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Review Article

The role of seaweed as a potential dietary supplementation for enteric methane mitigation in ruminants: Challenges and opportunities[☆]



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ABSTRACT

Seaweeds are macroalgae, which can be of many different morphologies, sizes, colors, and chemical profiles. They include brown, red, and green seaweeds. Brown seaweeds have been more investigated and exploited in comparison to other seaweed types for their use in animal feeding studies due to their large sizes and ease of harvesting. Recent *in vitro* and *in vivo* studies suggest that plant secondary compound-containing seaweeds (e.g., halogenated compounds, phlorotannins, etc.) have the potential to mitigate enteric methane (CH₄) emissions from ruminants when added to the diets of beef and dairy cattle. Red seaweeds including *Asparagopsis* spp. are rich in crude protein and halogenated compounds compared to brown and green seaweeds. When halogenated-containing red seaweeds are used as the active ingredient in ruminant diets, bromoform concentration can be used as an indicator of anti-methanogenic properties. Phlorotannin-containing brown seaweed has also the potential to decrease CH₄ production. However, numerous studies examined the possible anti-methanogenic effects of marine seaweeds with inconsistent results. This work reviews existing data associated with seaweeds and *in vitro* and *in vivo* rumen fermentation, animal performance, and enteric CH₄ emissions in ruminants. Increased understanding of the seaweed supplementation related to rumen fermentation and its effect on animal performance and CH₄ emissions in ruminants may lead to novel strategies aimed at reducing greenhouse gas emissions while improving animal productivity.

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1. Introduction

The livestock industry contributes 14.5% to 19% of global greenhouse gas (GHG) emissions (Johnson and Johnson, 1995;

Gerber et al., 2013) and accounts for approximately 11% of the GHG emissions in the US (Myhre et al., 2013; NASEM, 2018). Ruminant methane (CH₄) emission is a consequence of anaerobic carbohydrate fermentation by ruminal microbiota that produce carbon dioxide (CO₂) and hydrogen (H₂) in a reduction pathway used by methanogens (Morgavi et al., 2010). It is estimated that sheep, goats, and cattle lose 2% to 12% of ingested gross energy to CH₄ production depending on the diet (Johnson and Johnson, 1995). The ability of a CH₄ inhibitor to increase metabolizable energy in the ruminant diet and effectively reduce enteric CH₄ emissions is, therefore, an area of interest. Various dietary CH₄ interventions including ionophores, chemical compounds, legumes, essential oils, fats, probiotics, and plant secondary metabolites (e.g., halogenated, phlorotannins, tannins, saponins, iodine) have been investigated as methanogenesis inhibitors (Patra, 2012; Min et al., 2020). However, in some cases, the desired antimethanogenic effect may coexist

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with adverse effects such as decreasing dry matter intake (DMI) and feed efficiency (average daily gain: feed intake ratio).

Seaweed, otherwise known as macroalgae, are primitive non-flowering photosynthetic macrophytes. There are three distinct seaweed groups: green (chlorophyta), brown (phaeophyta), and red (rhodophyta). Worldwide seaweed production through aquaculture was over 30 million tonnes (fresh) in 2016 (FAO, 2018a,b; Rao et al., 2018). The capability of seaweed to promote well-being and health in livestock is facilitated to a great extent by bioactive secondary metabolites that are synthesized by some seaweed species (Abdul et al., 2016; Corona et al., 2016). Some of these secondary metabolites are responsible for antimethanogenic properties (Abecia et al., 2012; Roque et al., 2019a, b) but often health benefits come from various other nutrients (e.g., minerals, protein, and unsaturated fatty acids contents; Anderson et al., 2006; Cian et al., 2013). Recent in vitro and in vivo studies suggest that the halogenated compound-containing red seaweeds *Asparagopsis taxiformis* and *Asparagopsis armata* have the potential to reduce CH₄ production when added to grass- and grain-based diets (Roque et al., 2019a, b, Kinley et al., 2020; Min et al., 2021). Red seaweed is effective in the short-term (Mitsumori et al., 2012). The long-term feeding efficacy of red seaweed is still unknown. When seaweed is added to cattle diets, the effects on diet palatability, animal health, and reproduction, as well as milk and meat quality are not consistent. Furthermore, seaweeds occasionally accumulate heavy metals, iodine and other minerals; feeding contaminant-laden seaweeds could have negative effects on animal and human health (Makkar et al., 2016).

Phlorotannins (polymers of phloroglucinol) are mainly found in brown seaweeds (Li et al., 2011), which can positively or negatively impact rumen function and CH₄ production (Belanche et al., 2016; Huang et al., 2018). Supplementation of tannins at levels between 2% and 4% of dietary dry matter (DM) had positive effects in ruminants by increasing protein metabolism (Mueller-Harvey, 2006) and reducing bloat and enteric CH₄ emissions (Rochfort et al., 2008; Min et al., 2020). Although the processes by which tannins act are somewhat unknown, among the most accepted are substrate depression (McMahon et al., 2000), enzyme inhibition (Jones et al., 1994), and direct inhibition of selected rumen microorganisms (Scalbert, 1991). Some tannins can directly inhibit CH₄ production: an in vivo experiment in which ruminants (e.g., steers and lambs) were fed increasing doses of commercial brown seaweed (*Ascophyllum nodosum* meal; Tasco-14) had lower relative abundances of fecal *Escherichia coli* O157:H7 with no enhanced animal performance (Bach et al., 2008). Visser et al. (2017) identified that phlorotannins from *Laminaria digitata* decreased protein digestibility and CH₄ production (40%) during a 24-h in vitro ruminal fermentation. However, dietary supplementation with brown seaweed does not always have a positive impact on digestion and metabolism, as these effects are dependent on the particular strain of seaweed used. Belanche et al. (2016) observed no changes on in vitro CH₄ emissions when *L. digitata* or *A. nodosum* were included in the diet at 50 g/kg DM. Moneda et al. (2019) studied eight different seaweeds (Brown: *Alaria esculenta*, *L. digitata*, *Pelvetia canaliculata*, *Saccharina latissima*; Red: *Mastocarpus stellatus*, *Palmaria palmata* and *Porphyra* spp.; Green: *Cladophora rupestris*) that were included in an oat hay-based diet (1:1 oat hay:concentrate) at a rate of 50% and reported variable anti-methanogenic responses. Therefore, the use of brown seaweeds as CH₄ mitigation options can be an alternative to conventional feedstuffs in ruminant diets, but it is necessary to assess their nutritive value and effectiveness prior to use in commercial feeding operations.

It has been reported that bromoform-containing seaweed or commercially available bromochloromethane (BCM; 5 to 10 µmol/L) supplementation are some of the most effective inhibitors of

enteric CH₄ emissions because they interfere with methanogenesis (Wood et al., 1968; McCrabb et al., 1997; Goel et al., 2009). Furthermore, studies demonstrated that commercially available BCM supplementation or bromoform-containing seaweed significantly reduced CH₄ production (50% to 95%) and inhibited methanogenesis without negative effects on ruminal fermentation or animal growth performance (Tomkins and Hunter, 2004; Tomkins et al., 2009; Abecia et al., 2012; Kinley et al., 2016; Machado et al., 2018). However, most proposed mitigation strategies have shown inconsistent results among studies and may even lead to decreased DMI (McCrabb et al., 1997; Roque et al., 2019a), lower ruminal digestibility (Gojon-Baez et al., 1998; Machado et al., 2016; Tayyab et al., 2016), or altered rumen microbial community diversity including methanogen, bacteria, protozoa, and fungi populations (Goel et al., 2009; Mitsumori et al., 2012; Roque et al., 2019a, b). In addition, there is much variability in the anti-methanogenic potency between seasons and species of seaweed (Dubois et al., 2013; Sarojini et al., 2012; Pirian et al., 2017) and among animal species (McCrabb et al., 1997; Tomkins and Hunter, 2004; Li et al., 2018). Bromoform is the active ingredient in seaweed that causes the reduction in CH₄ emissions, although other compounds such as dibromochloromethane and dibromoacetic acid have also been detected at lower concentrations (Marshall et al., 1999; Mata et al., 2011; Machado et al., 2016, 2018). This paper reviews progress utilizing naturally occurring plant secondary compounds from select seaweed varieties as an active ingredient for anti-methanogenesis thereby reducing CH₄ emissions when ruminants are supplemented with seaweed.

2. Chemical composition and bioactive ingredients of seaweed

Nutritional and biochemical values of different seaweed have been studied by many researchers (Fleurence and Le Coeur, 1993; Ortega-Calvo et al., 1993; Rizk, 1997). The secondary compounds in seaweed contain various bioactive properties including anti-viral, anti-microbial, anti-tumor, anti-inflammatory, antioxidant, and many more (Table 1). Seaweeds are also the source of phytochemical compounds, including agar, carrageenan, and alginates (Cardozo et al., 2007; Rindi et al., 2011; Pal et al., 2014; Kolanjiathan et al., 2014; Neethu et al., 2017), these compounds are rich in valuable nutrients and have been used as a source of human food, various animal feeds, therapeutic agents, and fertilizer (Cardozo et al., 2007; Nunes et al., 2018). Seaweeds contain carbohydrates, proteins, minerals, vitamins, fats and oils, and amino acids (AA), and possess trace amounts of secondary compounds (e.g., phlorotannins, iodine, and halogenated compounds) in cell walls (McConnell and Fenical, 1977; El-Baroty et al., 2007; Pirian et al., 2017; Gaillard et al., 2018).

In general, compared to green and brown seaweed, red seaweed contains a high amount of crude protein (CP; Table 2) reaching 38.1% CP (e.g., *Porphyra* spp.) of the DM content of the plant. These results are consistent with other data (Cian et al., 2015). In contrast, green seaweed contains moderate amounts (15.3% to 18.6% CP DM), while brown seaweed exhibit much lower CP contents (6.0% to 16.6% DM; Table 2). But some species of green seaweed, such as *Ulva reticulata*, *Ulva lactuca*, *Ulva fasciata*, and *Enteromorpha*, were reported to have higher CP content (12% to 23% DM) compared to other species collected from the Gulf of Mannar coast, India (Abirami and Kowsalva, 2012). In addition, Pirian et al. (2017) reported that CP contents were 12.3% and 9.0% in green algae (*Caulerpa sertularioides*) and brown algae (*Colpomenia*) in Persian Gulf seaweed, respectively. In this regard, the CP content of red seaweed is comparable with that of high protein plant feeds such as soy and soybean meal (Kuiken and Lyman, 1949; Norziah and Ching, 2000).

