A Seminar Paper

On

Feeding Seaweed to Ruminant: A Potential Approach to Reduce Enteric Methane Emission and Increase Livestock Production

Course Title: Seminar

Course Code: ASN 698

Term: Winter 2022

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Feeding Seaweed to Ruminant: A Potential Approach to Reduce Enteric Methane Emission and Increase Livestock Production[1]

By

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ABSTRACT

Seaweeds are macro algae which mainly found in coastal areas have a good reputation as animal food because of their abundancy and easy harvest method. They may be red, brown or green. They are different in color, size, compositions. Thus, seaweeds are rich in essential nutrients and minerals which are useful to animal. That's why seaweed can be a good supplement to ruminant feeds as food scarcity is increasing day by day throughout the world. Besides seaweeds has some antimethanogenic properties which can be a great weapon to reduce enteric methane emission from ruminants. Red seaweed contains bromoform which can be used as antimethanogenic properties indicator. Brown seaweed contain phlorotannin which also has methane reducing activities. However, a lot of studies shows different inconsistent data about sea weeds antimethanogenic activity and its replacement as animal feed. In this work, we will review the exiting data regarding seaweeds nutrient composition and its antimerhanogenic properties. Increasing effort to this topic will surely develop the understanding about sea weed and its effects in animal production system and reducing enteric methane emission which will lead us to novel approach to mitigate greenhouse gas.

Keywords: Seaweed; feed supplement; methane reduction; livestock production; climate change

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[1]A seminar paper for the course ASN 698, winter'22.

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CHAPTER 1

INTRODUCTION

Due to overpopulation, shrinking land area and rapid growth of world economy the demand for livestock products are also increasing around the world (Thornton, 2010). But there are some serious shortages of animal feed for sustainable livestock production around the world. Most of the feeds available especially grains are exclusively used for human consumption (Huque and Sarker, 2014). That's why scientists are always in search of new feed alternatives to minimize the feed problem.

Seaweeds typically grow in aquatic environments and are commonly utilized by coastal communities for various purposes (Evans et *all.,* 2016). Seaweed is a beneficial option as an ingredient for livestock, with macro algae presenting variations in their proteins, minerals, lipids, and fibers. Their lipid content is typically low, with a concentration mainly of polyunsaturated fatty acids (PUFAs) and they are rich in polysaccharides. Algae have a relatively high protein quality compared to cereal and soy flour, with more than 75% of seaweed having higher proportions of total EAAs than wheat flour and 50% higher than soy flour and also higher than rice and corn (Maehre *et al.,* 2014). The proportion of EAAs such as methionine and lysine are comparable to traditional protein sources. Seaweeds are rich in essential minerals such as potassium, sodium, calcium, magnesium, and phosphorus, as well as trace elements like iron, manganese, copper, zinc, cobalt, selenium, and iodine (Corino *et al.,* 2019). Seaweed is a highly beneficial marine organism that also contains natural antioxidants, antimicrobials, vitamins such as A, B_1, B_{12}, C, D and E (Azenha *et al.,* 2019). Seaweeds are simple organisms that use photosynthesis to convert carbon dioxide into sugars and oxygen, utilizing sunlight as their energy source. Some of the most common edible varieties of seaweed *include Neopyropia/Porphyra/Pyropia spp., Undaria pinnatifida, Saccharina latissima*, *Palmaria palmata,* and *Chondrus crispus,* which are associated with a range of health benefits including reducing blood pressure, preventing blood clots, and serving as a valuable source of protein (Øverland *et al.,* 2019). Several species of algae with beneficial biological activity have been identified for use in diets for various animals. Seaweed is one of them.

The livestock industry is a significant contributor to global greenhouse gas emissions, accounting for 14.5% to 19% of emissions (Johnson and Johnson, 1995; Gerber *et al.*, 2013). This is due to ruminal methane (CH_4) emission from the fermentation of carbohydrates by ruminal microbiota in sheep, goats, and cattle, which results in a loss of 2% to 12% of ingested energy (Johnson and Johnson, 1995). So, strategies to reduce CH_4 enteric production may lead to a win-win situation to the environment and economy of the livestock sector to reduce CH_4 emissions and increase metabolizable energy in ruminant diets. Researchers have investigated various CH_4 inhibitors such as ionophores, chemical compounds, legumes, essential oils, fats, probiotics, and plant secondary metabolites like halogenated, phlorotannins, tannins, saponins, and iodine (Patra, 2012; Min *et al.*, 2020). Seaweed is proving to be one of the most promising ways to decrease methane production in the rumen, despite the use of various feed additives for this purpose. This is because seaweed contains diverse bioactive compounds, including halogenated and polyphenolic metabolites that can inhibit methanogenesis in the rumen (Kinley, R. D. *et al.*, 2020; Roque, B. M. *et al.*, 2019) *Asparagopsis taxiformis* a species of red seaweed, contains bromoform and dibromochloromethane, which inhibit enzymatic activities by binding to vitamin B_{12}. This chemical action is similar to coenzyme F430, which is a cofactor required for methanogenesis.

One particular species of brown seaweed has evolved to accumulate polyphenol compounds, including phlorotannins, as a defense mechanism against stress and herbivory (Belanche *et al*., 2016; Li *et al*., 2011). Previous research has shown that phlorotannins extracted from *Ascophyllum nodosum* have an antimethanogenic effect on the rumen without affecting microbial fermentation (Wang *et al.*, 2009).

So, considering the nutrient content and antimethanogenic properties seaweed can play a vital role in reducing enteric methane emissions and sustainable livestock production.

Objectives

1. To investigate the potentiality of seaweeds as feed supplements for ruminants
2. To understand the role of sea weed in methane mitigation from ruminants

CHAPTER 2

MATERIALS AND METHODS

This is a review paper. So, all of the data presented here are collected from secondary sources. Different published articles, papers in various journals and internet are used as a source of data. Then i developed this paper by the instructions from my respected major professor and course instructors. After that I compiled the paper and presented it in this form.

CHAPTER 3

REVIEW OF FINDINGS

3.1 Seaweed

Seaweeds are marine macroalgae growing along seashores, consisting of different colors (red, green, brown). It can be edible or nonedible based on their colors, size and species. Seaweeds composed of different bioactive and nutritional compounds which is important in medicine, therapeutics, human consumptions, animal feed etc.

