SEAWEED CULTIVATION IN VIETNAM FOR LIVESTOCK METHANE REDUCTION

A Top Sector Agri & Food SMP (Seed Money Project) Report

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Abstract

The environmental conditions of Vietnam support a large collection of seaweeds but only about 10 species are economically relevant. Although local information is scarce and somewhat conflicting, our report gives a review of important Vietnamese seaweed species that have potential use in reduction of livestock methane emissions. Current productivity status of local seaweed species are mostly lacking but discussed species may still be used in various industrial sectors as food, feed, medicine, cosmetics, fuel, textile and biostimulants. Strategies that reduce livestock emissions and seaweeds' toxicology profile are also discussed. The presence of sufficient quantities of bioactive compounds such as halocarbons (e.g., bromoform), phlorotannins, fatty acids, nitrate, sulphate, saponins, alkaloids and sulphated carbohydrates in seaweeds may play critical roles in reduction of enteric emissions via seaweed inclusion in livestock feed. The report ends by highlighting proposed species based on available local information, that should be further tested and validated with *in vitro* and *in vivo* experiments. Our top selected seaweed species include *Asparagopsis taxiformis, Sargassum spp*, *Ulva spp*, *E. dentriculatum*, *K. alvarezzi*, *C. lentillifera* and *Gracilaria spp*.

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1 General Introduction

The current environmental conditions (i.e., temperature, ocean current, rainfall, nutrient availability and salinity) of coastal regions in Vietnam are optimal for growing seaweeds, also known as macroalgae (Hong et al. 2007; Titlyanov et al. 2012). Latest literature puts the total number of macroalgae identified in Vietnam at 739 species (Van Nguyen et al. 2013; Phang et al. 2016). The most economically important seaweed species are *Sargassum*, *Gracilaria*, *Kappaphycus* and *Eucheuma*. Other Vietnamese seaweeds that are equally important because of their nutritional value and application are *Ulva*, *Caulerpa*, *Laurencia* and *Hydropuntia* (Hong et al. 2007). These species are directly consumed as food/ feed, provide raw materials for various industries (e.g., for production of medicines, cosmetics, textile, bioethanol) and may be used as crop biostimulants (Hong et al. 2007; Van Nguyen 2015).

The nutritional and biochemical composition of seaweeds vary widely depending on environmental conditions, life cycle and harvesting time. Seaweeds are rich sources of carbohydrates, fibres, macro- and micro- nutrients (Hong et al. 2007) but generally low in lipids, amino acids and proteins. Seaweeds have relatively high ash content: up to 30% of its total dry weight, compared to most arable crops that have between 5 to 15% (Sánchez-Machado et al. 2004). They contain chlorophylls and carotenoids, pigments that are responsible for their vibrant colours. Chlorophylls a, c, fucoxanthin, β-carotene and xanthophylls are found in brown seaweeds, green seaweeds contain chlorophylls a, b, β-carotene and xanthophylls, and chlorophylls a, d, β-carotene, phycoerythrin and phycocyanin exist in red seaweeds (Aryee et al. 2018). In terms of starch-like carbohydrates used for energy storage: brown seaweeds contain laminarin, alginate and fucose-sulphated-polysaccharides (e.g., fucoidan), green seaweeds contain ulvans while red seaweeds contain carrageenans, agarans, DL-galactan hybrids and sulphated mannans (Makkar et al. 2016). Seaweeds or their extracts, have potential as a new component for animal feed when used as an additive or supplement but have a lower perspective when used as a nutritional feed ingredient (Øverland et al. 2019; Bikker et al. 2020).