Table 1
Nutraceutical and pharmacological potential of some seaweeds.

Species	Compounds	Properties	References ¹
Red seaweed (Rhodophyta)			
<i>Asparagopsis taxiformis</i>	Alkaloides, flavonoids, anthraquinones, phenols, chlorophylls, halogenated compounds	Antioxidant, antiproliferative, free radical scavenging, antimethanogenesis	1, 2, 34, 35
<i>Asparagopsis armata</i>	Halogenated compounds	Antimicrobial, antitumor activity	3, 35
<i>Chondria armata</i>	Galactoglycerolipids	Antimicrobial	4, 5
<i>Corallina pilulifera</i>	Phlorotannins	Antioxidant and tyrosinase pathways	6, 7
<i>Corallina tamariscifolia</i>	Phlorotannins	Anti-inflammatory, antioxidant	8
<i>Euचेuma cava</i>	Phlorotannins, Lectins	Antioxidant, UV protection, Antibacterial, antiviral	9, 10, 11
<i>Laurencia pacifica</i>	Laurinterol, Bromophenols, Sesquiterpene	Antibacterial, antioxidant	12, 13, 14
<i>Gracilaria spp.</i>	Steroid, terpenoid, eiconoid	Antibacterial	14
<i>Rhodomella spp.</i>	Bromophenols	Antimicrobial activity	14
Green seaweed (Chlorophyta)			
<i>Cladophora glomerata</i>	Chlorophylls	Antioxidant, antibacterial	15, 16, 17
<i>Caulerpa sp.</i>	Flavonoids, phenols, saponin	Tyrosinase inhibitor	18
<i>Haematococcus lacustris</i>	Carotenoids	Antioxidant, anti-inflammatory	19, 20, 21
<i>Ulva lactuca</i>	Chlorophylls	Antibacterial, antioxidant	22, 23, 24
<i>Dunaliella tertiolecta</i>	Phenolics	Anti-aging	25
Brown seaweed (Ochrophyta)			
<i>Ascophyllum nodosum</i>	Phlorotannins	Anti-bacterial, inhibit rumen fermentation	26, 27
<i>Cystoseira tamariscifolia</i>	Phlorotannins	Anti-inflammatory	28
<i>Ecklonia cava</i>	Sulfated polysaccharide/Phlorotannins	Anti-viral, antioxidant, anti-inflammatory. Tyrosinase inhibition	29, 30, 31
<i>Ecklonia bicyclis</i>	Sulfated polysaccharide	Antiviral, antioxidant, anti-inflammatory	32, 33
<i>Himantalia elongata</i>	Volatile fatty acids	Antioxidant, antimicrobial	34
<i>Laminaria digitata</i>	Iodine	Control iodine deficiency disorders and animal weight gain	35

¹ Sources: 1 = Nunes et al. (2018), 2 = Neethu et al. (2017), 3 = Horta et al. (2019), 4 = Al-Fadhli et al. (2006), 5 = Fabrowska et al. (2015), 6 = Thomas and Kim (2013), 7 = Stengel et al. (2011), 8 = Ferreres et al. (2012), 9 = Heo et al. (2009), 10 = Ko et al. (2011), 11 = Samarakoon and Jeon (2012), 12 = Fenical (1976), 13 = Liu et al. (2011a), 14 = Kasanah et al. (2015), 15 = Spears (1988), 16 = Borowitzka (2013), 17 = Christaki et al. (2013), 18 = Demais et al. (2007), 19 = Goldberg (1943), 20 = Spears (1988), 21 = Lanfer-Marquez et al. (2005), 22 = Goldberg (1943), 23 = Spears (1988), 24 = Delaunay and Voile (2011), 25 = Norzagaray-Valenzuela et al. (2017), 26 = Wang et al. (2008, 2009a, b), 27 = Kannan et al. (2019), 28 = Ferreres et al. (2012), 29 = Robic et al. (2009), 30 = Samarakoon and Jeon (2012), 31 = Heo et al. (2009), 32 = Chizhov et al. (1999), 33 = Wijesingha and Jeona (2012), 34 = Plaza et al. (2010), 35 = He et al. (2002).

The CP content of seaweeds varies between species and also among seasonal periods (Mishra et al., 1993; Castro-Gonzalez et al., 1994; Fleurence, 1999; Guiry and Guiry 2014; Pirian et al., 2017). Therefore, seaweeds are an interesting potential source of food protein, and animal feed. However, research is needed to ascertain the appropriate seaweed type and feeding rate so that animal productivity is not negatively impacted.

Due to their high polysaccharide content, seaweeds have a high level of neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Lahaye, 1991). Red seaweed generally contains higher levels of NDF (27.2% to 43.1% DM) than green (15.3% to 18.6% DM) and brown seaweed (16.6% to 22.0% DM; Table 2). Unlike land plants (which have cell walls made of mainly cellulose, hemicellulose, and lignin), the cell walls of seaweeds consist principally of alginates, with some cellulose, xylan, and xyloglucan (Rogers and Perkins, 1968). Regardless of this structural difference, the varied active polysaccharide components in seaweed polysaccharides are hydrolyzed and fermented by carbohydrate-active enzymes in the ruminant digestive system (Hehemann et al., 2010). The opportunity to reduce enteric CH₄ with seaweed supplementation is a hot topic. There has been rising interest seaweed use for livestock feed, as the bioavailability of polysaccharides in some seaweed can result in CH₄ reduction potential (Morais et al., 2020). Further animal nutrition studies are needed to evaluate both the nutritional benefit of seaweed supplementation and the efficacy of polysaccharide bioactivities at mitigating enteric CH₄ emissions, as well as to determine any potential unfavorable effects on animal health, economics, or productivity.

2.1. Secondary metabolites

Seaweeds have an extended history of use as livestock feed. Seaweed has a greatly variable chemical composition, depending on the seaweed species, seasons, and environment (Makkar et al., 2016). Commonly, the most studied phytochemicals in seaweeds are phlorotannins and halogenated compounds. Studies of the effects of feed iodine and iodine adversaries on iodine status in animals could help to advance understanding of human iodine nutrition and physiology (Laurberg et al., 1998). The ocean is the primary source of iodine, containing between 50 and 60 µg/L (NRC, 2005). In both humans and livestock, iodine deficiency reduces the level of thyroid hormones resulting in hypothyroidism, goiter formation, depression of metabolism, growth, and a high rate of stillbirths (Schone and Rajendram, 2009). These intakes prevent iodine deficiency, facilitate a high performance (e.g., weight gain and low feed:gain ratio), maintain adequate iodine stores (>0.50 mg/g thyroid), and sustain thyroid function (Schone and Rajendram, 2009). Animal nutrition societies generally recommend iodine intakes in the range of 0.5–0.80 mg/kg feed for growing calves and dairy cattle (Table 3). Lactating dairy cattle need more dietary iodine because over 10% of iodine intake may be excreted in milk, depending upon milk production rate (Miller et al., 1975). Based on published literature, however, maximum tolerable levels (mg/kg diet) suggested for iodine are cattle, 50; sheep, 50; swine, 400; chicken and turkey, 300 (NRC, 1980). Additionally, iodine requirements may also be affected by animal genetic variances, temperature, and environment. Cattle, sheep, and

Table 2
Chemical composition of seaweed species (all values on DM basis)¹.

Type	Red seaweed			Green seaweed	Brown seaweed			
Species	<i>Porphyra</i> spp.	<i>Aparagopsis taxiformis</i>	<i>Asparagopsis armata</i>	<i>Ulva</i> sp.	<i>Ascophyllum nodosum</i>	<i>Macrocystis</i> Sp.	<i>Laminaria</i> Sp.	<i>Costaria costata</i>
Nutrients, %								
CP	24.6–38.1	17.8	18.3	15.3–18.6	6.0–8.3	10.1	9.8–16.6	7.8
NDF	43.1	36.9	27.2	22.8–26.2	20.9–22.0	19.9	16.6	–
ADF	6.6	11.6	10.9	7.6–8.7	13.1	12.6	Na	–
Either extract	0.3–0.5	0.4	0.32	1.2	3.9	0.6	0.8	–
Ash	6.5–8.7	–	10.0	7.7–23.2	22.0–22.5	32.9	29.9–31.5	–
Minerals, %								
Ca	4.4	3.8	4.47	2.9	1.0–3.0	14.1	0.08	0.12
P	3.8	0.2	0.27	0.27	0.1–0.2	2.9	–	–
Na	4.1	6.6	9.36	2.0–3.3	2.4–4.0	36.5	25.3	4.16
Mg	4.9	0.8	1.38	1.7	0.5–1.09	39.2	5.5	0.96
Minerals, mg/kg								
Fe	2.2	6.2	1.188	1.24	134.0	117.0	233.2	–
Mn	–	0.1	0.63	0.10	10–50	11.0	6.2	1.48
Zn	0.15	0.24	0.07	0.05	35–100	12.0	111.7	10.8
Cu	0.51	0.87	–	7.07	4.0–15	2.0	14.9	6.4
S	–	4.5	–	–	2.0–2.3	–	–	–
Iodine	1.5	1.71–3.37	0.6–1.8	0.9	0.01–0.1	ND	0.9	0.03
Bromoform ²	–	1,723	1,320	150 ³	2.7 ³	150 ³	49.7	–
Phlorotannins	–	5.0–6.0 ⁴	5.0 ⁵	1.0–2.0 ⁵	20–14	21	2.0	2.0

DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDN = total digestible nutrient.