3.2 Nutrient composition of different seaweeds

Sea weeds have various bioactive properties including anti-viral, anti-microbial, anti-tumor, anti-inflammatory, antioxidant, and many more. 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).

Table 1. Nutraceutical and pharmacological potential of some seaweeds

Species	Compounds	Properties
Red seaweed		
Asparagopsis taxiformis	Alkaloids, Flavonoids, Phenols, Chlorophylls, Halogenated compound	Antioxidants, Free radical scavenging, Antimethanogenesis
Asparagopsis armata	Halogenated compound	Antimicrobial and antitumor properties
Laurencia pacifica	Bromophenols, Laurinterol	Antibacterial, Antioxidants
Green seaweed		
Ulva lactuca	Chlorophylls	Antibacterial, Antioxidants
Haematococcus lacustris	Carotenoids	Antioxidants, Anti-inflammatory

Brown seaweed		
Ascophyllum nodosum	Pholorotanins	Antibacterial, inhibit rumen fermentation
Laminaria digitata	Iodine	Control iodine disorder and weight gain

Source: (Nunes *et al.,* 2018)

Compared to green and brown seaweed, red seaweed generally has a higher crude protein (CP) content, with some species like *Porphyra spp*. containing as much as 38.1% CP in the dry matter (Cian et *al.,* 2015). Green seaweed has moderate CP levels, ranging from 15.3% to 18.6% DM, while brown seaweed has lower CP contents, ranging from 6.0% to 16.6% DM. However, some species of green seaweed, such as Ulva reticulate, *Ulva lactuca*, *Ulva fasciata,* and *Enteromorpha,* have been found to have higher CP content of between 12% to 23% DM compared to other species (Abirami and Kowsalva, 2012). Similarly, studies have shown that green algae (*Caulerpa sertulariodes*) and brown algae *(Colpomenia*) have CP contents of 12.3% and 9.0% respectively in Persian Gulf seaweed (Pirian *et al.,*2017). The CP content of red seaweed is similar to that of high protein plant feeds like soy and soybean meal (Kuiken and Lyman, 1949; Norziah and Ching, 2000).

The protein content of seaweeds varies depending on the species and seasonal period s (Mishra *et al.,* 1993; Castro-Gonzalez *et al.,* 1994; Fleurence, 1999; Guiry and Guiry 2014; Pirian *et al.,* 2017) Seaweeds have potential as a food protein and animal feed, but more research is needed to determine the appropriate type and feeding rate to avoid negative effects on animal productivity. Seaweeds have high levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) due to their high polysaccharide content (Lahaye, 1991). Red seaweed generally has higher levels of NDF than green and brown seaweed. Seaweeds have a different cell wall structure compared to land plants, consisting mainly of alginates with some cellulose, xylan and xyloglucan. However, the active polysaccharide components in seaweed are still fermentable in the ruminant digestive system (Hehemann *et al.,* 2010). Seaweed supplementation has potential to reduce methane emissions l (Morais *et al.*, 2020) but more studies are needed to evaluate its nutritional benefits and any potential negative effects on animal health and productivity

Table 2. Nutrient composition of different seaweed (On DM basis)

Type	Red Seaweed		Green Seaweed	Brown seaweed	
Species	*Porphyra spp*	*Asparagopsis taxiformis*	*Ulva Spp.*	*Macrocystis spp.*	*Laminaria spp.*
Nutrients %					
CP	24.6-38.1	17.8	15.3-18.6	10.1	9.8-16.6
NDF	43.1	36.9	22.8-26.2	19.9	16.6
ADF	6.6	11.6	7.6-8.7	12.6	-
EE	0.3-0.5	0.4	1.2	0/6	0.8
Ash	6.5-8.7	-	7.7-23.2	32.9	29.9-31.5
Minerals %					
Ca	4.4	3.8	2.9	14.1	0.08
P	3.8	0.2	0.27	2.9	-
Na	4.1	6.6	2.0-3.3	36.5	25.3
Mg	4.9	0.8	1.7	39.2	5.5

Source: (Abirami *et al.*, 2013)

3.3 Secondary metabolites

Seaweeds have a long history of being used as feed for livestock. However, the chemical composition of seaweed can vary greatly depending on the species, seasons, and environment where they grow (Makkar *et al.*, 2016). The most commonly studied phytochemicals in seaweed are phlorotannins and halogenated compounds. Iodine is an important element found in seawater and both humans and animals require it for proper thyroid function. Iodine deficiency can lead to hypothyroidism, goiter formation, and other health problems (Laurberg *et al.*, 1998). Adequate iodine intake is necessary to maintain thyroid function, facilitate high performance, and prevent deficiency.

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 dibromochloromethane (0.158 mg/g DM), bromochloroacetate (0.088 mg/g DM), and dibromoacetate (0.009 mg/g DM; Table 2). The other seaweeds, including *Macrocystis* *pyrifera*, *Ulva sp*., *Eisenia arborea*, *Laminaria farlowii*, *Egregia menziesii*, and *Cystoseira osmundacea*, produce negligible amounts of halogenated compounds (Table 2) such as chloromethane, bromomethane, methyl iodide, bromomethane bromide, and bromoform (Dembitsky and Tolstikov, 2003). Phlorotannins, which are a type of polyphenol compound, are commonly found in all types of seaweed, but they are most abundant in brown seaweed. The concentration of phlorotannins in brown seaweed can range from 20 to 140 grams per kilogram of dry weight. 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

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

Item	USA	UK	Germany
Dairy cattle	0.50	0.80	0.50
Growing calves/bulls	0.50	0.12	0.25

(Source: Min et al. 2021)

ration was increased from 51.5% to 64.9% DM (Leupp *et al.*, 2005). These effects are possibly related to changes in the rumen microbiome community diversity in cattle as reported by (Ushakova *et al.*,2006). Furthermore, several studies have indicated that feeding beef cattle with seaweeds containing phlorotannins can reduce the excretion of foodborne pathogens like *E. coli*. (Wang *et al.*, 2009; Evans and Critchley, 2014; Huang *et al.*, 2018). The outcomes of these studies have verified that including tannins in the diet of ruminants could be a beneficial strategy for decreasing the levels of foodborne pathogens in their digestive system. As a result, the risk of carcass contamination can be reduced, which can ultimately improve the safety of the food (Min *et al.*, 2007; Huang *et al.*, 2018).

