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

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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.

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). Ruminal 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 ruminal 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
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 diet are low dietary (DM) had positive effects in rumen 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 (Asphodelle nodosum meal; Tasco-14) had no relative abundance of fecal Escherichia coli O157:H7 with no enhanced animal performance (Bac 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 effect 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 identified at lower concentrations (Marshall et al., 1999; Briska 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; Kolanjathan 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 (Gian 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 38.1% CP (e.g., Colpomenia solida, Enteromorpha, and Enteromorpha). It was reported to have higher CP content (12% to 23% DM) compared to other species collected from the Gulf of Mannar coast, India (Abirami and Kowsala, 2012). In addition, Pirian et al. (2017) reported that CP contents were 12.3% and 9.0% in general algae (Carpagia (superioris) 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).
Nutritional studies are needed to evaluate both the nutritional benefits 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 phytotoxins in seaweeds are phlorotannins and halogenated compounds. Studies of the effects of feed iodine and iodine antagonists 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 and minimize the risk of hypothyroidism in the livestock species and condition (Makkar et al., 2016). Animal nutrition societies generally recommend iodine intake in the range of 0.5–0.8 mg I/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).

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 (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.

Table 1: Nutraceutical and pharmacological potential of some seaweeds.

| Species                  | Compounds                              | Properties                                   | References |
|--------------------------|----------------------------------------|----------------------------------------------|------------|
| **Red seaweed (Rhodophyta)** |                                        |                                              |            |
| *Asparagopsis taxiformis* | Alkaloids, flavonoids, anthraquinones, | Antioxidant, antiproliferative, free          | 1, 2, 34, 35 |
|                          | phensols, chlorophylls, halogenated     | radical scavenging, antimethanogenesis       |            |
| *Asparagopsis armata*     | Halogenated compounds                  | Antimicrobial, antitumor activity            | 3, 35      |
| *Chondria armata*         | Galactoglycerolipids                    | Antimicrobial                               | 4, 5       |
| *Corallina pilulifera*    | Phlorotannins                           | Antioxidant and tyrosinase pathways          | 6, 7       |
| *Corallina tamariscifolia*| Phlorotannins                           | Anti-inflammatory, antioxidant               | 8          |
| *Eucheuma cava*           | Phlorotannins, Lectins                  | Antioxidant, UV protection, antibacterial    | 9, 10, 11  |
| *Laurencia pacifica*      | Laurinterol, Bromophenols, sesquiterpene| Antibacterial, antioxidant                   | 12, 13, 14 |
| *Gracilaria spp.*         | Steroid, terpenoid, eiconoid            | Antibacterial                               | 14         |
| *Rhodomelæa spp.*        | Bromophenols                            | Anti-microbial                              | 14         |

**Green seaweed (Chlorophyta)**

| Species                  | Compounds                              | Properties                                   | References |
|--------------------------|----------------------------------------|----------------------------------------------|------------|
| *Cladophora glomerata*   | Chlorophylls                           | Antioxidant, antibacterial                    | 15, 16, 17 |
| *Caulerpa sp.*           | Flavonoids, phenols, saponin           | Tyrosinase inhibitor                         | 18         |
| *Haematococcus lacustris*| Carotenoids                            | Antioxidant, anti-inflammatory               | 19, 20, 21 |
| *Ulva lactua*            | Chlorophylls                           | Antibacterial, antioxidant                   | 22, 23, 24 |
| *Dunaliella tertiolecta* | Phenolics                              | Anti-aging                                   | 25         |

**Brown seaweed (Ochrophyta)**

| Species                  | Compounds                              | Properties                                   | References |
|--------------------------|----------------------------------------|----------------------------------------------|------------|
| *Ascophyllum nodosum*     | Phlorotannins                           | Anti-bacterial, inhibit rumen fermentation    | 26, 27     |
| *Cystoseira tamariscifolia*| Sulfated polysaccharide/Phlorotannins  | Anti-inflammatory                            | 28         |
| *Ecklonia cava*           | Sulfated polysaccharide                 | Anti-viral, antioxidant, anti-inflammatory, tyrosinase inhibition | 29, 30,31  |
| *Ecklonia bicyle*         | Sulfated polysaccharide                 | Antiviral, antioxidant, anti-inflammatory    | 32, 33     |
| *Himanthalia elongata*    | Volatile fatty acids                    | Control iodine deficiency disorders and animal weight gain | 34         |
| *Laminaria digitata*      | Iodine                                  | Antioxidant, antimicrobial                    | 35         |