¹ Sources: Abirami and Kowsalva (2012), Abudabos et al. (2013), Aminina et al. (2020), Anderson et al. (2006), Applegate and Gray (1995), Arasaki and Arasaki (1983), Baardseth (1970), Belanche et al. (2016), Cian et al. (2013), El-Baroty et al. (2007), Farley (2012), Hind et al. (2014), Holdt and Kraan (2011), Imbs et al. (2009), Leyton et al. (2016), Machado et al. (2016), Marino et al. (2016), Nunes et al. (2018), Roque et al. (2019a, b), Nunes et al. (2018), Ragan and Jensen (1978), Roque et al. (2019b).

² Bromoform contents are µg/g DM, unless stated otherwise ng/g fresh weight.

³ Minor level of bromoform productions (ng/g of fresh weight): Manley et al. (1992), Carpenter and Liss (2000).

⁴ Minor levels of total phenolic compounds (Nunes et al., 2018).

⁵ Minor levels of condensed tannins (Kafhi et al., 2020; Mihaila, 2020).

goats display a significant reduction in thyroid hormone production during the summer (ARC, 1980). Iodine feed supplements are needed to produce thyroid hormones, maintain metabolism, and facilitate reproduction, growth, and development of the body (NRC, 2005). Iodates [Ca(IO₃)₂ × 6H₂O; Ca(IO₃)₂], iodides (NaI; KI; EU, 2003, 2005), and seaweeds (Table 2) are recommended for feed supplements (NRC, 2005). However, the iodine content in some red and brown seaweeds is high reaching up to 3.7 mg/kg of DM (Table 2). In certain brown seaweeds, the concentration of iodine can reach very high levels, in particular, the genus *Laminaria* and *Saccharina japonica* (as *Laminaria japonica*) had the highest iodine content of 5.6 and 3.04 mg/kg DM, respectively (Misurcova, 2011; Holdt and Kraan, 2011). Therefore, the seaweed content of animal feeds may need to be limited to a maximum of 10% of the diet. There is a need to determine the mechanisms involved in iodine metabolism, particularly the interaction of iodine with other

Table 3
Required and recommended iodine supplementation of feed of cattle, pigs, and poultry in the US, UK, and Germany (mg/kg feed dry matter)¹.

Item	US (NRC, 1985, 1996, 1998, 2001, 2005)	UK (AFRC, 1981)	Germany (GfE, 1995, 1999, 2001, 2003, 2006)
Dairy cattle	0.50	0.80	0.50
Growing calves/bulls	0.50	0.12	0.25
Sows	0.16	0.50	0.60
Growing pigs	0.16	–	0.15
Laying hens	0.32–0.49	–	0.50
Broiler chickens	0.35	–	0.50

US NRC = National Research Council (NRC) of the United States; GfE = Gesellschaft für Ernährungsphysiologie; AFRC = Agricultural Food Research Council.

¹ Data for sheep with 0.100 to 0.80 mg/kg dry matter (NRC, 1985) and goats with 0.30 to 0.80 mg/kg feed dry matter (GfE, 2003) were not included above.

nutrients such as selenium, bromine, and iron (NRC, 2005). Required and recommended iodine supplementation of fed cattle, pigs, and poultry are presented in Table 3.

Polyphenol compounds like phlorotannins were frequently reported in all genera of seaweeds but their presence tends to be highest in brown seaweed ranging from 20 to 140 g/kg DM (Table 2). Results of previous studies showed that when *A. nodosum* seaweed meal (brown seaweed; Tasco) was added (10 g/d; DM basis) to TMR rations in cannulated steers, the digestibility of that dietary ration was increased from 51.5% to 64.9% DM (Leupp et al., 2005). Results by Wang et al. (2008) proposed that not only can prebiotics alter the microbiota of the rumen but that phlorotannins found in *A. nodosum* may play a role in altering the fermentation patterns in the rumen of cattle. Williams et al. (2009a), reported that the rate of fiber (e.g., NDF) digestibility in situ by rumen-cannulated steers was increased by *A. nodosum* (Tasco) treatment. These effects are possibly related to changes in the rumen microbiome community diversity in cattle as reported by Ushakova et al. (2006). In addition, numerous studies have reported that phlorotannins containing seaweeds fed to beef cattle can decrease the shedding of foodborne pathogens such as *E. coli* O157:H7 (Wang et al., 2009a,b; Evans and Critchley, 2014; Huang et al., 2018). These results confirmed that feeding tannin-containing diets could be a useful method to decrease the presence of foodborne pathogens in the ruminant digestive tract thereby reduce the risk of carcass contamination and hence enhance food safety (Min et al., 2007; Huang et al., 2018).

The bromoform contents of red seaweed such as *Asparagopsis* spp. (Table 2) were lower than the polyphenol content, 1.32 to 1.72 mg/g DM. The seaweeds *A. taxiformis* and *A. armata*, are distributed across tropical and temperate marine ecosystems and contain high levels of halogen-containing (F, Cl, Br, and I) compounds including bromoform (CHBr₃; 1.723 µg/g DM), followed by

dibromochloromethane (CHBr₂Cl; 0.158 µg/g DM), bromochloroacetate (C₂H₂BrClO₂; 0.088 µg/g DM), and dibromoacetate (C₂H₂Br₂O₂; 0.009 µg/g DM; Table 2). The other seaweeds, including *Macrocystis pyrifera*, *Ulva* sp., *Eisenia arborea*, *Laminaria farlowii*, *Egria menziesii*, and *Cystoseira osmundacea*, produce negligible amounts of halogenated compounds (Table 2) such as chloromethane (CH₃Cl), bromomethane (CH₃Br), methyl iodide (CH₃I), bromomethane bromide (CH₃Br₂), and bromoform (Manley et al., 1992; Dembitsky and Tolstikov, 2003).

2.2. AA

Seaweed species and the season of the collection are the most common factors affecting both seaweed protein and AA composition (Fleurence, 1999). The protein content reported in brown seaweed is mostly low in comparison with green (10% to 26%) and red seaweed species (35% to 47%) with protein contents comparable to protein-rich foods such as soybean meal (Garcia-Vaquero and Hayes, 2016). The proteins from seaweeds have relatively high levels of the AA glycine (Gly), alanine (Ala), arginine (Arg), glutamic (Glu), and aspartic (Asp) acids whereas methionine (Met), cysteine (Cys), iso-leucine (Isol), and histidine (His) appear in a lower amount (Table 4). Glutamic (10.0 to 12.7 g/100g of protein) and aspartic acid (6.9 to 12.2 g/100 g of protein), which have acidic side chains at neutral pH, in seaweeds represent 10.0 to 12.78 g/100 g of protein. Commonly, all the six species of seaweeds (Table 4) are rich in essential and non-essential AA and showed a balanced sulfur-containing AA profile comparable to that of FAO (1918) and soybean meal, except *A. nodosum*. Red seaweed contained high levels of CP (17.8% to 38.1% DM) and sulfur-containing AA in that protein. Therefore, seaweeds might be important sources of proteins with a high level of essential AA. It was found that seaweeds could be a complementary source of food proteins for human and animal nutrition. However, Pirian et al. (2017) reported that essential AA concentrations such as leucine (7.6 to 8.8 g/100g of protein), alanine (3.4 to 4.9 g/100 g of protein), threonine (3.1 to 4.4 g/100 g of

protein) are varied in the seaweed species, including *Ulva linza*, *Sargassum vulgare* and *Gracilaria corticata* collected from the Persian Gulf coast-line. Seasonal changes also affected the content of total AA profiles in *Laminaria* and *Ulva* seaweeds (Gaillard et al., 2018). However, seaweeds may contain non-protein-nitrogen (N; e.g., amine, amides, amino sugars, nitrates), resulting in a possible overestimation of their protein content (Misurcova, 2011, 2012). The accurate value of the N-to-protein conversion factor should be determined for each seaweed genera from the total N content based on AA composition and the distribution of N in protein and other nonprotein N compounds (Fujihara et al., 2001; Ezeagu et al., 2002; Lourenco et al., 1998; Salo-Vaananen and Koivistoinen, 1996). In different genera of green, brown, and red seaweed the assessments of N-to-protein conversion factors have been provided. The average rate of the N-to-protein conversion factor is 5.13 for green, 5.38 for brown, and 4.92 for red seaweeds (Lourenco et al., 2002). Data obtained from a previously published study (Lourenco et al., 2002) indicated that seaweed has the potential to become widely used as alternative feed ingredients for sustainable ruminant production.

2.3. Lipids and fatty acids

Brown seaweed normally has the greatest total lipid content, followed by green and red seaweeds (Gosch et al., 2012). In recent years, lipid composition in seaweeds has raised considerable interest due to their high content of unsaturated fatty acids (USFA). Seaweed lipids generally comprise long-chain fatty acids, especially polyunsaturated fatty acids (PUFA) with 18- and 22- carbon (C) atoms, depending on species. The average contribution of saturated and unsaturated fatty acids contents is presented in Table 5. Saturated fatty acids (SFA) and USFA varied among seaweed species and the yielding of SFA/USFA ratio was accounted to 0.35, 0.83, 0.93, 0.90, and 0.33 for *A. taxiformis*, *Porphyra dioica*, *Ulva rigida*, *Codium tomentosum*, and *A. nodosum*, respectively (Table 5). Among the selected seaweed species, both *A. taxiformis* and *A. nodosum* were the most abundant in USFA. It has been reported that high levels of

Table 4
Amino acid (AA) composition (g/100g of protein) of various seaweed species¹.