3.4 Amino acid content of various seaweeds

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 acid (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. Seaweeds, such as *Ulva linza, Sargassum vulgare* and *Gracilaria corticate* have varying concentrations of essential amino acids (AA) such as leucine, alanine, and threonine, which can be affected by seasonal changes (Pirian *et al.*, 2017). However, the protein content of seaweed may be overestimated as it may contain non-protein nitrogen compounds such as amines, amides, amino sugars, and nitrates (Misurcova, 2012). To accurately determine protein content, the N-to-protein conversion factor should be determined based on AA composition and the distribution of N in protein and nonprotein N_2 compounds for each seaweed genus the distribution of N in protein and other nonprotein N_2 compounds (Fujihara *et al.,* 2001; Ezeagu *et al.*, 2002). Conversion factors for green, brown, and red seaweeds have been provided (Lourenco *et al.,* 2002), indicating the potential for seaweed to be used as an alternative feed ingredient for sustainable ruminant production.

Table 4: Amino acid (AA) composition (g/100g of protein) of various seaweed species

Type	**Red Seaweed**		**Green Seaweed**		**Brown seaweed**	
Species	*Porphyra columbina*	*Asparagopsis taxiformis*	*Ulva Spp.*	*Ascophyllum nodosum*	*Macrocystis pyrifera*	*Laminaria digitata*
Essential AA						
Methionine	1.68	2.32	1.6-6.7	0.7	2.05	1.49
Cysteine	1.89	4.32	2.1-5.9	Trace	3.5	1.96
Valine	5.85	6.19	4.4-6.9	3.7	4.45	6.01
Isoleucine	2.71	5.09	2.6-3.7	2.8	3.20	2.61
Leucine	7.38	8.25	5.2-6.7	4.6	5.76	4.45
Phenylalanine	3.7	5.86	3.5-4.6	2.3	3.37	2.83
Tyrosine	2.55	3.67	1.4-3.0	0.9	2.68	1.74

Histidine	1.26	1.48	2.0-3.01	1.3	1.30	2.38
Lysine	6.01	4.32	3.8-4.4	4.9	5.05	4.77
Threonine	5.91	5.86	3.8-9.4	2.8	4.78	3.41
Non-essential AA						
Serine	6.16	5.93	4.2-6.4	3.0	4.44	2.45
Arginine	6.19	7.15	4.5-5.0	8.0	3.83	2.96
Glutamic Acid	10.5	10.89	3.5-12.7	10.0	13.83	2.86
Aspartic Acid	12.2	12.24	7.9-12.4	6.9	10.04	4.69
Proline	3.96	5.15	0.2-8	2.6	3.73	1.91
Glycine	8.87	5.15	5.4-7.7	5.0	4.83	3.31
Alanine	12.54	7.35	5.94	5.3	11.43	4.51

Source: (Angell *et al.* 2012)

3.5 Lipid content of different seaweed

Seaweeds can be categorized into brown, green, and red based on their lipid content, with brown seaweed containing the highest amount, followed by green and red seaweeds (Gosch *et al.*, 2012). The composition of seaweed lipids has garnered attention in recent years due to their high content of unsaturated fatty acids (USFA), including polyunsaturated fatty acids (PUFA) with 18- and 22-carbon atoms, which vary by species. The proportion of saturated and unsaturated fatty acids in seaweeds also differs among species. For instance, the ratio of SFA/USFA was found to be 0.35, 0.83, 0.93, 0.90, and 0.33 for *A. taxiformis*, *Porphyra dioica, Ulva rigida*, *Codium tomentosum*, and *A. nodosum*, respectively. *A. taxiformis* and *A. nodosum* were the species with the highest concentration of USFA among those studied. (Table 5 presents the average contribution of saturated and unsaturated fatty acids contents for each species).

The proportion of USFA in seaweeds varied significantly, with *A. nodosum* and *A. taxiformis* having the highest content of USFA. Some seaweeds also contain significant amounts of omega-3, omega-6, and other PUFA, which have potential benefits for animal and human health (Table 5; Holdt and Kraan, 2011; van Ginneken *et al.*, 2011). These fatty acids may improve the quality of meat and milk, boost immune function, and enhance reproductive performance (Moallem, 2018). Therefore, PUFA is considered an important nutrient for animal health and well-being.

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

Item	Red		Green		Brown
	Asparagopsis taxiformis	Porphyra dioica	Ulva rigida	Codium tomentosum	Ascophyllum nodosum
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

Source: (Cian *et al.*, 2013)

3.6 Sea weed for ruminant feed

The high demand for animal feed protein and the need for alternative sources to traditional soybean and animal protein feeds, as well as regulations related to livestock feeding, have affected the use of seaweed in ruminant feeds. Various studies have examined the impact of adding small amounts of various macroalgal species to animal feed and evaluating the potential prebiotic activity and improved animal performance. These studies have mainly focused on the effects on bovine, caprine, and other ruminant nutrition. There is limited information available on the use of green seaweeds in ruminant feed. *Ulva lactuca*, for example, can be included in the diet of male lambs at a maximum of 20% without any negative effect on palatability (Arieli *et all.*, 1993). It is suitable to use with feeds that have a high energy/low protein content, such as cereal grains. Another green seaweed, *Chaetomorpha linum* (Chlorophyta), was also fed to growing lambs with a 20% seaweed meal (Misurcova *et al.*, 2011). However, it had a slightly depressing effect on growth and feed conversion ratio, possibly due to its high ash content. Red seaweed has been more extensively studied in bovine feed compared to other ruminants. The use of a 70% concentrate of *Phymatolithon calcareum*, also known as *Lithothamnion* *calcareum*, at a ratio of 0.5 g/kg has shown success in buffering rumen pH, but did not improve fiber digestion or modify rumen fermentation (Montañez-Valdez *et al.*, 2011).