This report gives an overview of local economically relevant seaweed species present in Vietnam that have potential use in reducing methane emissions by inclusion in livestock feed. The strategies by which livestock emissions can be reduced that may be utilised by seaweeds inclusion in feed, and the toxicology profile of seaweeds are discussed in section 2. Section 3 outlines the economically relevant Vietnamese seaweeds indicating information on current productivity status and their potential use. It should be noted that literature on current production levels of local seaweeds is scarce and sometimes conflicting, thus contributing to the knowledge gaps amongst the different species in the report. The last section summarises species proposed for inclusion in livestock feed highlighting our top 10 seaweed species that may be tested for their potential in mitigating methane emissions, first with *in vitro* experiments and validated with *in vivo* experiments in livestock.

2 Introduction

2.1 Reducing carbon footprint of livestock with seaweed-inclusive feed

Livestock produces ~ 7.1 gigatons (GT) of carbon dioxide equivalents (CO_2-eq) greenhouse gasses (GHG) worldwide per year, with methane (CH_4) attributing to 44% (3.1 GT) of the total GHG emissions produced by livestock (Gerber et al. 2013). Beef and dairy cattle alone contribute ~ 2.5 and 2.1 GT CO_2-eq per year respectively, and are the two largest producers of GHG worldwide in the livestock sector (Gerber et al. 2013). Methane is mainly formed in the rumen of cattle (approximately 80%), and excreted via exhalation or belching which is also known as enteric methane emissions. The rumen inhabits a rich ruminal microbiota consisting of symbiotic relationships between archaea, bacteria, fungi and protozoa, that are involved in fermentation of ingested feed. During this fermentation process, different (intermediary) compounds are produced that facilitate the symbiotic relationships, thus enabling microbial growth. Primary fermenters hydrolyse starch, cell wall carbohydrates and proteins into simple sugars and amino acids. Both primary and secondary fermenters convert the resulting simple sugars and amino acids into volatile fatty acids (VFA), ammonia (NH_3), CO_2 and hydrogen (H_2) (McAllister et al. 1996). Several types of methanogens can convert CO_2 and H_2 into CH_4 (McAllister et al. 1996). The produced VFA, mainly acetate, butyrate and propionate, are mostly absorbed by the rumen wall and are an important energy source for the ruminant (Boadi et al. 2004).

In general, several strategies can be identified to reduce CH_4 production by dairy cattle namely: 1) changing VFA profiles to reduce acetate and butyrate and increase propionate production (e.g., dietary change from fibre rich to starch rich), 2) offering alternative H_2 sinks (e.g., nitrate and sulphate), 3) the use of anti-microbial properties (e.g., tannins), 4) inhibition of specific enzymes (e.g., halogenated metabolites), and 5) addition of dietary lipids (Dijkstra et al. 2011; Hristov et al. 2013; Patra et al. 2017; van Lingen et al. 2018; Abbott et al. 2020). The use of seaweeds to reduce enteric methane production mainly focus on strategies 2, 3 and 4 due to their biochemical composition. Seaweeds generally have low fat content, high levels of fibres (i.e., cell wall carbohydrates) and minimal amounts of starch. Starch is absent in brown and red seaweeds but present in some green seaweed species. The storage molecule of brown and red seaweeds is laminarin and floridean starch respectively. Sulphated carbohydrates like carrageenans, agar, fucose-sulphated-polysaccharides (FSP, e.g., fucoidan) and ulvans may potentially act as alternative H_2 sinks, however this effect has not yet been studied in detail. In addition, brown seaweeds can be rich in polyphenols, specifically phlorotannins that make up 2-8% of total polyphenols on dry weight (dry product) basis (Jiménez-Escrig et al. 2001; Zubia et al. 2009; Cofrades et al. 2010; Heffernan et al. 2014; Moreira et al. 2017). Phlorotannins from *Laminaria digitata* can decrease methane production in an *in vitro* gas production setting without negatively affecting total gas production at inclusion levels < 40 g/ kg (Vissers et al. 2018). Importantly, the use of *Asparagopsis spp.* in the diet of cattle can reduce enteric methane production up to 80-90% (Kinley et al. 2020; Roque et al. 2021), sparking huge global interest for scientists, companies and governments. Bromoform ($CHBr_3$) was identified as the most likely bioactive compound responsible for methane reduction and it is present in sufficient quantities in *Asparagopsis taxiformis* (Machado et al. 2016). However other halogenated metabolites (e.g., dibromochloromethane) that have similar effects have also been identified (Machado et al. 2016). Other seaweeds such as *Ulva spp*, *Macrocystis pyrifera* and *Pterocladia capillacea* produce $CHBr_3$ but not up to (high) quantities as *Asparagopsis taxiformis* (Carpenter and Liss 2000; Abbott et al. 2020).