Type	Red seaweed		Green seaweed		Brown seaweed		Soybean meal
Species	<i>Porphyra columbina</i>	<i>Asparagopsis taxiformis</i>	<i>Ulva</i> Spp.	<i>Ascophyllum nodosum</i>	<i>Macrocystis pyrifera</i>	<i>Laminaria digitata</i>	N × 6.25
Essential AA							
Methionine	1.68	2.32	1.6–6.7	0.7	2.05	1.49	1.4
Cysteine	1.89	4.32	2.01–5.9	trace	3.5	1.96	1.38
Valine	5.85	6.19	4.4–6.7	3.7	4.45	6.01	5.34
Iso-leucine	2.71	5.09	2.6–3.7	2.8	3.20	2.61	5.31
Leucine	7.38	8.25	5.2–6.7	4.6	5.76	4.45	7.58
Phenylalanine	3.7	5.86	3.5–4.68	2.3	3.27	2.82	5.08
Tyrosine	2.55	3.67	1.4–3.0	0.9	2.68	1.74	3.35
Histidine	1.26	1.48	2.0–3.01	1.3	1.30	2.38	2.33
Lysine	6.01	4.32	3.8–4.4	4.9	5.05	4.77	6.65
Threonine	5.91	5.86	3.8–9.4	2.8	4.78	3.41	3.90
Non-essential AA							
Serine	6.16	5.93	4.2–6.4	3.0	4.44	2.45	5.18
Arginine	6.19	7.15	4.5–5.0	8.0	3.83	2.96	7.72
Glutamic acid	10.5	10.89	13.5–12.7	10.0	13.83	3.86	18.4
Aspartic acid	12.2	12.24	7.9–12.4	6.9	10.04	4.69	14.14
Proline	3.96	5.15	0.0–2.8	2.6	3.73	1.91	5.99
Glycine	8.87	5.15	5.4–7.7	5.0	4.83	3.31	5.54
Alanine	12.54	7.35	5.98.7	5.3	11.43	4.51	4.54

N = nitrogen.

¹ Sources: Anderson et al. (2006), Angell et al. (2012), Castro-Gonzalez et al. (1994), Cian et al. (2013), Imbs et al. (2009), Arieli et al. (1993), Dawczynski et al. (2007), Kolb et al. (2004), Makkar et al. (2016), Kuiken and Lyman (1949), Ortiz et al. (2006). *Porphyra columbina* was collected from Punta Maqueda, Argentina (Cian et al., 2013); *Asparagopsis taxiformis* and *Ulva* spp. were collected from shallow reefs at Nelly Bay, Magnetic Island; Anderson et al., 2006); *Ascophyllum nodosum* was harvested off the coast of Nova (Angell et al., 2012); The samples of *Macrocystis pyrifera* was collected in summer in Bahía Tortu-gas, Baja California Sur (Castro-Gonzalez et al., 1994); *Laminaria digitata* was collected in Troitsa Bay of the Peter the Great Bay of the Sea of Japan (Kolb et al., 2004; Imbs et al. (2009). Soybean meals (mean value of 20 strains of soybean meals) were obtained through the cooperation of the United States Regional Soybean Laboratory at Urbana (Kuiken and Lyman, 1949).

monounsaturated fatty acids (MUFA) and PUFA were also found in *S. vulgare* (35.1% and 21.5%) and *U. linza* (30% and 21%), respectively (Pirian et al., 2017). Furthermore, average USFA contents varied from 26% of the total fatty acid content in *U. rigida* to 75.0% in *A. nodosum* (Table 5). Certain seaweeds (red and brown) also contain high levels of omega-3, omega-6, and other PUFA (Table 5; Holdt and Kraan, 2011; van Ginneken et al., 2011) which could aid meat and milk qualities, immune systems, and reproduction rates through improved conception rates and reduced pregnancy losses (Moallem, 2018). Therefore, PUFA is believed to be an essential nutritional component in humans and animals, playing an important role in improved animal health.

3. The effect of seaweed on methanogenesis

Anti-methanogenic, halogenated compounds (e.g., BCM) in seaweed, have been reported to inhibit enteric CH₄ emissions when fed to ruminants (Table 6), but limited studies have assessed how seaweed supplementation might impact the ruminal microbiota and methanogenesis. The addition of red seaweed and BCM has been reported to depress CH₄ production both in vivo and in vitro (Tables 6 and 7). In steers and dairy cattle fed forage- and grain-based diets, the addition of BCM (<0.6 g/100 kg BW) or red seaweed of *Asparagopsis* spp. (<1.0% OM basis) decreased CH₄ production by 50.0% to 99.5%, while feeding seaweed also decreased DMI (38.0%) in dairy cattle (Table 6). These results are consistent with other data. Dairy cattle fed the red seaweed, *A. armata*, supplemented diet (0.5% and 1.0% inclusions; OM basis) had reduced DMI and milk yield (kg/d) by up to 38.0% and 13.5%, respectively (Roque et al., 2019a, Table 6). Recently, however, Kinley et al. (2020) reported that the low levels of red seaweed (*A. taxiformis*; 0.05% to 0.2% OM) in a beef total mixed ration (TMR)-based diet reduced enteric CH₄ emissions by up to 98% without any reduction of DMI in beef cattle (Table 6). These results were consistent with the previous study reported in Angus-Hereford beef steers fed a high-forage-based TMR diet compared to low-forage-based TMR diets (Roque et al., 2020). McCrabb et al. (1997) reported a reduction in DMI (7.4%) of forage-based diet contained BCM (1.2% of DM BCM) for steers fed low and medium-quality alfalfa hay diets over 10 to 12 weeks. These results indicate that increasing BCM supplementation or BCM-containing seaweed (ranging from 0.5% to 1.0% of DM) progressively decreased DMI in beef and dairy cattle. However, DMI was not different between treatment groups in sheep, lactating dairy goats (Table 6), and

steers fed concentrate-based diet (Tomkins et al., 2009). Li et al. (2018) reported the effects of five dietary inclusion levels of *A. taxiformis* (0%, 0.5%, 1%, 2%, and 3% OM) on CH₄ emissions when fed to sheep consuming a high fiber diet for 72 h. It appears that dairy or beef cattle seem to have palatability issues when *Asparagopsis* seaweed supplementation was included up to 1.0% of DM in a diet, or more than 0.3 g bromoform/100 kg BW (Tomkins and Hunter, 2004; Tomkins et al., 2009), respectively, compared to sheep (Orpin et al., 1985; Hansen et al., 2003).

With high potency and wide-spectrum efficacy against rumen methanogens, a red seaweed (*Asparagopsis* spp.) represents a promising natural intervention strategy for reducing enteric CH₄ emissions from ruminants if animal production levels can be maintained. Such a conclusion is supported by in vitro data (Table 7). Recent studies suggest that the red seaweed *A. taxiformis* has the potential to reduce CH₄ emission from beef cattle by up to 95% (Table 7). Inclusion of 5% *A. taxiformis* (OM basis) in a dairy ration resulted in a 95% reduction in CH₄ emissions with no negative impacts on rumen fermentation (Roque et al., 2019). This in vitro experiment was similar to in vivo results reported from Kinley et al. (2016) and Machado et al. (2016), in which a strong anti-methanogenic activity of *A. taxiformis* was observed when included in the diet at 22.7 g/kg DM. Both *A. taxiformis* and *A. armata* supplementation, used at dietary inclusion levels at 0%, 2%, and 4% as-fed basis in an anaerobic in vitro study, increased total gas, butyrate, and valerate production ($P < 0.01$), while production of CH₄ (mg/g DM), acetate, propionate, acetate/propionate ratios and in vitro dry matter digestibility (% DM) were reduced ($P < 0.01$) as both red seaweed supplementation increased (Min et al., 2021). Therefore, it may be possible to suppress methanogenesis both directly and indirectly by the addition of red seaweeds.

Among the 17 seaweed species tested in vitro (Fig. 1), *Cladophora patentiramea* (green seaweed), *Dictyota* (brown seaweed), and *Asparagopsis* (red seaweed) had the strongest effects, inhibiting CH₄ production by 69.7%, 93.1%, and 99.0%, respectively (Machado et al., 2014). Molina-Alcaide et al. (2017) and Moneda et al. (2019) observed similar effects of anti-methanogenic activities for brown (*P. canaliculata*) and red (*M. stellatus*) seaweeds in ruminant diets at 200 g/kg DM. However, Belanche et al. (2016) reported no changes in vitro CH₄ production when brown seaweeds (*L. digitata* or *A. nodosum*) were included in the diet at 50 g/kg DM. The data suggest that inhibition of methanogenesis varies among seaweed species and their secondary metabolites (Lanigan, 1972; Ungerfeld et al., 2004).

Table 5
Fatty acids (FA, % DM) profile of red, green, and brown seaweed species¹.

Item	Red		Green		Brown
	<i>Asparagopsis taxiformis</i>	<i>Porphyra dioica</i>	<i>Ulva rigida</i>	<i>Codium tomentosum</i>	<i>Ascophyllum nodosum</i>
C14:00	3.77	23.3	20.2	22.3	9.4
C16:00	3.73	18.3	2.1	4.9	13.4
C18:00	1.18	4.9	2.9	2.6	0.76
C18:1	3.52	3.3	9.5	11.1	27.8
C18:2n-6	7.75	1.7	1.5	—	7.47
C20:1	—	0.6	1.2	—	0.22
C20:2n-6	1.38 (C20:3)	0.6	1.2	—	5.05
C20:4n-6	1.19 (C20:4)	2.7	—	4.5	17.24
C20:5n-3	1.6 (C20: 5)	20.5	1.4	7.9	7.24
C22:6	32.77	—	—	—	—
SFA	23.17	37.5	24.1	30.2	25.1
MUFA	19.52	22.5	13.0	16.8	31.5
PUFA	46.97	22.6	13.0	16.8	43.5
Ave. USFA	66.49	45.1	26.0	33.6	75.0
SFA:USFA	0.35	0.83	0.93	0.90	0.33

SFA = saturated fatty acids; USFA = unsaturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.

¹ Sources: Cian et al. (2013), Lorenzo et al. (2017), Lopes et al. (2020), Mellouk et al. (2017). Major fatty acids were presented in this Table.

Table 6
In vivo studies of methane (CH₄) emissions from seaweed and commercial bromochloromethane (BCM) supplementation.