1 Sources: 1 = Nunes et al. (2018), 2 = Neethu et al. (2017), 3 = Horta et al. (2019), 4 = Al-Fadhl 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 = Samarakoong and Jeon (2012), 12 = Fenical (1976), 13 = Liu et al. (2011a), 14 = Kasanahe 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 = Samarakoong and Jeon (2012), 31 = Heo et al. (2009), 32 = Chizhov et al. (1999), 33 = Wijesinghe and Jeon (2019), 34 = Plaza et al. (2010), 35 = He et al. (2002).
Table 2
Chemical composition of seaweed species (all values on DM basis).

| Type            | Red seaweed | Asparagopsis taxiformis | Asparagopsis armata | Brown seaweed |
|-----------------|-------------|-------------------------|---------------------|---------------|
| Species         | Porphyra spp. |                        |                      | Asparphyllum nodosum | Macrocystis Sp. | Laminaria Sp. | Costaria Costata |
| 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                |                |
| Ether extract   | 3.5–6.5     | 0.4                     | 0.8                 | 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                     | 4.4                 | 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                     | 1.18                | 1.24          | 134.0            | 117.0        | 233.2             |                |
| Mn              | 0.1         | 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           |
| Bromoform2      | –           | 1.723                   | 1.320               | 15012        | 2.71             | 15012        | 49.7              |                |
| Phlorotannins   | –           | 5.0–6.01                | 5.0                 | 1.0–2.01      | 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.

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),

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

| 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                                | 0.80            | 0.50                                        |
| 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 Ernährungsp生理ology; 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.

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

3 Minor level of total phenolic compounds (Nunes et al., 2018).

4 Minor levels of condensed tannins (Kafi et al., 2020; Carpenter and Liss, 2000).

5 Minor level of total phenolic compounds (Nunes et al., 2018).

6 Data for pigs with 0.100 to 0.80 mg/kg dry matter.

7 Data for beef cattle with 0.100 to 0.80 mg/kg dry matter.

8 Data for dairy cattle with 0.100 to 0.80 mg/kg dry matter.

9 Data for sheep with 0.100 to 0.80 mg/kg dry matter.

10 Data for goats with 0.100 to 0.80 mg/kg dry matter.

11 Data for poultry with 0.100 to 0.80 mg/kg dry matter.

12 Data for swine with 0.100 to 0.80 mg/kg dry matter.

13 Data for livestock with 0.100 to 0.80 mg/kg dry matter.

14 Data for ruminants with 0.100 to 0.80 mg/kg dry matter.

15 Data for poultry with 0.100 to 0.80 mg/kg dry matter.

16 Data for swine with 0.100 to 0.80 mg/kg dry matter.

17 Data for ruminants with 0.100 to 0.80 mg/kg dry matter.

18 Data for poultry with 0.100 to 0.80 mg/kg dry matter.
dibromochloromethane (CHBr₂Cl; 0.158 µg/g DM), bromochloroacetate (C₂H₂BrClO₂; 0.088 µg/g DM), and dibromoacetate (C₂H₆Br₂O₂; 0.009 µg/g DM; Table 2). The other seaweeds, including *Macrocystis pyriforma, Ulva sp.*, *Eisenia arborea, Laminaria farlowii, Egregia 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).