2.2 Digestibility and toxicology profile

Seaweeds can be rich sources of minerals and vitamins and thus provide nutritional and nutraceutical (i.e., medicinal) benefits when consumed (Morais et al. 2020). For example, the seaweed *Sargassum* is rich in potassium, albumin and iodine representing 12.82, 7.95 and 0.03% of total dry weight respectively (Hong et al. 2007). Another genus *Caulerpa* is a rich source of calcium and magnesium (up to 5.9% and 4.1% of dry weight respectively), such that consumption of less than 15 g of fresh *Caulerpa* meet requirements of recommended daily intake (de Gaillande et al. 2017). Manganese and iron are also present in high quantities in *Caulerpa*, although with high variability depending on the species (de Gaillande et al. 2017). Most seaweed species easily absorb and store minerals including heavy metals such as lead, mercury and cadmium, that are toxic even when consumed in trace quantities. The trade-off between the amount of seaweed needed to provide sufficient nutrition and the accumulation of heavy metals in the seaweed may limit its recommendation as food and feed (Morais et al. 2020). For example, although the presence of copper and selenium in *Caulerpa* are useful resources to meet demands of nutrient deficiency when consumed, it can easily cause metal toxicity when the seaweed is consumed in high quantities i.e., at amounts recommended for macro- or other micronutrients supplementation (de Gaillande et al. 2017).

In both review papers of Øverland et al. (2019) and Bikker et al. (2020) several (positive) effects on e.g., health and production of different seaweed species are described for livestock production, however the application is limited by the lower digestibility and high ash content of the seaweeds. In recent years, application of seaweed to reduce enteric methane produced by ruminants has gained traction. Although addition of a higher concentration (10% of substrate organic matter) of *Asparagopsis* positively reduced methane emissions, at this concentration feed digestibility was significantly impaired (Kinley et al. 2016). Similar observations have been reported with *in vivo* experiments. *Asparagopsis* can reduce enteric methane production up to 80% in the diet of ruminants with inclusion at levels as low as 0.5 to 3% of the organic matter, but also cause reductions in feed intake and potential damage to the ruminal wall (Li et al. 2018; Roque et al. 2019; Muizelaar et al. 2021; Stefenoni et al. 2021). Therefore, *Asparagopsis* should be included at lower quantities (≥ 0.5% of organic matter) where they are easily digestible and still have methane inhibiting properties. Bromoform ($CHBr_3$) is the most abundant natural compound present in *Asparagopsis* (Machado et al. 2016), raising safety concerns. A recent study on the safety and transfer of $CHBr_3$ from *Asparagopsis* indicates deposition in the milk and urine of dairy cattle, at levels potentially higher than allowed safety standards for $CHBr_3$ (and other trihalomethanes) in drinking water (Muizelaar et al. 2021). Therefore, indicating a need to further analyse the long-term effect of bromoform on the animals' well-being, as well as on the effect of continuous consumption of such milk.

3 Local Seaweed species

A general overview of economically important seaweed species local to Vietnam and the methane-reducing *A. taxiformis* is discussed below. Current productivity data on local macroalgae is not up to date and somewhat conflicting.