In caprine feed, there is more research on the inclusion of seaweed. Orkney sheep from the North Ronaldsay Island mostly feed on brown seaweed, particularly species like *Laminaria digitata*, *Laminaria hyperborea*, and *Saccharina latissima* (Phaeophyceaea), which account for 90% of their summer feed. Orkney sheep also consume other seaweed species, including *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus sp.* (brown seaweed), *Palmaria palmata* (red seaweed), and some green algae. Additionally, the supplementation of the brown seaweed *Ascophyllum nodosum* in feedlot cattle was found to reduce fecal shedding of *Escherichia coli* (Makkar *et al.,* 2016). Some studies have suggested that adding up to 30% of *Macrocystis pyrifera* to goat feed does not negatively affect digestibility, degradability, or ruminal fermentation parameters such as pH and ammoniac nitrogen. In fact, it can increase rumen pH, water intake, and urine excretion (Makkar *et al.,* 2016). Similarly, species from the *Genus Sargassum* have also been studied and can be introduced at up to 30% in the diets of growing sheep and goats without depressing intake, growth performance, and diet digestibility. Using *Sargassum sp*. meal in the diet can help limit the decrease in rumen pH resulting from acidogenic diets (Marín *et al.,* 2009). There have been some studies on using mainly brown and red seaweed as feed for ruminants, but the data is limited to a few isolated cases and is insufficient to initiate research and development work for developing new products for the ruminant feed market. More research is needed to support the use of seaweed as a feed supplement for ruminants. It would be helpful to investigate the bioavailability of a mixture of *Ulva lactuca* and one or more of the red seaweed options to determine whether it can be used as a prebiotic, combining the advantages of both species.

3.7 Effects of seaweed in mitigation of enteric methane

Studies have shown that certain seaweeds contain compounds, such as BCM, that can inhibit methane emissions in ruminants (Table 6). However, there is limited research on how seaweed supplementation affects the ruminal microbiota and methane production. When red seaweed or BCM were added to the diets of cattle, methane production was significantly reduced, but there was also a decrease in dry matter intake (DMI) and milk yield (McCrabb *et al.,* 1997; Roque *et al*., 2019; Kinley *et al*., 2020; Li *et al*., 2018). However, a recent study found that low levels of red seaweed supplementation (0.05%-0.2% of DM) (table 6 and 7) in beef cattle diets reduced methane emissions without affecting DMI (Kinley *et al*., 2020). Increasing levels of

BCM supplementation or seaweed containing BCM progressively decreased DMI in cattle, but not in sheep, lactating dairy goats, or steers fed a concentrate-based diet.

Table 6. *In-vivo* studies of methane (CH_4) emissions from seaweed and commercial bromochloromethane (BCM) supplementation

Animal	Basal diet	Treatment	DMI, kg/d	CH_4 production
Beef steers	Feedlot TMR (total mixed ration)	BCM, g/100 kg of BW		CH_4, g/kg DMI
		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}
		Rate of change, %	−11.3	−95.2
Beef steers	Alfalfa hay	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
		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	*A. armata*, %, OM basis		CH_4, g/kg DMI
		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	*A. taxiformis*, %, OM basis		CH_4, g/kg DMI
		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
Sheep	High-fiber pellet	*A. taxiformis*, %, OM basis		CH_4, g/kg DMI
		0 (control)	1.0	15.0^{a}
		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
Sheep	Feedlot TMR	BCM, g/100 kg of BW		CH_4, % of GE intake
		0 (control)	1.0	6.1^{a}
		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
Dairy goats	Alfalfa + concentrate	BCM, g/100 kg of BW		CH_4, g/kg DMI
		0 (control)	0.99	29.95
		0.3	1.04	19.9
		Rate of change, %	0.5	−33.6

(Sources: Hunter and Tomkins, 2004)

Palatability issues were observed in dairy and beef cattle when seaweed supplementation exceeded certain levels, but not in sheep (Li *et al.*, 2018). A red seaweed called *Asparagopsis spp.* has shown great potential in reducing methane emissions from ruminants, without negatively impacting animal production. In vitro studies have demonstrated that including 5% *A. taxiformis* in dairy rations can result in a 95% reduction in methane emissions (Goel *et al.*, 2009), and similar results have been observed in in vivo experiments (Roque *et al.*, 2019).

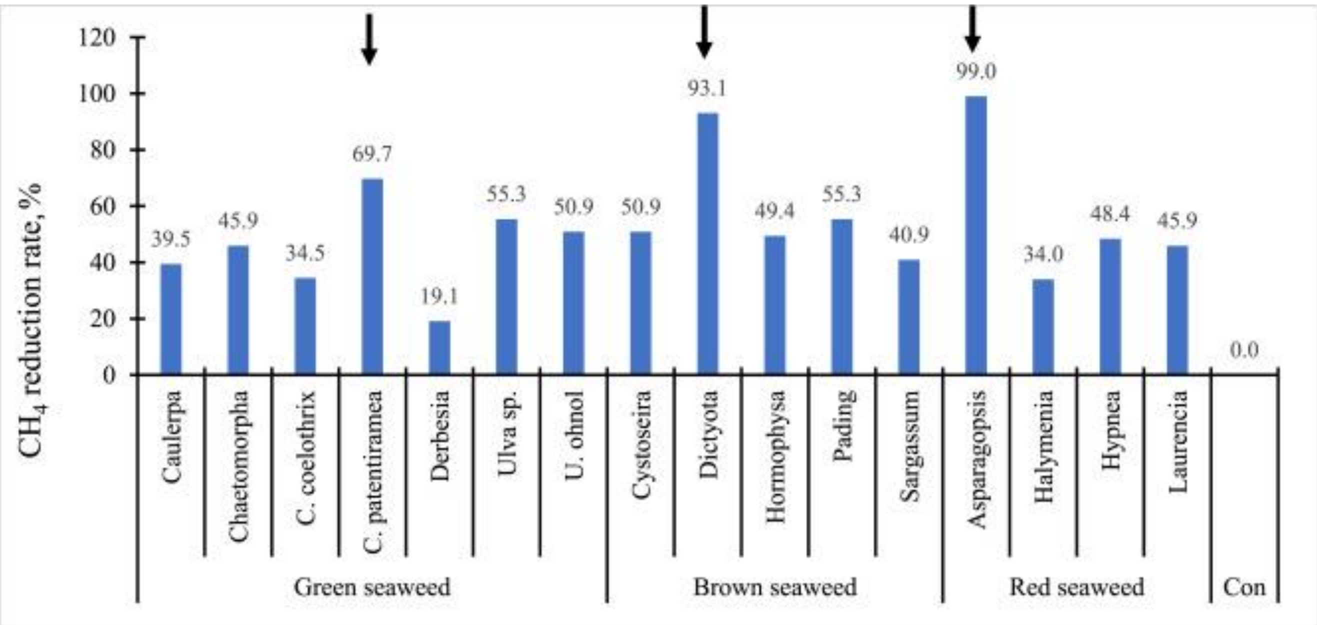
Other seaweed species, such as green and brown seaweeds, have also been found to inhibit methane production, but to a lesser extent than red seaweeds (Belanche *et al.*, 2016). However, the degree of inhibition may vary depending on the seaweed species and their secondary metabolites. Overall, the use of seaweeds as a natural intervention strategy for reducing methane emissions in ruminants shows promise.