Animal	Basal diet	Treatment	DMI, kg/d	CH ₄ production	Reference ¹
Beef steers	Feedlot TMR (total mixed ration)	BCM, g/100 kg of BW		CH ₄ , g/kg DMI	1
		0 (control)	6.2 ^b	8.7 ^a	
		0.15	7.4 ^a	3.8 ^{ab}	
		0.30	5.6 ^b	1.4 ^b	
		0.60	5.5 ^b	0.8 ^b	
Beef steers	Alfalfa hay	Rate of change, %	-11.3	-95.2	2
		BCM, g/100 kg of BW		CH ₄ , mL/min	
		0 (control)	8.1 ^a	205.5 ^a	
		1.2	7.5 ^b	0.24 ^b	
		Rate of change, %	-7.4	-90.6	
Beef steers	Feedlot TMR	BCM, g/100 kg of BW		CH ₄ , g/kg DMI	3
		0 (control)	10.4	20.0 ^a	
		0.98	10.3	0.1 ^b	
		Rate of change, %	-0.96	-99.5	
Dairy cows	Dairy TMR	<i>A. armata</i> , %, OM basis		CH ₄ , g/kg DMI	4
		0 (control)	27.9 ^a	15.0 ^a	
		0.5	24.9 ^b	12.0 ^b	
		1.0	17.3 ^c	7.5 ^b	
		Rate of change, %	-38.0	-50.0	
Beef steers	Feedlot TMR	<i>A. taxiformis</i> , %, OM basis		CH ₄ , g/kg DMI	5
		0 (control)	8.4	10.4	
		0.05%	8.0	10.0	
		0.10%	10.3	6.2	
		0.2%	8.8	0.2	
		Rate of change, %	0.4	-98.0	
		<i>A. taxiformis</i> , %, OM basis		CH ₄ , g/kg DMI	
Sheep	High-fiber pellet	0 (control)	1.0	15.0 ^a	6
		0.5	1.1	12.7 ^{ab}	
		1.0	1.0	7.0 ^b	
		2.0	1.1	5.6 ^c	
		3.0	1.0	2.9 ^c	
		Rate of change, %	0.0	-80.7	
		<i>A. taxiformis</i> , %, OM basis		CH ₄ , % of GE intake	
Sheep	Feedlot TMR	0 (control)	1.0	6.1 ^a	7
		0.15	1.0	1.0 ^b	
		0.3	1.0	0.9 ^b	
		0.45	1.0	0.8 ^b	
		Rate of change, %	0.0	-86.9	
		BCM, g/100 kg of BW		CH ₄ , g/kg DMI	
Dairy goats	Alfalfa + concentrate	0 (control)	0.99	29.95	8
		0.3	1.04	19.9	
		Rate of change, %	0.5	-33.6	
		BCM, g/100 kg of BW		CH ₄ , g/kg DMI	

DMI = dry matter intake; TMR = total mixed ration; *A. armata* = *Asparagopsis armata*; *A. taxiformis* = *Asparagopsis taxiformis*; GE = gross energy.

^{a, b, c} Values in a column with different superscript letters were significantly different ($P < 0.05$).

¹ Sources: 1 = Tomkins and Hunter (2004); 2 = McCrabb et al. (1997), 3 = Johnson et al. (1972), 4 = Roque et al. (2019a), 5 = Kinley et al. (2020), 6 = Li et al. (2018), 7 = Sawyer et al. (1974), 8 = Abecia et al. (2012).

In addition, bromoform or BCM concentration could be used as an indicator of anti-methanogenic properties, when red seaweeds are used as the active ingredient in ruminant diets (Fig. 2). A polynomial correlation between the concentration of bromoform and in vitro CH₄ emissions shows enteric CH₄ production decreases curvilinearly with increasing bromoform concentration (Fig. 2). Independent of the fit, BCM does not reduce CH₄ production until somewhere around 0.25 mg/g OM and then linearly decreases until CH₄ production is 0 when BCM concentration ranges from 0.8 to 0.9 mg/g OM. It has been found that BCM in red seaweed inhibits methanogen populations in both batch- and continuous-culture systems (Goel et al., 2009). However, most of the research was conducted with freeze-dried seaweed without considering other post-harvest processing methods. Vucko et al. (2017) assessed in vitro influences of different processing methods of *A. taxiformis* in a factorial design based on rinsing (unrinsed vs. dip rinsed/submerged), freezing (frozen vs. not frozen), and drying (freeze-dried vs. kiln-dried/dehydrated) on CH₄ production and the concentration of bromoform. *A. taxiformis* that had been frozen and freeze-dried, irrespective of rinsing, was the most effective at

inhibiting CH₄ emissions. Of these, the unrinsed treatment had the highest bromoform concentration (4.4 mg/g DM) followed by either oven-dried or dehydrated without freezing.

Seaweeds are particularly abundant in their production of haloperoxidase enzymes, and these particular molecules play influential roles in shaping biotic interactions and in marine chemical ecology (Thapa et al., 2020). However, the bromoform is somewhat soluble in water and readily evaporates into the air during handling (e.g., rinsing, freezing, or drying), possibly allowing more to volatilize (EPIC, 2020). The supplementation of green seaweed, *Oedogonium* (0.2 g OM) to different basal diets (1 g OM) decreased CH₄ emission at different rates, by approximately 40% (Dubois et al., 2013), 30% (Machado et al., 2014), and 15% (Machado et al. 2016), when Rhodes grass, Finders grass (*Iseilema* spp.), and Rhodes grass hay were used as basal ingredients, respectively. Therefore, interrelationships between seaweed species and different dietary ingredients are unclear and need to be further studied.

The plant secondary metabolites and the interactions between anti-methanogenic compounds and bioactive ingredients in

Table 7
In vitro studies of methane (CH₄) emissions from red seaweed or bromochloromethane (BCM) supplementation.

System	Basal diet	Treatment	CH ₄ production	Reference ¹			
Ankom	Rhodes grass (<i>Chloris gayana</i>)	<i>A. taxiformis</i> , % DM	CH ₄ , mL/g OM	1			
		Control (no seaweed)	22.2 ^a				
		0.5	19.6 ^b				
		1.0	3.4 ^c				
		5.0	<0.05 ^c				
		10.0	<0.05 ^c				
		Rate of change, %	-99.8				
		Rhodes grass	<i>Oedogonium</i> sp., % DM		CH ₄ , mL/g OM	2	
			Control (no seaweed)		22.2 ^a		
			10.0		20.9 ^a		
50.0	18.4 ^b						
100	6.1 ^c						
Rate of change, %	-72.5						
Batch	Grass-hay	BCM, μmol/L	CH ₄ , mL/100 mL	2			
		Control (no BCM)	15.8 ^a				
		5.0	3.5 ^b				
		10.0	1.1 ^b				
		Rate of change, %	-93.0				
Batch	Meadow hay/corn silage	Seaweeds, 25% DM	CH ₄ , mL/g DM	3			
		Control (no seaweed)	1.75 ^a				
		<i>Ulva</i> sp. (green)	1.30 ^b				
		<i>L. ochroleua</i> (brown)	1.98 ^a				
		<i>S. latissima</i> (brown)	1.81 ^a				
		<i>Gigartina</i> sp. (red)	1.17 ^b				
		<i>G. vermiculopphylla</i> (red)	1.07 ^b				
		Rate of change, %	-38.9				
		CC	Dairy TMR		<i>A. taxiformis</i> , 5% OM	CH ₄ , mL/g OM	4
					Control (no seaweed)	12.08 ^a	
5.0	0.59 ^b						
Rate of change, %	-95.1						

A. taxiformis = *Asparagopsis taxiformis*; DM = dry matter; OM = organic matter; *L. ochroleua* = *Laminaria ochroleua*, *S. latissima* = *Saccharina latissima*; *G. vermiculopphylla* = *Gracilaria vermiculopphylla*; CC = continuous system; TMR = total mixed ration.

^{a, b, c} Values in a column with different superscript letters were significantly different ($P < 0.05$).

¹ Sources: 1 = Machado et al. (2015b), 2 = Goel et al. (2009), 3 = Maia et al. (2016), 4 = Roque et al. (2019b).

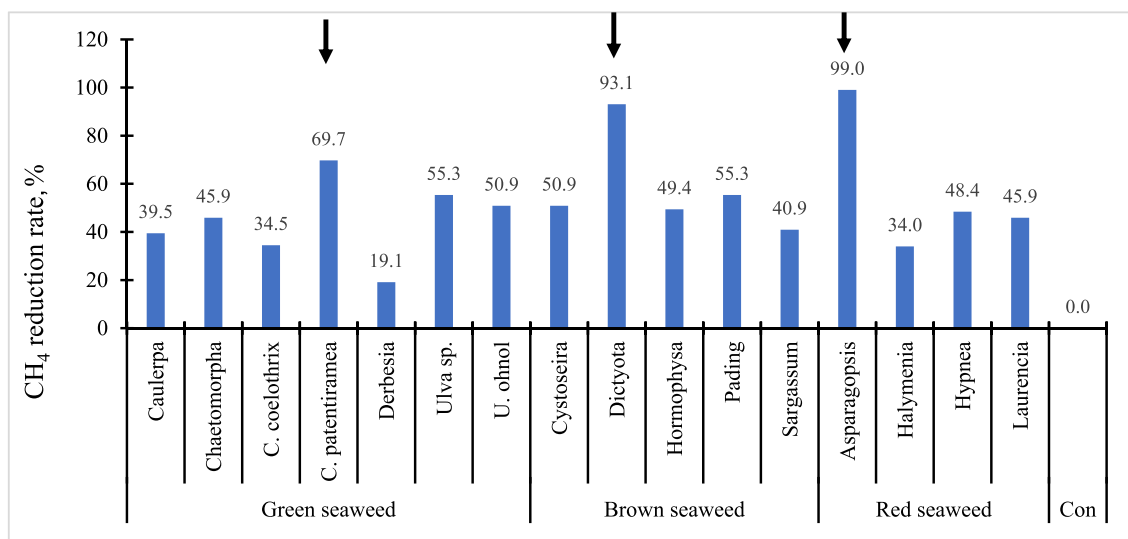


Fig. 1. Various seaweed species (0.2 g OM/seaweed species; green-, brown-, and red-seaweed) and in vitro methane (CH₄) production (mL/g OM) (Adapted from Machado et al., 2014). Con = Control (1 g of Flinders grass + 0.2 g of decorticated cottonseed meal as a positive control, OM basis). Samples of 1 g of Flinders grass + 0.2 g of all other seaweeds (OM basis) were used in this study. *C. coelothrix* = *Cladophora coelothrix*; *C. patentiramea* = *Cladophora patentiramea*; *U. ohnoi* = *Ulva ohnoi*. Pooled rumen fluid as an in vitro inoculum was collected from three fistulated steers (*Bos indicus*) fed Flinders grass (*Iseilema membranacea*) hay. An arrow indicates a lower CH₄ production from seaweed species.

