *Animals*, **13**, 1643.

Thapa H R, Lin Z, Yi D, Smith J E, Schmidt E W, Agarwal V. 2020. Genetic and biochemical reconstitution of bromoform biosynthesis in *Asparagopsis* lends insights into seaweed reactive oxygen species enzymology. *ACS Chemical Biology*, **15**, 1662–1670.

Thauer R K. 1998. Biochemistry of methanogenesis: A tribute to marjory stephenson. *Microbiology*, **144**, 2377–2406.

Thauer R K. 2012. The Wolfe cycle comes full circle. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 15084–15085.

Thorsteinsson M, Lund P, Weisbjerg M R, Hellwing A L F, Hansen H H, Nielsen M O. 2022. Enteric methane emissions of dairy cows supplemented "Compound X" in a dose-response study. In: *8th International Greenhouse Gas & Animal Agriculture Conference*. Orlando, USA. pp. 263.

Tomkins N W, Colegate S M, Hunter R A. 2009. A bromochloromethane formulation reduces enteric methanogenesis in cattle fed grain-based diets. *Animal Production Science*, **49**, 1053–1058.

Whittington T. 2022. Reality check: seaweed as a feedstock for cattle likely to be nothing more than a 'niche market' by 2030 [Internet]. [2023-7-5]. https://www.countryman.com.au/countryman/opinion/a-reality-check-on-seaweed-as-a-feedstock-for-cows-c-5238740

Ungerfeld E M. 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: A meta-analysis. *Frontiers in Microbiology*, **6**, 37.

Ungerfeld E M. 2020. Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. *Frontiers in Microbiology*, **11**, 589.

Ungerfeld E M, Rust S R, Boone D R, Liu Y. 2004. Effects of several inhibitors on pure cultures of ruminal methanogens. *Journal of Applied Microbiology*, **97**, 520–526.

Vijn S, Compart D P, Dutta N, Foukis A, Hess M, Hristov A N, Kalscheur K F, Kebreab E, Nuzhdin S V, Price N N, Sun Y, Tricarico J M, Turzillo A, Weisbjerg M R, Yarish C, Kurt T D. 2020. Key considerations for the use of seaweed to reduce enteric methane emissions from cattle. *Frontiers in Veterinary Science*, **7**, 597430.

Vucko M J, Magnusson M, Kinley R D, Villart C, de Nys R. 2017. The effects of processing on the in vitro antimethanogenic capacity and concentration of secondary metabolites of *Asparagopsis taxiformis*. *Journal of Applied Phycology*, **29**, 1577–1586.

Wagner T, Ermler U, Shima S. 2016. The methanogenic CO_2 reducing-and-fixing enzyme is bifunctional and contains 46 (4Fe–4S) clusters. *Science*, **354**, 114–117.

Wang Y, Xu Z, Bach S J, McAllister T A. 2008. Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on *in vitro* ruminal digestion of mixed forage or barley grain. *Animal Feed Science and Technology*, **145**, 375–395.

Wasson D E, Cueva Welchez S F, Stefenoni H A, Fetter M E, Lage C F A, Melgar A, Raeisaenen S E, Silvestre T M, Yarish C, Hristov A N. 2022a. The macroalgae *Asparagopsis taxiformis* decreases dry matter intake and milk production in dairy cows. In: 8*th International Greenhouse Gas & Animal Agriculture Conference.* Orlando, USA. pp. 284.

Wasson D E, Yarish C, Hristov A N. 2022b. Enteric methane mitigation through *Asparagopsis taxiformis* supplementation and potential algal alternatives. *Frontiers in Animal Science*, **3**, 999338.

WMO (World Meteorological Organization). 2018. *Scientific Assessment of Ozone Depletion: 2018*. Geneva, Switzerland.

Wongnate T, Sliwa D, Ginovska B, Smith D, Wolf M W, Lehnert N, Raugei S, Ragsdale S W. 2016. The radical mechanism of biological methane synthesis by methyl-coenzyme M reductase. *Science*, **352**, 953–958.

Wood J M, Kennedy F S, Wolfe R S. 1968. The reaction of multi-halogenated hydrocarbons with free and bound reduced vitamin B12. *Biochemistry*, **7**, 1707–1713.

Zhu P, Li D, Yang Q, Su P, Wang H, Heimann K, Zhang W. 2021. Commercial cultivation, industrial application, and potential halocarbon biosynthesis pathway of *Asparagopsis* sp. *Algal Research*, **56**, 9.

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