Table 7. *In-vitro* studies of methane (CH_4) emissions from red seaweed or bromochloromethane (BCM) supplementation

System	Basal diet	Treatment	CH_4 production
Ankom	Rhodes grass (*Chloris gayana*)	*A. taxiformis*, % DM	CH_4, mL/g OM
		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	*Oedogonium* sp., % DM	CH_4, mL/g OM
		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
		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
		Control (no seaweed)	1.75[a]
		Ulva sp. (green)	1.30[b]
		L. ochroleua (brown)	1.98[a]
		S. latissima (brown)	1.81[a]
		Gigartina sp. (red)	1.17[b]
		G. vermiculopphylla (red)	1.07[b]
		Rate of change, %	−38.9
CC	Dairy TMR	*A. taxiformis*, 5% OM	CH_4, mL/g OM
		Control (no seaweed)	12.08[a]
		5.0	0.59[b]
		Rate of change, %	−95.1

(Source: Machado et al. 2015)

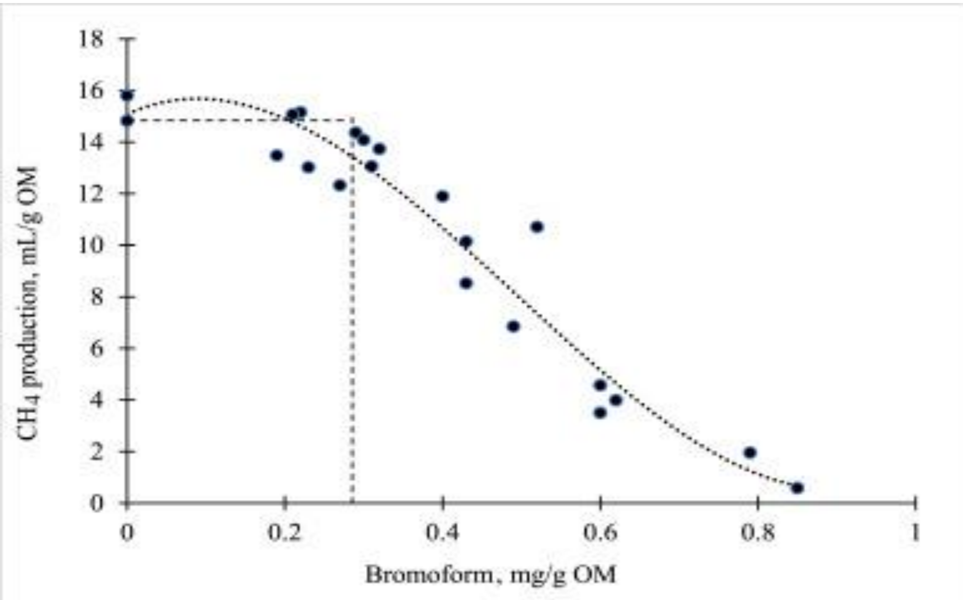
Bromoform or BCM concentration can serve as an indicator of the anti-methanogenic properties of red seaweeds used in ruminant diets. The concentration of bromoform shows a curvilinear decrease in enteric CH_4 production with increasing concentrations (Goel *et al.*, 2009). BCM inhibits methanogen populations in batch- and continuous-culture systems, with the strongest effects observed at concentrations ranging from 0.8 to 0.9 mg/g OM. Freeze-drying of seaweed has been found to be the most effective post-harvest processing method for inhibiting CH_4 emissions, and freezing and freeze-drying of *A. taxiformis* had the highest bromoform concentrations (Vucko *et al.*, 2017). Irrespective of rinsing, the unrinsed treatment had the highest bromoform concentration, followed by oven-drying or dehydrating without freezing.Haloperoxidase enzymes are abundantly produced by seaweeds and play important roles in marine chemical ecology and biotic interactionsy (Thapa *et al.,* 2020). Studies have shown that supplementation of different basal diets with green seaweed, Oedogonium (0.2 g OM), can lead to reductions in methane emissions 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



(Source: Machado et al., 2014)

Figure 1. Various seaweed species (0.2 g OM/seaweed species; green-, brown-, and red-seaweed) and in vitro methane (CH_4) production (mL/g OM).

However, there is a need for further investigation into the interrelationships between different seaweed species and dietary ingredients.This refers to the process in the rumen where a group of microorganisms digest dietary carbohydrates, resulting in the production of volatile fatty acids (VFA), carbon dioxide (CO_2), and hydrogen (H_2) as shown in (Figure 3). During rumen fermentation, hydrogen gas (H_2) is produced through the re-oxidation of cofactors such as NADH, NADPH, and FADHr (Stams and Plugge, 2009). Methanogenic archaea in the rumen use CO_2 and H_2 as substrates for their metabolism, which is the primary pathway for methanogenesis (Ellis *et al.*, 2008). Through interspecies H_2 transfer, methanogens actively affect the metabolism of rumen fermentative and acetogenic bacteria. In addition to using H_2 and CO_2, other groups of methanogens can also use formate, acetate, methanol, methylamines, and alcohol (Ellis *et al.,* 2008). There are three pathways for methanogenesis: hydrogenotrophic, methylotrophic, and acetoclastic, all of which require the methyl-coenzyme M reductase (MCR) gene cluster for CH_4 production (Ferry and Kastead, 2007; Conrad, 2009). The addition of seaweed to the diet can alter the pathway of fermentation, reducing CH4 production. This process is summarized in (Figure 3). In addition, bromoform compounds found in Gracilaria sp. (red seaweed) are the effect of the reduction in the methanogen population (Prayitno *et al.*, 2018). It is also reported that bromoform-rich red seaweed inhibited the work of methanogens specifically. BCM would reduce the activity of coenzyme cobalamin (vitamin B_{12}) and coenzyme MCR (Denman et al., 2007). Therefore, halogen-rich seaweed could be a useful tool for the mitigation of enteric GHG emissions as a potential antimethanogenic agent.