3.1 *Asparagopsis*

Asparagopsis is a genus of red seaweed and *A. taxiformis* which is native to Vietnam, is an important seaweed specie for enteric methane reduction (Morais et al. 2020). Although important, ocean-based cultivation techniques for the specie is still under development. Experimental activities for large-scale cultivation of *A. taxiformis* is currently carried out by our project partners Greener Grazing and SeaTech Energy.

3.1.1 Production levels

Productivity status of this specie is in Vietnam is presently unknown. Difficulties associated with large-scale cultivation of *A. taxiformis* also contributes here.

3.1.2 Use

The inclusion of *A. taxiformis* in cattle and sheep feed at organic matter rate of 5% and 3% respectively reduced enteric methane emissions by 95% and 80% respectively, but raised some concerns on the livestock well-being (Li et al. 2018; Roque et al. 2019). Addition of *A. taxiformis* at lower levels of 0.2% was more effective: inhibiting methane emissions by 98% without any negative effects observed in the cattle (Kinley et al. 2020). Other uses of *Asparagopsis* is for food seasoning and as a bioactive compound for skin care products and cosmetics.

3.2 *Gracilaria*

The genus *Gracilaria* are a group of red algae. About 19 species of *Gracilaria* have been reported in Vietnam and they are distributed along the entire coast. They grow in intertidal zones attached to stony substrates or unattached in brackish water lagoons or ponds (Titlyanov et al. 2012).

3.2.1 Production levels

In 2004, the total cultivation area used for production of *Gracilaria spp* was 9 800 ha resulting in 40 000 to 50 000 wet tons annually. They are typically harvested between January to September or between October to April, depending on the region (Van Nguyen 2015). A 1.5-decade old report indicates that only *G. asiatica*, *G. heteroclada* and *G. tenuistipitata* (now annotated as *Agarophyton tenuistipitatum*) were cultivated in Vietnam (Hong et al. 2007). Both *G. tenuistipitata* and *G. asiatica* have an average annual productivity of 1.5 to 4 dry tons/ ha while *G. heteroclada* is widely cultivated yielding up to 200 dry tons/ ha annually (Van Nguyen 2015).

3.2.2 Use

Species of *Gracilaria* are consumed as food or used as a source of agar: containing between 32% to 79% of agarose and having agar strength up to 1121 g/ cm^2 (Titlyanov et al. 2012; Buschmann et al. 2017). They may be used as fish supplements providing both nutritional and nutraceutical benefits for the fish (Morais et al. 2020). Integrated shrimp-tilapia-*Gracilaria* farming was recently adopted in Hoang Phong, Thanh Hoa province as an intervention for climate change and to promote smart aquaculture management practises (Tran et al. 2020). Adoption of the mixed aquaculture system may be beneficial for farmers, increasing overhead farm profit by 73.6% compared to current aquaculture strategies (Tran et al. 2020). *Gracilaria* is one of the genera of seaweeds (the others are *Sargassum* and *Kappaphycus*) currently exported (mainly to) China (Titlyanov et al. 2012) thus, further commercialisation should be boosted. It also has promising use as a biostimulant. Addition of 5% *Gracilaria edulis* sap increased maize yield by ~ 30% in optimal fertilizer conditions (Singh et al. 2015). Furthermore, the addition of 7.5% of the seaweed sap was able to reduce fertilizer application by 50% while still keeping maize productivity levels at an optimum (Singh et al. 2015).

3.3 *Kappaphycus*

Kappaphycus are species of red seaweed.

3.3.1 Production levels

Four species of *Kappaphycus* (*K. cottonii*, *K. inermis*, *K. alvarezzi* and *K. striatus*) are found in Vietnam. *Kappaphycus alvarezzi* and *K. striatus* originating from the Philippines, are cultivated experimentally in Vietnam (Van Nguyen, 2015). Annual production of *K. cottonii* is around 40 wet tons annually and the yearly combined production of *K. cottonii* and *K. striatus* is up to 3 000 dry tons (Hong et al. 2007; Van Nguyen 2015). Even though results from cultivation of *K. alvarezzi* was promising i.e., similar productivity levels to those achieved in the Philippines, it is still not adopted by the local communities (Hong et al. 2007; Van Nguyen 2015). Therefore, highlighting a need for the Vietnamese government to implement strategies that stimulate mainstream adoption of seaweed farming.