seaweed are proposed in Fig. 3. The dietary carbohydrates that have been digested by a group of rumen microbiota in the rumen with the production of volatile fatty acids (VFA), carbon dioxide (CO₂), and hydrogen (H₂) (Fig. 3). During rumen fermentation, H₂ is

emitted into the rumen through the re-oxidation of the various cofactors (NADH, NADPH, and FADH). The produced CO₂ and H₂ are the key substrates utilized by methanogenic archaea, which is widely accepted as the major methanogenesis pathway in the

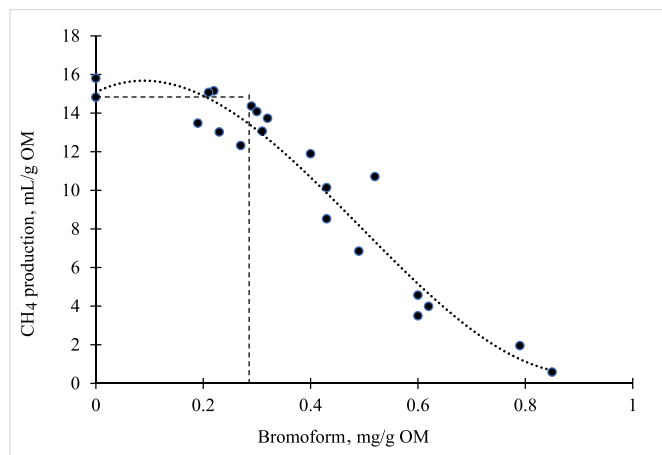


Fig. 2. In vitro correlation between the concentration of halogenated compounds (bromoform or bromochloromethane (BCM; mg/g OM) and the methane (CH₄) emissions (mL/g OM) in the *Asparagopsis taxiformis* (cut off: < 1.0% of bromoform or BCM (mg/g OM)). Adapted from Goel et al. (2009) and Vucko et al. (2017). OM = organic matter.

rumen (Ellis et al., 2008). This is an active process, in which methanogens effectively affect the metabolism of rumen fermentative and acetogenic bacteria via interspecies H₂ transfer (Stams and Plugge, 2009). In addition, other groups of methanogens also use formate, acetate, methanol, methylamines, and alcohol (Ellis et al., 2008). Methanogenic archaea use one of three pathways for methanogenesis: (1) hydrogen-dependent and CO₂-reducing, or hydrogenotrophic (most common); (2) methylotrophic; and, (3) acetoclastic (Berghuis et al., 2019). All three pathways require the gene cluster for CH₄ production known as methyl-coenzyme M reductase (MCR; Ferry and Kestead, 2007; Conrad, 2009).

The schematic diagram of dietary manipulation with seaweed addition, which alters the pathway of fermentation to reduce CH₄, is summarized in Fig. 3. One of the halogenated compounds such as bromoform or chloroform (CHCl₃) is identified to block the function of corrinoid enzymes and to inhibit MCR (Oremland and Capone, 1998). The CHCl₃ (10 μmol/L) can inhibit the production of CH₄ from both H₂/CO₂ and acetate, which means the acetoclastic and hydrogenotrophic methanogens can all be inhibited (Scholten et al., 2000; Liu et al., 2011b). In addition, bromoform-rich *Asparagopsis* spp. is known as an inhibitor of methanogenesis by serving as competitive inhibitors (or analogs) of the MCR, preventing the final catalysis step (Goel et al., 2009). The mode of action is cross-reacting with reduced vitamin B₁₂ and inhibiting the cobamide-dependent methyl-transferase step of methanogenesis (Wood et al., 1968; Chalupa, 1977). However, fluoroacetate (FCH₂COO⁻) only inhibits acetoclastic methanogenesis (Chidthaisong and Conrad, 2000). Therefore, these halogenated compounds block the function of specific enzymes and inhibit MCR together with methyl group transfer in methanogenesis (Fig. 3; Wood et al., 1968; Ellermann et al., 1988; Liu and Whitman, 2008; Yu and Smith, 2000; Ungerfeld et al., 2004; Attwood and McSweeney, 2008; Frey, 2010; Hedderich and Whitman, 2013; Allen et al., 2014; Costa and Leigh, 2014). In addition, bromoform compounds found in *Gracilaria* sp. (red seaweed) are the effect of the reduction in the methanogen population (Prayitno et al., 2018). The same authors reported that bromoform-rich red seaweed inhibited the work of methanogens specifically. Denman et al. (2007b) reported BCM would reduce the activity of coenzyme cobalamin (vitamin B₁₂) and coenzyme MCR. Therefore, halogen-rich seaweed could be a useful tool for the mitigation of enteric GHG emissions as a potential anti-methanogenic agent.

4. The effect of seaweed on ruminal fermentation

The in vitro DM digestibility of various seaweeds (brown, red, and green) has been evaluated (Tayyab et al., 2016; Gojon-Baez et al., 1998; Gaillard et al., 2018). After 72 and 96 h in vitro rumen incubation, the DM or OM digestibility of seaweed species (e.g., *M. pyrifera* and *Sargassum* spp.) varied between 27.9% and 94.6% DM (Tayyab et al., 2016; Gojon-Baez et al., 1998). Inclusion greater than 10% (DM basis) of *A. taxiformis* or *Oedogonium* reduced in vitro OM digestibility of Rhodes grass hay (Machado et al., 2016). Inclusion of *A. armata* in lactating dairy cows fed a dairy TMR diet reduced DMI (Roque et al., 2019a, b) and protein digestibility (Tayyab et al., 2016). However, the in vivo trials of Castro et al. (2009) and Marin et al. (2009), with the inclusion of up to 30% of different seaweed species, *M. pyrifera* and *Sargassum* sp., did not negatively impact digestibility. In addition, North Ronaldsay sheep demonstrated a dietary preference among seaweed species, preferring brown seaweed (*Laminaria digitate* and *Laminaria hyperborean*) (Hansen et al., 2003; Orpin et al., 1985). However, a direct comparison of results between studies is limited due to the use of different methodologies to determine digestibility, different levels of red seaweed inclusion, various plant secondary compounds, and the nature of the basal substrates.

Decreasing methanogenesis could free molecular H₂ for use in pathways that yield rumen fermentation end products (e.g., VFA) that provide an additional energy supply to the host animal, thus increasing the efficiency of feed utilization for growth and milk production. Effects of BCM or seaweed supplementation on rumen fermentation and animal performance are presented in Table 8. In the present study (Table 8), BCM supplementation (0.29 to 0.30 g/100 kg BW) in steers fed various diets decreased acetate (2.0% to 29.4%) and the acetate-to-propionate (A/P) ratio (4.3% to 14.2%) and increased the propionate concentration (3.4% to 11.1%). Furthermore, Bromoform in red seaweed or BCM supplementation markedly reduced A/P ratio in both in vitro and in vivo and resulted in improved average daily gain (ADG; < 21%) or feed efficiency (gain-to-feed [G/F] ratio; 7.6% to 15.4%) in beef steers or dairy cattle (Table 8). These results are consistent with others (Machado et al., 2015b). Their research indicated that total VFA was not affected by *A. taxiformis* treatment, compared with the control. In the presence of *A. taxiformis* (2% OM), however, the concentration of acetate and A:P ratio was decreased by 20%; whereas, propionate concentration was increased by 50%. If ruminal VFA production promotes less acetate production relative to propionate (i.e., lower A/P ratio), the net balance of H₂ in the rumen decreases, resulting in reduced CH₄ formation (van Nevel and Demeyer, 1996), which confirms similar responses in fermentation patterns of ruminants where CH₄ production was inhibited with various halogenated compounds of CH₄ (Trei et al., 1971, 1972; Cole and McCroskey 1975), and other anti-methanogenic agents such as ionophores (Goodrich et al., 1984) and plant tannins (Min et al., 2019). Previous results suggested that moderate levels (<1% DM) of *A. taxiformis* supplementation directly affects propionate production in the rumen (Mitsumori et al., 2012; Roque et al., 2020). When BCM (0.3g/100 kg body weight) was fed to cannulated Brahman-crossbred (*Bos indicus*) beef steers, the total enteric CH₄ emission was reduced by 30% with a subsequent increase in propionate, iso-butyrate, valerate, and iso-valerate concentrations (Denman et al., 2007a, b). These results were consistent with an average decrease of 34% in the number of methanogens enumerated by *mcrA*-targeted real time-PCR. These same authors reported that alternative methanogens (e.g., *Methanococcus*, *Methanosarcina*, and *Methanococcus*) are established following the suppression of major methanogens such as *Methanobacterium* by BCM (Denman et al., 2007a, b; Kobayashi, 2010). However, constraining one biochemical response in a complex system such as