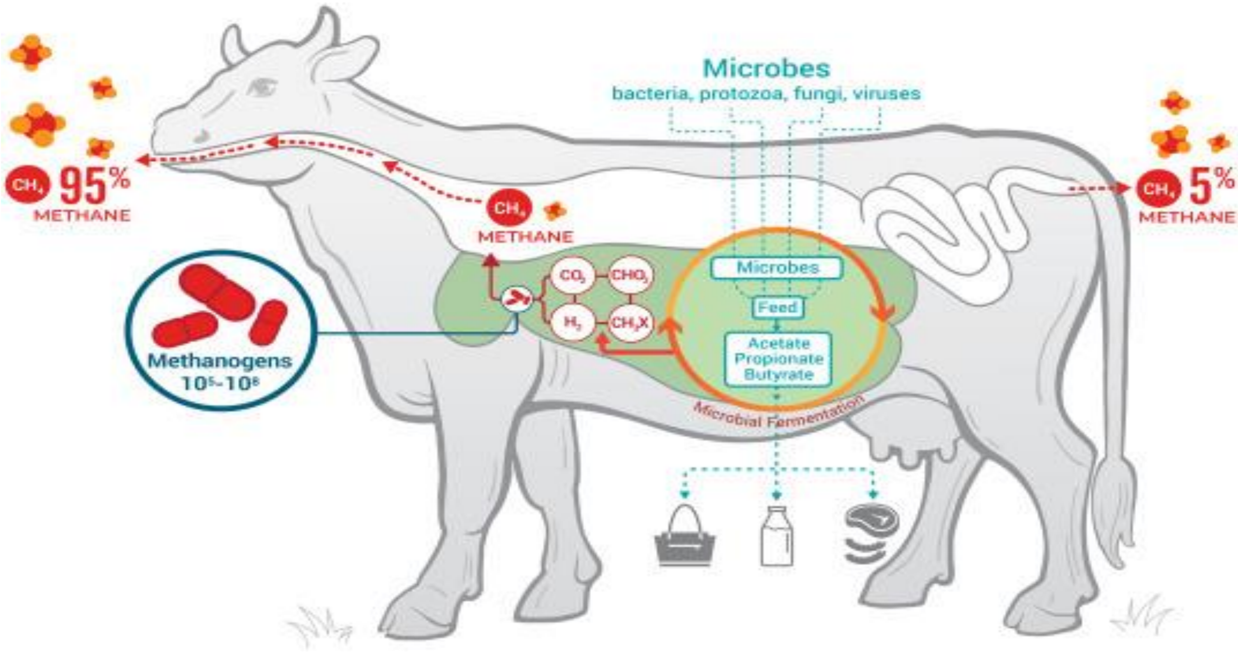


(Source: Goel *et al.,* 2009)

Figure 2. In vitro correlation between the concentration of halogenated compounds (bromoform or bromochloromethane (BCM; mg/g OM) and the methane (CH_4) emissions (mL/g OM) in the *Asparagopsis taxiformis* (cut off: < 1.0% of bromoform or BCM (mg/g OM)

3.8 Effects of sea weed in rumen fermentation

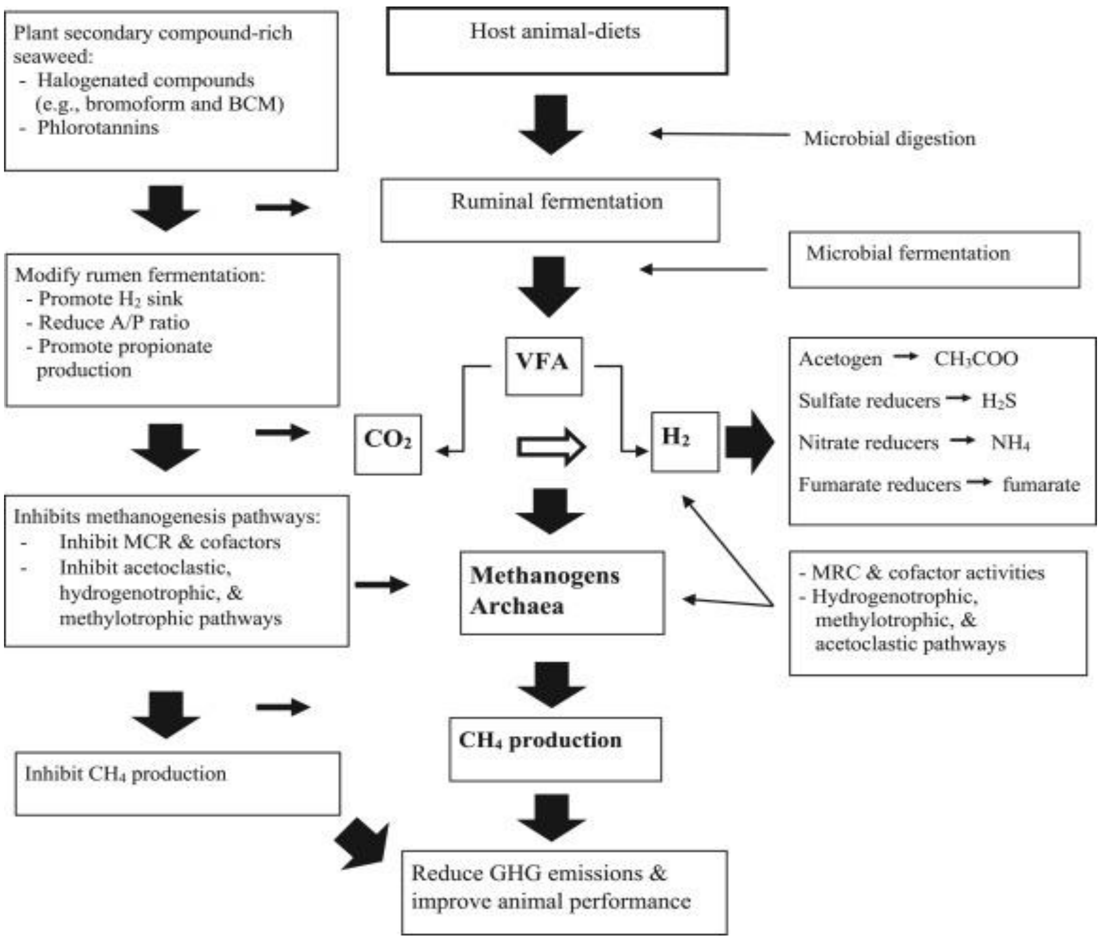
Studies have evaluated the in vitro dry matter (DM) digestibility of seaweeds, including brown, red, and green species, with varying results (Tayyab *et al.*, 2016; Gojon-Baez *et al*., 1998; Gaillard *et al.*, 2018). The DM or organic matter (OM) digestibility of seaweed species, such as *M. pyrifera* and *Sargassum spp*., after in vitro rumen incubation for 72 and 96 hours, ranged from 27.9% to 94.6% DM (Tayyab *et al.*, 2016; Gojon-Baez *et al.*, 1998). However, the inclusion of certain seaweeds, such as *A. taxiformis* or *Oedogonium*, at more than 10% reduced in vitro OM digestibility (Machado *et al.*, 2016). In lactating dairy cows, the inclusion of *A. armata* in their diet reduced dry matter intake (DMI) (Roque *et al.*, 2019) and protein digestibility (Tayyab *et al.*, 2016). However, in vivo trials (Castro *et al.*,2009) with the inclusion of up to 30% of different seaweed species did not negatively affect digestibility. Reducing methane production in the rumen could increase the availability of molecular hydrogen (H_2) for pathways that yield fermentation end products like volatile fatty acids (VFA) (Table 8). This would provide additional energy for the animal, leading to more efficient feed utilization and better growth and milk production. Studies on the effects of supplementation with bromochloromethane (BCM) or seaweed on rumen fermentation and animal performance are summarized in (Table 8).