### Table 4

Amino acid (AA) composition (g/100g of protein) of various seaweed species.

| Type               | Red seaweed       | Green seaweed | Brown seaweed      | Soybean meal |
|--------------------|-------------------|---------------|--------------------|--------------|
|                    | *Porphyra columbina* | *Asparagopsis taxiformis* | *Ulva Spp.* | *Ascosiphum nodosum* | *Macrocystis pyriforma* | *Laminaria digitata* | 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          | 2.0-5.9            | trace        | 3.5                        | 1.96                      | 1.38         |
| Valine             | 5.85              | 6.19          | 4.4-6.7            | 3.7          | 5.76                        | 4.45                      | 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          | 10.44                       | 4.06                      | 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          | 5.8-8.7            | 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), Phorphyra 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); Ascosiphum nodosum was harvested off the coast of Nova (Angell et al., 2012); The samples of Macrocystis pyriforma was collected in summer in Bahía Tortugás, Baja California Sur (Castro-Gonzalez et al., 1994); Laminaria digitata was collected in Trout 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 Ginniken 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, Kinley et al. (2020) reported that the low levels of red seaweed (*A. taxiformis*; 0.05% to 0.2% DM) 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 (<0.2% OM) 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/propanate 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 patens* (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. canalliculata*) 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).

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**Table 5**

| Item   | Red                        | Green                               | Brown                        |
|--------|----------------------------|-------------------------------------|------------------------------|
|        | *Asparagopsis taxiformis*  | *Porphyra dioica*                   | *Asphyllum nodosum*          |
|        |                            | *Ulva rigida*                       |                              |
|        |                            | *Codium tomentosum*                 |                              |
| C14:00 | 3.77                       | 23.3                                | 9.4                          |
| C16:00 | 3.73                       | 18.3                                | 13.4                         |
| C18:00 | 1.18                       | 4.9                                 | 2.6                          |
| C18:1  | 3.52                       | 3.3                                 | 2.6                          |
| C18:2n-6 | 7.75                     | 1.7                                 | 4.9                          |
| C20:1  | 0.6                        | 1.2                                 | 4.9                          |
| C20:2n-6 | 1.38 (C20:3)          | 0.6                                 | 0.22                         |
| C20:4n-6 | 1.19 (C20:4)           | 2.7                                 | 5.05                         |
| C20:5n-3 | 1.6 (C20: 5)       | 20.5                                | 27.8                         |
| C22:6  | 32.77                      | 1.4                                 | 7.24                         |
| SFA    | 23.17                      | 24.1                                | 25.1                         |
| MUFA   | 19.52                      | 13.0                                | 31.5                         |
| PUFA   | 46.97                      | 13.0                                | 43.5                         |
| Ave. USFA | 66.49                  | 26.0                                | 75.0                         |
| SFA/USFA | 0.35                    | 0.93                                | 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.
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 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).

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

| Animal          | Basal diet              | Treatment                  | DMI, kg/d | CH4 production | Reference |
|-----------------|-------------------------|----------------------------|-----------|----------------|-----------|
| Beef steers     | Feedlot TMR (total mixed ration) | BCM, g/100 kg of BW | 0 (control) | 6.2b | 8.7* | 1 |
|                 |                         |                            | 0.15  | 7.4a | 3.8ab |
|                 |                         |                            | 0.30  | 5.6b | 1.4b |
|                 |                         |                            | 0.60  | 5.5b | 0.8b |
| Rate of change, % |                         |                            | −11.3 | −95.2 | |
| Beef steers     | Alfalfa hay             | BCM, g/100 kg of BW        | 0 (control) | 8.1a | 205.5a | 2 |
|                 |                         |                            | 1.2    | 7.5b | 0.24b |
| Rate of change, % |                         |                            | −7.4   | −90.6 | |
| Beef steers     | Feedlot TMR             | BCM, g/100 kg of BW        | 0 (control) | 10.4 | 20.0* | 3 |
|                 |                         |                            | 0.98   | 10.3 | 0.1b  |
| Rate of change, % |                         |                            | −0.96  | −99.5 | |
| Dairy cows      | Dairy TMR               | A. armata, % OM basis      | 0 (control) | 27.9a | 15.0* | 4 |
|                 |                         |                            | 0.5    | 24.9b | 12.0b |
|                 |                         |                            | 1.0    | 17.3b | 7.5b  |
| Rate of change, % |                         |                            | −38.0  | −50.0 | |
| Beef steers     | Feedlot TMR             | A. taxiformis, % OM basis  | 0 (control) | 8.4  | 10.4  | 5 |
|                 |                         |                            | 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 | |
| Sheep           | High-fiber pellet       | A. taxiformis, % OM basis  | 0 (control) | 1.0  | 15.0* | 6 |
|                 |                         |                            | 0.5    | 1.1  | 12.7ab |
|                 |                         |                            | 1.0    | 1.0  | 7.00  |
|                 |                         |                            | 2.0    | 1.1  | 5.60  |
|                 |                         |                            | 3.0    | 1.0  | 2.90  |
| Rate of change, % |                         |                            | 0.0    | −80.7 | |
| Sheep           | Feedlot TMR             | BCM, g/100 kg of BW        | 0 (control) | 1.0  | 6.1*  | 7 |
|                 |                         |                            | 0.15   | 1.0  | 1.00  |
|                 |                         |                            | 0.3    | 1.0  | 0.90  |
|                 |                         |                            | 0.45   | 1.0  | 0.80  |
| Rate of change, % |                         |                            | 0.0    | −86.9 | |
| Dairy goats     | Alfalfa + concentrate   | BCM, g/100 kg of BW        | 0 (control) | 0.99 | 29.95 | 8 |
|                 |                         |                            | 0.3    | 1.04 | 19.9  |
| Rate of change, % |                         |                            | 0.5    | −33.6 | |