3.3.2 Use

In Vietnam, *Kappaphycus* species are commonly used for food and as a source of carrageenan. Carrageenans serve as thickening agents for food and for clarification of wine or beer. In the pharmaceutical industry, carrageenans are used as stabilisers, binders, emulsifiers and for creating dental moulds. They also serve as raw materials for production of cosmetics and for textile printing (FAO 2018; Pereira 2018). *K. alvarezzi* is also cultivated as a source of agar in Vietnam (Charoensiddhi et al. 2020). Cultivation of *K. striatus* may be preferred to *K. alvarezzi* because the former specie is more resilient to the seaweed disease ‘ice-ice’, even though it has a lower growth rate than *K. alvarezzi* (Hung et al. 2019). In addition, both *K. alvarezzi* and *K. striatus* are sources of lectin that may be used for the purification of glycoproteins, and for blood typing and protein-carbohydrate affinity studies in clinical settings (Le et al. 2009; Hung et al. 2019).

Cultivation of *K. alvarezzi* may aid maintenance of ecological balance and for bioremediation of coastal areas by significantly reducing nutrient deposits of ammonia, nitrite, nitrate, phosphate and phosphorus (Hayashi et al. 2017). *Kappaphycus*-based biostimulants that boost arable crop yield provides a new platform for alternative use. Application of *K. alvarezzi*-derived biostimulants significantly increased yields of maize and soybean (Rathore et al. 2009; Singh et al. 2015).

3.4 *Eucheuma*

Eucheuma are a group of red macroalgae that is sometimes extended to the genus Kappaphycus e.g., the previously identified *Eucheuma cottonii* was found to be *Kappaphycus alvarezzi*. Both *E. arnoldii* and *E. denticulatum,* species native to Vietnam are red seaweeds like *Kappaphycus*. Limited Vietnam-based information on the genus is available, hence further research is needed.

3.4.1 Production levels

Information on production status of *Eucheuma* in Vietnam is unavailable in current literature.

3.4.2 Use

In Vietnam, *Eucheuma* is currently only consumed as food but has potential as a source of carrageenan for industrial use (Titlyanov et al. 2012).

3.5 *Caulerpa*

Caulerpa is a genus of green macroalgae.

3.5.1 Production levels

Although *C. lentillifera* was introduced to Vietnam about a decade ago, it is still not commercialised (Van Nguyen 2015). The specie is mainly cultivated in experimental farms using the 'off-bottom trays' technique developed by the Nha Trang Oceanography Institute and Tri Tin Company Limited. This method of cultivation leads to harvest of *C. lentillifera* after 2 weeks (de Gaillande et al. 2017).

3.5.2 Use

Caulerpa is mainly consumed as food (Van Nguyen 2015; de Gaillande et al. 2017).

3.6 *Ulva*

Ulva is a green macroalgae occurring naturally in Vietnam.

3.6.1 Production levels

Ulva cultivation is still in its trial phase in Vietnam.

3.6.2 Use

In Vietnam, species of *Ulva* are consumed as food or used in traditional medicine. *Ulva* is filled with minerals, vitamins, soluble dietary fibres, insoluble dietary fibres (glucans) and relatively rich in proteins, and may be used as an animal feed (Morais et al. 2020). Studies on the inclusion of low quantities (1 or 2 % of total feed) of *Ulva lactuca* in rabbit feed indicated improvement in growth and reproductive performance of the animals without any side effects. The addition of *Ulva* species at higher quantities (≥ 10%) showed no significant changes in rabbit growth or productivity and had negative impact on faecal matter, therefore a maximum inclusion rate of 5% of the seaweed was recommended (reviewed in Makkar et al., 2016).