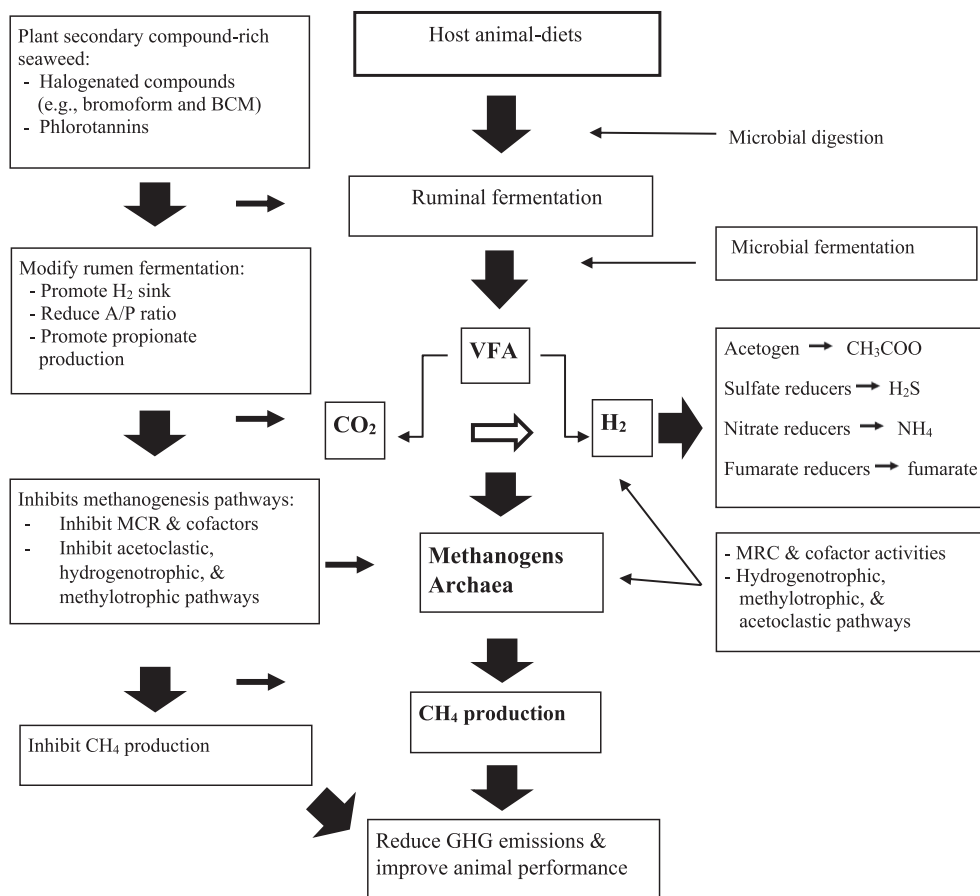


Fig. 3. Proposed schematic microbial fermentation of plant secondary compound (e.g., bromoform, BCM)-rich seaweed and methane (CH₄) reduction pathways in the rumen. Three major pathways of methanogenesis are known: hydrogenotrophic, methylotrophic, and acetoclastic pathways. A/P ratio = acetate-to-propionate ratio; BCM = bromochloromethane, MCR = methyl CoM reductase; VFA = volatile fatty acids; GHG = global greenhouse gas. Sources: Wood et al. (1968), Baptiste et al. (2005), Denman et al. (2007), Attwood and McSweeney (2008), Frey (2010), Allen et al. (2014), Patra (2012, 2016), Machado et al. (2016), Danielsson et al. (2017), Machado et al. (2018), and Roque et al. (2020).

that in the rumen may result in many other interconnected effects, one of which is the inhibition of fiber digestion because of changes in the microbial digestion (van Nevel and Demeyer 1995). Despite this research, mechanisms of associative effects of seaweeds and methanogenesis are not well understood.

5. The effect of seaweed on animal performance and carcass traits

Brown seaweed (*A. nodosum*) is one of the most used and studied seaweed species in livestock industries (Allen et al., 2001a, b; Makkar et al., 2016). *A. nodosum* is a plentiful source of bioactive ingredients such as iodine, minerals, PUFA, vitamins, and phlorotannins (Ragan and Glombitza, 1986; Cvetkovic et al., 2004; Antaya et al., 2015; Makkar et al., 2016). Phlorotannins have the potential benefits of inhibiting ruminal proteolysis (Wang et al., 2008; Zhou et al., 2018) and foodborne pathogens (Connan et al., 2004; Belanche et al., 2016; Zhou et al., 2018; Huang et al., 2018). Although previous studies evaluated the effects of brown seaweed on milk production, heat stress, and animal health in dairy cows (Pompeu et al., 2011; Antaya et al., 2015), the effects of long-term seaweed supplementation on nutrient utilization and plant secondary metabolism are not clear in dairy and beef cattle. Although previous studies evaluated the effects of BCM supplementation on ADG or feed efficiency (G:F ratio) in beef steers (Table 8), the effects of long-term seaweed supplementation on nutrient utilization and animal performance (e.g., ADG and milk production) are not clear

in dairy and beef cattle. It has been reported that no differences or variable responses were found in animal performance (ADG) and carcass quality in Angus-Hereford beef steers along with no significant differences in milk yield and milk components (e.g., fat, protein, lactose, and solid not fat) in Jersey cows fed TMR diets with low levels of *A. taxiformis* (0.25% to 0.5% DM) and *A. nodosum* (113 g/d), respectively (Antaya et al., 2019; Roque et al., 2020). Likewise, no significant effects of 10% seaweed meal (*A. nodosum* and *Laminaria cloustoni*) as a percentage of DM in the diet were observed in Ayrshire dairy cows on milk yield or fat percentage (Burt et al., 1954). In addition, blood concentrations of cortisol, glucose, fatty acids, and thyroxine did not change with feeding control diet or brown seaweed (*A. nodosum*) supplementation (Antaya et al., 2019).

In contrast, the ability of bromoform-containing red seaweed (e.g., *A. taxiformis* and *A. armata*) to reduce CH₄ emissions while improving animal production in ruminants was reported. In multiparous Holstein dairy cow fed a grain-based diet, the addition of a high level of bromoform-containing *A. armata* (1% OM; 1.32 mg/g DM of bromoform) supplementation decreased CH₄ emission (67.2%), DMI (38%), and milk production (11.6%), but no significant changes in body weight and milk composition were noted (e.g., fat, protein, lactose, solid-not fat and bromoform concentration [0.11 vs. 0.15 µg/L]) between cows in the control group compared with those that received the low level of *A. armata* (0.5% OM) inclusion (Roque et al., 2019a). Abecia et al. (2012) reported that milk production was greater (36%) for dairy goats in the BCM-

Table 8
Effect of bromochloromethane (BCM) and seaweed supplementation upon the in vitro and in vivo ruminal fermentation profiles, average daily gain (ADG), and feed efficiency (gain:feed [G:F] ratio) in ruminants.

Item	Acetate, %	Propionate, %	A/P ratio	ADG, kg/d	G:F ratio	Reference ¹
In vivo						
Steers (Brahman-crossbred)						
Control (no BCM)	79.6	17.0	4.7	0.5	0.08	1
BCM (0.3 g BCM/100 kg BW)	78.0	17.6	4.5	0.5	0.09	
Rate of change, %	-2.0	3.4	-4.3	0.0	11.1	
Steers (Brahman)						
Exp. 1 (n = 11): Angleton grass-based diet						
Control (no BCM)	61.6 ^a	21.7 ^b	2.9 ^a	0.23	0.012	
0.29 g BCM/100 kg BW	59.4 ^b	24.4 ^a	2.5 ^b	0.22	0.013	
Rate of change, %	-3.6	11.1	-13.8	-4.34	7.6	
Exp. 2 (n = 8): Rhodes gras-based diet						
Control	64.8	18.6	3.5	0.59	0.033	
0.29 g BCM/100 kg BW	45.7	20.8	3.0	0.62	0.039	
Rate of change, %	-29.4	10.6	-14.2	4.8	15.4	
Steers (Brahman-crossbred)						
Control (no BCM)	-	-	-	1.4	0.18	3
0.3 g BCM/100 kg BW	-	-	-	1.5	0.19	
Rate of change, %	-	-	-	6.7	5.3	
Holstein steers, g/100 kg BW						
Control (no BCM)	-	-	4.75 ^a	0.56 ^b	0.05	4
0.18 g BCM/100 kg BW	-	-	2.27 ^b	0.71 ^a	0.07	
Rate of change, %	-	-	-52.2	21.1	28.6	
Sheep (<i>A. taxiformis</i>), % DM						
0 (control)	65.0	20.8	3.19	-	-	5
0.5	56.3	27.7	2.10	-	-	
1.0	54.4	31.5	1.76	-	-	
2.0	55.0	30.8	1.86	-	-	
3.0	54.5	32.0	1.77	-	-	
Wether						
Control (no BCM)	51.9	26.8	1.94	-	-	6
2.5, mg BCM/kg BW	53.6	24.6	2.18	-	-	
3.0, mg BCM/kg BW	49.3	28.1	1.75	-	-	
Dairy goats						
Control (no BCM)	61.4	11.1 ^b	5.71 ^a	-6.1	-	7
3.0 mg BCM/kg BW	60.3	15.5 ^a	3.92 ^b	-6.6	-	
In vitro						
<i>A. taxiformis</i> , % OM						
0 (control)	66.4 ^a	22.5 ^c	3.0 ^a	-	-	8
0.5	57.2 ^a	27.9 ^b	2.1 ^b	-	-	
1.0	47.4 ^b	33.2 ^b	1.4 ^b	-	-	
5.0	31.5 ^b	46.8 ^a	0.7 ^c	-	-	
10.0	29.1 ^b	46.7 ^a	0.6 ^c	-	-	
BCM, % OM						
0 (control)	74.0 ^a	19.4 ^b	3.8	-	-	8
1.0	69.5 ^b	20.8 ^b	3.4	-	-	
5.0	61.9 ^b	26.8 ^b	2.4	-	-	
10.0	57.4 ^b	29.6 ^a	2.0	-	-	
25.0	57.4 ^b	29.3 ^a	2.0	-	-	
<i>A. taxiformis</i> , % OM						
0 (control)	75.0 ^a	19.2 ^b	3.9 ^a	-	-	9
2	60.4 ^b	28.7 ^a	2.1 ^b	-	-	
Macroalgae ² , 0.2 g OM/g of grass						
Freshwater algae	65.5	24.7	2.7	-	-	10
Green seaweed	64.6	25.9	2.5	-	-	
Brown seaweed	63.3	29.3	2.7	-	-	
Red seaweed	59.3	28.4	2.1	-	-	

BW = body weight; DM = dry matter; *A. taxiformis* = *Asparagopsis taxiformis*; A/P ratio = acetate-to-propionate ratio.

^{a, b, c} Values in a column with different superscript letters were significantly different ($P < 0.05$).

¹ Sources: 1 = Denman et al. (2007b), 2 = McCrabb et al. (1997), 3 = Tomkins and Hunter (2004), 4 = Johnson et al. (1972), 5 = Li et al. (2018), 6 = Sawyer et al. (1974), 7 = Abecia et al. (2012), 8 = Machado et al. (2015a), 9 = Machado et al. (2016), 10 = Machado et al. (2014).