(Source: Glasson *et al.*, 2022)

Figure 3. Ruminant fermentation processes and products, and microbial contributors.

BCM supplementation in steers reduced acetate and the acetate-to-propionate ratio (A/P), while increasing propionate concentration. Similarly, supplementation with red seaweed or BCM decreased the A/P ratio and resulted in improved average daily gain or feed efficiency in beef steers and dairy cattle. These findings are consistent with previous research s (Machado *et al.*, 2015) indicating that supplementing with *A. taxiformis* can decrease the A/P ratio and increase propionate concentration. Decreased acetate production relative to propionate reduces the net balance of H_2 in the rumen, leading to reduced methane formation (Van Nevel and Demeyer, 1996).



(Source: Roque *et al.*, 2021)

Figure 4. Schematic diagram of microbial fermentation of plant secondary compound (e.g., bromoform, BCM)-rich seaweed and methane (CH_4) reduction pathways in the rumen.

Other studies have also found that inhibiting methane production with halogenated compounds, ionophores (Goodrich *et al.*, 1984), or plant tannins (Min *et al.*, 2019) can alter fermentation patterns (Mitsumori *et al.,* 2012; Roque e*t al.,* 2020) and lead to increased propionate concentration. When fed to beef steers, BCM reduced enteric methane emissions and increased the concentration of propionate, iso-butyrate, valerate, and iso-valerate. This was accompanied by a decrease in the number of methanogens and an increase in alternative methanogens like Methanomicrobium, Methanosarcina, and Methanococcus.

3.9 The effect of seaweed on animal production and meat quality

A. nodosum is a popular seaweed species used in livestock industries (Allen *et al.,* 2001; Makkar *et al.,* 2016) due to its bioactive ingredients such as iodine, minerals, PUFA, vitamins, and phlorotannins. Phlorotannins have potential benefits in inhibiting ruminal proteolysis and foodborne pathogens (Cvetkovic *et al.*, 2004; Antaya *et al.*, 2015; Makkar *et al.*, 2016). While previous studies have 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 animal performance in dairy and beef cattle are not clear. Although BCM supplementation has shown positive effects on ADG and feed efficiency in beef steers, the effects of long-term seaweed supplementation on nutrient utilization and animal performance are not well understood in dairy and beef cattle. Previous studies have reported no significant differences in animal performance and milk yield in Jersey cows and Angus-Hereford beef steers fed diets with low levels of *A. taxiformis* and *A. nodosum (*Antaya *et al.,* 2019; Roque *et al.,* 2020). Previous studies have shown mixed results regarding the effects of seaweed supplementation on animal performance in dairy and beef cattle. Some studies have reported no significant effects of brown seaweed *(A. nodosum*) supplementation on milk yield, fat percentage, or blood concentrations of cortisol, glucose, fatty acids, and thyroxine in dairy cows (Antaya *et al*., 2019). Similarly, Ayrshire dairy cows fed a diet containing 10% seaweed meal did not show any significant changes in milk yield or fat percentage.

On the other hand, red seaweed (e.g., *A. taxiformis and A. armata*) containing bromoform has been reported to decrease methane emissions while improving animal production in ruminants. However, high levels of *A. armata* supplementation (1% OM) decreased milk production in Holstein dairy cows fed a grain-based diet.

In dairy goats, a diet containing brown seaweed (BCM) resulted in greater milk production due to higher proportions of short-chain fatty acid (e.g., propionate). Similarly, Sahiwal cows fed *Sargassum wightii* seaweed had significantly higher milk yield and 4% fat corrected milk

(Singh *et al*., 2015). Overall, the effects of long-term seaweed supplementation on nutrient utilization and animal performance in dairy and beef cattle remain unclear and require further investigation. It is found that supplementing a grain-based diet with 2% *A. nodosum* (as a percentage of dry matter) led to an increase in carcass marbling scores (Anderson *et al.,*2006) and a 39.6% increase in the percentage of English crossbred steers (n=32) and heifers (n=32) that graded as choice. This may explain the slightly improved average daily gain (1.52 vs. 1.45 kg/day; P=0.06) observed in steers fed a corn-based diet with 2% *A. nodosum.*

Studies have shown that feeding beef steers with tall fescue grass that has been sprayed with a seaweed extract solution or supplementing their diets with a proprietary brown seaweed meal can lead to an increase in carcass marbling and USDA quality grade (Allen *et al.,* 2001). The use of brown seaweed or its extract has also been linked to improved animal health, immune function, antioxidant levels, heat stress tolerance, meat shelf-life, color, and marbling score in beef cattle (Behrends *et al.,* 2000; Allen *et al.,* 2001; Montgomery *et al.,* 2001; Saker *et al.,* 2001). However, the mechanisms behind these effects are not yet fully understood. Previous research has focused on the benefits of seaweed supplementation for animal health, food safety, and carcass characteristics (Fike *et al.,* 2001; Montgomery *et al.,* 2001; Braden *et al.,* 2007), but further investigation is needed to understand how it works in beef cattle diets. Studies have shown a linear increase in milk iodine levels in multiparous Jersey cows fed increasing amounts of brown seaweed during the winter season (Antaya *et al.,* 2015). However, further research is needed to determine the impact of seaweed type, inclusion rate, and feeding duration on milk production, milk composition profiles, and animal performance, including average daily gain and feed efficiency.