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

Values in a column with different superscript letters were significantly different (P < 0.05).

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).
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 seaweed are proposed in Fig. 3. The dietary carbohydrates that have been digested by a group of rumen microbiota in the rumen with

![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 seaweed (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. A arrow indicates a lower CH₄ production from seaweed species.](image)

Table 7

| System   | Basal diet       | Treatment                        | CH₄ production | Reference |
|----------|------------------|----------------------------------|----------------|-----------|
| In vitro studies of methane (CH₄) emissions from red seaweed or bromochloromethane (BCM) supplementation. | | | | |
| Ankom    | Rhodes grass (Chloris gayana) | A. taxiformis, % DM                | CH₄, mL/g OM   | 1         |
|          |                  | Control (no seaweed)              | 22.2           |           |
|          |                  | 0.5                               | 19.6           |           |
|          |                  | 1.0                               | 3.4            |           |
|          |                  | 5.0                               | <0.05          |           |
|          |                  | 10.0                              | <0.05          |           |
|          |                  | Rate of change, %                 | −99.8          |           |
| Rhodes grass | Oedogonium sp., % DM                | CH₄, mL/g OM   | 2         |
|          |                  | Control (no seaweed)              | 22.2           |           |
|          |                  | 10.0                              | 20.9           |           |
|          |                  | 50.0                              | 18.4           |           |
|          |                  | 100                               | 6.1            |           |
|          |                  | Rate of change, %                 | −72.5          |           |
| Batch    | Grass-hay        | BCM, µmol/L                       | CH₄, mL/100 mL | 2         |
|          |                  | Control (no BCM)                  | 15.8           |           |
|          |                  | 5.0                               | 3.5            |           |
|          |                  | 10.0                              | 1.1            |           |
|          |                  | Rate of change, %                 | −93.0          |           |
| Batch    | Meadow hay/corn silage | Seaweeds, 25% DM                | CH₄, mL/g DM   | 3         |
|          |                  | Control (no seaweed)              | 1.75           |           |
|          |                  | Ulva sp. (green)                  | 1.30           |           |
|          |                  | L. ochroleus (brown)              | 1.98           |           |
|          |                  | S. latissima (brown)              | 1.81           |           |
|          |                  | Gigartina sp. (red)               | 1.17           |           |
|          |                  | G. vermiculophylla (red)          | 1.07           |           |
|          |                  | Rate of change, %                 | −38.9          |           |
| CC       | Dairy TMR        | A. taxiformis, 5% OM              | CH₄, mL/g OM   | 4         |
|          |                  | Control (no seaweed)              | 12.08          |           |
|          |                  | 5.0                               | 0.52           |           |
|          |                  | Rate of change, %                 | −95.1          |           |

* A. taxiformis – Asparagopsis taxiformis; DM – dry matter; OM – organic matter; L. ochroleus – Laminaria ochroleus; S. latissima – Saccharina latissimi; G. vermiculophylla – Gracilaria vermiculophylla; CC – continuous system; TMR – total mixed ration.