² Data was presented as an average mean value from the fresh-water algae (3 species), green seaweed (7 species), brown seaweed (6 species), and red seaweed (4 species) species.

containing diet due to higher proportions of short-chain fatty acid (e.g., propionate; Park et al., 2007), although the compositions of milk components (fat, protein, lactose, casein, and total solids) were not affected by BCM treatment. Moreover, seaweed supplemented (*Sargassum wightii*) Sahiwal cows had significantly higher milk yield and 4% fat corrected milk (Singh et al., 2015) indicating a potential for optimizing the level of seaweed supplementation to dairy cattle.

According to Anderson et al. (2006), the addition of 2% *A. nodosum* (% DM of seaweed) to a grain-based diet increased carcass marbling scores and increased the percent grading choice by 39.6% of English crossbred steers ($n = 32$) and heifers ($n = 32$). This could explain the improved ADG (1.52 vs. 1.45 kg/d; $P = 0.06$) that was observed in steers fed a corn-based diet with 2% *A. nodosum* supplementation during two 14-d (28-d) feedlot feeding trials, compared to the control diet (Anderson et al., 2006).

It has been reported that beef steers grazing tall fescue (*Festuca arundinacea*) grass that had been sprayed with a seaweed extract solution (Tasco-EX; extracted from *A. nodosum*, Nova Scotia, Canada) had more carcass marbling at harvest in retail cuts than control steers (Allen et al., 2001b). Additionally, Tasco-14, a proprietary brown seaweed meal (Acadian Seaplants Ltd., Dartmouth, Nova Scotia, Canada) has been found to increase marbling score and USDA quality grade in feedlot cattle when supplemented in two 14 d periods (28-d) before slaughter (Braden et al., 2007). Brown seaweed (*A. nodosum*) or supplementation with its extract has had a positive effect on animal health, heat stress tolerance, immune function, increased antioxidant levels, and enhances meat shelf-life, color, and marbling score in beef cattle (Zaki et al., 1994; Behrends et al., 2000; Allen et al., 2001a, b; Montgomery et al., 2001; Saker et al., 2001). Although previous studies evaluated the effects of brown seaweed supplementation to improve animal health, food safety, and carcass characteristics (Fike et al., 2001; Montgomery et al., 2001; Braden, 2003; Braden et al., 2007), the mechanisms involved in seaweed supplementation are currently not well understood for beef cattle diets.

Even though brown seaweed supplementation has the potential to mitigate iodine deficiency in humans via milk consumption (Brito, 2017), there are concerns of excess iodine intake particularly for children (IOM, 2001; Zimmermann et al., 2005). Currently, no conclusive standards exist for iodine levels in milk, but a maximum of 500 µg/L has been advised (EFSA, 2012). A linear increase in milk iodine, which averaged 177, 602, 1,015, and 1,370 µg/L in multiparous Jersey cows fed, respectively, 0, 57, 113, and 170 g/d of brown seaweed (*A. nodosum*) was observed in cows during the winter season (Antaya et al., 2015). Additional research is needed to determine if seaweed type, inclusion rate, and feeding duration impact milk production, milk composition profiles, and animal performance (ADG and feed efficiency).

6. Rumen microbiome adaptation to seaweed

The North Ronaldsay sheep consume a variety of seaweed species (*P. palmata*, *A. esculenta*, *A. nodosum*, *Fucus* sp., and *Laminaria* spp.), but due to animal dietary preference and availability, *Laminaria* spp. accounts for approximately 90% of their total diet (Hansen et al., 2003). North Ronaldsay sheep fed a diet containing *L. digitata* seaweed had rumen microbial communities that differed greatly in ciliate protozoa (e.g., *Dasytricha ruminantium* species) and bacterial populations (*Streptococcus bovis*, *Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, and lactate-utilizing bacterial species) compared to those on a pasture-based diet (Greenwood et al., 1983; Orpin et al., 1985). This is similar to findings of Eadie (1957) and Mitsumori et al. (2012) who reported decreased relative abundance of methanogen, protozoa, and fungi populations when sheep and goats were fed diets containing brown seaweed (*Laminaria* sp.) or BCM (0, 0.5, 2.0, and 5 g/100 kg BW) supplementation. These results, however, are inconsistent with other data (Belache et al., 2016). Their research indicates that tannin-rich (phlorotannins) brown seaweed (*A. nodosum* and *Laminaria digitata*) had no substantial effect on rumen fermentation (VFA, ammonia), feed digestibility, or CH₄ emissions. These same authors reported that the richness of total bacteria, anaerobic fungi, biodiversity indices, and abundances of the main bacterial and methanogen genera were also unaffected by brown seaweed supplementation (Belanche et al., 2016). Likewise, both *A. taxiformis* and *A. armata* have strong activity against ruminal gram-negative and gram-positive bacteria (Paul et al., 2006; Salvador et al., 2007). Besides, *A. taxiformis* has confirmed antimethanogenic activity in in vitro ruminal fermentation studies (Machado et al., 2015b, 2016). Recently, 16S ribosomal RNA (rRNA) gene amplicon

sequencing showed that the relative abundance of methanogens in the fermentation bottles incubated with *A. taxiformis* (1,723 µg bromoform/g DM; Machado et al., 2016) decreased significantly compared to control diets, but this reduction in methanogen richness along with CH₄ production was significant when averaged throughout the experiment (Roque et al., 2019b). This suggests that *A. taxiformis* has a direct effect on the metabolic functionality of rumen methanogens whereas its impact on microbiome congregation, specifically methanogen abundance, is hindered. It strongly inhibits the production of CH₄ when added at a dose of 2% of the OM incubated (Roque et al., 2019b), demonstrating that these red seaweeds are active against archaea for the microbial production of CH₄. These results demonstrated that the impact of seaweed on the rumen microbial community differs according to seaweed species.

Goel et al. (2009) reported that the populations of total bacteria and protozoa were not affected when BCM was added to in vitro batch cultures, but methanogenesis and growth of methanogens were reduced. There was a concomitant decrease in the relative abundance of major methanogens (*Methanobacteriales*, *Methanomassiliococcales*, and *Methanomicrobiales*) although bacterial communities were similar (Machado et al., 2018). The relative abundance of methanogen that received supplementation with *A. taxiformis* (5% OM) were significantly decreased compared to the control diet in the continuous culture system (Roque et al., 2019b). These results, along with recent studies, are in close agreement with the microbial community changes in vitro and in vivo studies (Goel et al., 2009; Mitsumori et al., 2012). In contrast, BCM (3.0 mg/100 kg BW) supplementation did not inhibit the population of bacterial, protozoa, and methanogenic archaea in lactating dairy goats over 57-d although CH₄ emissions were reduced by 33% (Abecia et al., 2012). The disparity in results between Abecia et al. (2012) and Mitsumori et al. (2012) might be explained by the duration of the trial (57-d vs. 8-d feeding trials) and the final concentration of BCM (up to 3 mg BCM vs. 5.0 mg BCM/100 kg body weight) in the diets. The increased duration of the Abecia et al. (2012) study may have provided time for the microbial ecosystem to adapt to the dietary treatment. Williams et al. (2009b) reported that methanogens take longer than 4-weeks to adapt to dietary changes, compared with approximately 15-d for the rumen bacterial community as a whole. Additional research is needed to determine if the duration of feeding BCM impacts the ruminal microbiota population and if methanogenic adaptation occurs.

7. Benefits and challenges of seaweed

Promoting seaweed as a dietary supplement for adaption-based climate change animal production strategies requires a value-added outcome for cattle producers. In recent years, seaweed has been studied as a promising and sustainable feedstock for the livestock industry for the following reasons:

- 1) Seaweed can be used to provide an alternative source of nutrients. Seaweeds are a source of various nutritious compounds including proteins, lipids, vitamins, fatty acids, AA, carbohydrates, minerals, and other nutraceuticals. Seaweed also contains bioactive compounds such as anti-methanogenic, antioxidant, anti-inflammatory, anti-bacterial, or anti-viral agents (Pal et al., 2014; Pirian et al., 2017; Gaillard et al., 2018; Nunes et al., 2018; Roque et al., 2020).
- 2) Dietary supplementation with seaweed biomass would allow for the delivery of phlorotannins or halogenated CH₄ analogs as a holistic approach for the mitigation of enteric CH₄ emissions and animal health compared to the use of extracts or metabolites (Tomkins et al., 2009; Machado et al., 2018; Wang et al., 2009a, b; Kinley et al., 2020). Therefore, seaweed could be a

useful tool for mitigation of enteric GHG emissions without detrimental effects on ruminal fermentation. Although, additional research is needed to determine the seaweed inclusion rate that has positive impacts on animal performance, intake, efficiency, carcass traits, fatty acids profiles in milk and meat, and ruminal health (e.g. anti-inflammatory).

8. Summary of findings

Current research findings support the hypothesis that certain seaweeds decreased CH₄ emissions. However, the available supply of this seaweed (specially bromoform-rich red seaweed) is a dilemma and there are some concerns over its sustainable production and potential negative impacts on the rumen digestibility and health impacts of bromoform. Seaweeds may be alternative feed ingredients for sustainable ruminant production. Although there have been several recent advances in our knowledge of anti-methanogenesis using seaweeds, there are still significant gaps in the in vitro and in vivo experiments to date (e.g., dairy and feedlot cattle performance). Moreover, reducing enteric CH₄ emissions is challenging, and any improved mitigation strategy needs to be sustainable, practical, and economically feasible, thus ensuring the functional capacity of the rumen microbiome for ruminal fermentation and improved animal productivity. Future research will need to address the unsolved issues in existing animal performance, rumen microbiome changes, reproduction performance, immune-related animal health, and milk composition and milk quality. Additionally, to attain a comprehensive consideration of the methanogenesis responsible for the significant reduction of CH₄, and its probable long-term influence on ruminants, rumen fluid metabolomic profiles associated with feed efficiency and the host animal are warranted.

Author contributions

B.R. Min and **D. Brauer** designed the model, the computational framework, analyzed the data, and wrote the manuscript. **D. Parker** and **H. Waldrip** assisted with data analysis and helped the overall directions. **C. Lockard**, **K. Hales**, **A. Akbay**, and **S. Augyte** provided critical feedback and helped shape the analysis and manuscript. All authors discussed the results and commented on the manuscript.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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