3.10 Effects of seaweed in rumen microbial density

North Ronaldsay sheep primarily consume *Laminaria spp*. seaweed, which constitutes around 90% of their diet (Hansen *et al.,* 2003). Studies have shown that feeding these sheep a diet containing *L. digitate* seaweed leads to significant changes in their rumen microbial communities, particularly in ciliate protozoa and bacterial population. However, other studies have reported that brown seaweed supplementation, such as *A. nodosum* and L. digitate, had no substantial effect on rumen fermentation, feed digestibility, or methane emissions (Belanche *et al*., 2016). These studies also found that the main bacterial and methanogen genera were unaffected by brown seaweed supplementation. Additionally, *A. taxiformis* and *A. armata* have been found to have strong activity against ruminal gram-negative and gram-positive bacteria

(Paul *et al.,* 2006; Salvador *et al.,* 2007). Recent studies have shown that *A. taxiformis* has confirmed antimethanogenic activity in in vitro ruminal fermentation studies (Machado *et al.,* 2016). Specifically, when added at a dose of 2% of the organic matter incubated, it strongly inhibits the production of CH_4 (Roque *et al.,* 2019). While 16S ribosomal RNA gene amplicon sequencing has shown that the relative abundance of methanogens in fermentation bottles incubated with *A. taxiformis* decreased significantly, this reduction in methanogen richness and CH_4 production was only significant when averaged throughout the experiment (Roque *et al.,* 2019). These findings suggest that *A. taxiformis* has a direct effect on the metabolic functionality of rumen methanogens, rather than on the microbiome congregation, specifically methanogen abundance (Goel *et al.,*2009). Overall, the impact of seaweed on the rumen microbial community differs depending on the species of seaweed. Studies have shown that supplementation with *A. taxiformis* has led to a decrease in the relative abundance of major methanogens, while bacterial communities remained similar (Machado *et al.,* 2018). These findings are consistent with other in vitro and in vivo studies (Goel *et al.,* 2009; Mitsumori *et al.,* 2012). However, one study found that supplementation with BCM did not inhibit the population of bacterial, protozoa, and methanogenic archaea in lactating dairy goats over a longer feeding trial period (Abecia *et al*., 2012). It is possible that the duration of the study allowed the microbial ecosystem to adapt to the dietary treatment (Williams *et al.* 2009) and further research is needed to determine the impact of feeding duration on the ruminal microbiota population and methanogenic adaptation.

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 ruminant

Item	Acetate, %	Propionate,%	A/P ratio	ADG, kg/d	G:F ratio
In vivo					
Steers (Brahman-crossbred)					
Control (no BCM)	79.6	17.0	4.7	0.5	0.08
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
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
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 (*A. taxiformis*), % DM					
0 (control)	65.0	20.8	3.19	–	–
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	–	–
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	–
3.0 mg BCM/kg BW	60.3	15.5[a]	3.92[b]	−6.6	–
In vitro					
A. taxiformis, % OM					
0 (control)	66.4[a]	22.5[c]	3.0[a]	–	–
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	–	–
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	–	–
A. taxiformis, % OM					
0 (control)	75.0[a]	19.2[b]	3.9[a]	–	–
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	–	–
Green seaweed	64.6	25.9	2.5	–	–
Brown seaweed	63.3	29.3	2.7	–	–
Red seaweed	59.3	28.4	2.1	–	–

(Source: Min *et al.*, 2021)

3.11 Benefits of using seaweed

1. To promote seaweed as a dietary supplement for animal production in the context of climate change, there needs to be added value for cattle producers. Seaweed has gained attention in recent years as a potential sustainable feedstock for livestock due to its rich nutritional content, including proteins, lipids, vitamins, fatty acids, amino acids, carbohydrates, minerals, and nutraceuticals.

2. Additionally, seaweed contains bioactive compounds such as anti-methanogenic, antioxidant, anti-inflammatory, anti-bacterial, and anti-viral agents. By adding seaweed biomass to animal feed, phlorotannins or halogenated methane analogs can be delivered as a holistic approach to reducing enteric methane emissions and improving animal health, as opposed to using extracts or metabolites.

3.12 Challenges of using seaweed

1. Because of its high mineral content, excess amount of raw seaweed consumption causes mineral toxicity to the animals. So before using proper treatment of seaweeds needed to be done.
2. As seaweeds are unconventional type of feed ingredient, it’s a matter of concern to make farmers convenient to use it as a regular feed item.
3. Many more research is needed to establish sea weed as a daily feed ingredient.

CHAPTER 4

CONCLUSION

Seaweeds are becoming popular due to their potential as a source of food supplements and livestock production. They contain protein, dietary fibers, and phytochemicals that can enhance the nutritional value of animal feeds. Seaweeds can help meet the demand for renewable and sustainable energy sources without compromising on food and land resources because they grow quickly, yield high biomass, and have high productivity at no extra cost compared to other traditional biomass feedstock like corn or soybean.

But there are still gaps in our knowledge, particularly in dairy and feedlot cattle performance, rumen microbiome changes, and animal health. The supplementation strategy should also be sustainable, practical, and economically feasible while ensuring the functionality of the rumen microbiome and improving animal productivity.

Recent studies suggest that certain seaweeds may decrease CH_4 emissions in ruminants. Some species of seaweed also shows good efficiency in reducing enteric methane emissions. However, the availability and sustainability of these seaweeds, particularly those rich in bromoform, are concerning. Metabolomic profiles associated with feed efficiency and animal host are also necessary to gain a comprehensive understanding of the methanogenesis responsible for reducing CH_4 emissions. Future research needs to address these unsolved issues, as well as the long-term impact of methanogenesis on ruminants.

So, we can conclude that, seaweeds have a great potentiality in supplementation of feeds and mitigation of enteric methane emissions but lots of efforts should be done to use seaweed in successful livestock production.

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