* a, b, c Values in a column with different superscript letters were significantly different (P < 0.05).

* Sources: 1 = Machado et al. (2015b), 2 = Goel et al. (2009), 3 = Masa et al. (2016), 4 = Roque et al. (2019b).
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 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) aceticlastic (Berghuis et al., 2019). All three pathways require the gene cluster for CH₄ production known as methyl-coenzyme M reductase (MCR; Ferry and Kaste, 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 aceticlastic and hydrogenotrophic methanogenic processes can all be inhibited (Scholtten 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, fluorooacetate (FCH₂COO⁻) only inhibits aceticlastic methanogenesis (Chidhasiong 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). In addition, bromoform compounds found in Gracilariopsis 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 that bromoform 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. arnuta 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 Marín 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 hyperborea) (Hansen et al., 2003; Orpin et al., 1985). However, a direct comparison of results from in vivo 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 rumin 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 rumin decreases, resulting in reduced CH₄ formation (van Nevel and Demeyer, 1997), 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-butryate, 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., Methanomicrobium, 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 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 (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.
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-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

### 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 |
|------|------------|---------------|-----------|-----------|-----------|-----------|
| 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      | 2         |
| Rate of change, % | -2.0       | 3.4           | -4.3      | 0.0       | 11.1      |           |
| Steers (Brahman) |            |               |           |           |           |           |
| Exp. 1 (n = 11): Anglegrass based diet |            |               |           |           |           |           |
| Control (no BCM) | 61.6       | 21.7          | 2.9       | 0.23      | 0.012     | 3         |
| 0.29 g BCM/100 kg BW | 59.4       | 24.4          | 2.5       | 0.22      | 0.013     |           |
| Rate of change, % | -3.6       | 11.1          | -13.8     | -4.3      | 7.6       |           |
| Exp. 2 (n = 8): Rhodes grass based diet |            |               |           |           |           |           |
| Control | 64.8       | 18.6          | 3.5       | 0.59      | 0.033     | 4         |
| 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) |            |               |           |           |           |           |
| 0.3 g BCM/100 kg BW |            |               |           |           |           |           |
| Rate of change, % |            |               |           |           |           |           |
| Holstein steers, g/100 kg BW |            |               |           |           |           |           |
| Control | 47.4       | 20.8          | 3.0       | 0.62      | 0.039     | 5         |
| 0.18 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      |           |
| Sheep (A. taxiformis), % DM |            |               |           |           |           |           |
| 0 (control) | 65.0       | 20.8          | 3.19      | -         | -         | 6         |
| 0.5 | 56.1       | 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      | -         | -         | 7         |
| 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          | 5.71      | -6.1      | -         | 8         |
| 3.0 mg BCM/kg BW | 60.3       | 15.4          | 3.02      | -6.6      | -         |           |

**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).

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).

* 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.

(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-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 performance (ADG and feed efficiency) in ciliate protozoa (e.g., Dasytricha ruminantium, B. ruminantium), but due to animal dietary preference and availability, great differences in ciliate protozoa (e.g., Dasytricha ruminantium, B. ruminantium) were observed in cows during the winter season (Antaya et al., 2015). Additional research is needed to determine if seaweed supplementation is 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, 1015, and 1370 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. digitate 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, 165 ribosomal RNA (rRNA) gene amplicon sequencing showed that the relative abundance of methanogens in the fermentation bottles incubated with A. taxiformis (1,723 μg bromofom/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 methanogen community and growth of methanogens were reduced. There was a concomitant decrease in the relative abundance of major methanogens (Methanobacteriales, Methanomassiliicoccales, 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 considerate 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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