Ulva has been recently identified as a potential candidate to produce bioethanol due to its high carbohydrate content (Trung et al. 2019). The presence of ulvan (a sulphated carbohydrate) in these species make it a useful raw material for the pharmaceutical industry. Ulvans contain xylose, glucose, rhamnose, sulphate and uranic acid, and have anti-inflammatory, anticoagulant, antiviral, anticancer and antihyperlipidemic effects when administered in high dosages (Hong et al. 2011; Pereira 2018).

3.7 *Hydropuntia*

Hydropuntia is a red macroalgae.

3.7.1 Production levels

Hydropuntia. edulis and *H. eucheumatoides* are the common species of *Hydropuntia* present in Vietnam. The algae are typically harvested between March to September (Titlyanov et al. 2012). Current productivity levels of local *Hydropuntia* remains unknown.

3.7.2 Use

In Vietnam, *Hydropuntia* is consumed as food, either cooked directly or used to produce agar (as a food additive).

3.8 *Sargassum*

The genus *Sargassum* is a type of brown seaweed.

3.8.1 Production levels

Although at least 60 species of Sargassum exist in Vietnam, only about 10 species commonly occur. These species are: *S. carpophyllum*, *S. aquifolium*, *S. duplicatum*, *S. glaucescens*, *S. graminifolium*, *S. henslowianum*, *S. mcclurei*, *S. oligocystum*, *S. polycystum*, and *S. vachellianum* (Titlyanov et al. 2012). The cultivation period of these species occur between November to June and harvest is performed between March to June (Titlyanov et al. 2012). Annual production levels of *Sargassum* in the 1900s was estimated at 20 000 wet tons but only about 2.5% of the macroalgae was harvested. Current demand for *Sargassum* due to exportation to China has resulted in entire biomass removal causing sharp decline in the overall productivity of local Sargassum beds (Titlyanov et al. 2012).

3.8.2 Use

In Vietnam, some species of the *Sargassum* are directly consumed as food (a source of potassium and protein) while others are used in traditional medicine (Hong et al. 2007; Titlyanov et al. 2012). They also serve as organic fertilizers for crop cultivation in Vietnam (Titlyanov et al. 2012). The presence of phycocolloids alginate and fucoidan in these seaweeds, provide starting material for food, pharmaceutical, cosmetics and textile industries. (FAO 2018; Hong et al. 2018). Experiments with rats, indicated that methanolic extracts from *S. swartzii* exhibited acute and chronic anti-inflammatory effects similar to those observed when reference drugs indomethacin and prednisolon were administered (Hong et al. 2011). The positive effect although observed only under administrations of higher seaweed concentrations (i.e., 175 and 350 mg/ kg of seaweed compared to 25 mg/ kg of indomethacin and 5 mg/ kg of prednisolon respectively) did not cause (short-term) toxicity (Hong et al. 2011). Thus, indicating its potential as alternative anti-inflammatory medicine.

Addition of *Sargassum dentifolium* (inclusion at 3 or 6% of total chicken feed) was beneficial to layers, improving egg quality (Al-Harthi and El-Deek 2012; Makkar et al. 2016). Cultivation of *Sargassum* beds may mitigate CO_2 emissions by serving as short-term blue carbon sinks that significantly contribute to long-term carbon sequestration (McLeod et al. 2011; Sondak et al. 2017). Production of biochar from *Sargassum* may also facilitate long-term carbon sequestration (Farrelly et al. 2013; Sondak et al. 2017).

3.9 *Laurencia*

Laurencia is a red macroalgae present in Vietnam.

3.9.1 Production levels

Information on local production levels is unavailable.

3.9.2 Use

Laurencia has high amounts of certain nutrients and bioactive compounds (*L. obtusa* has a rich supply of K^+) making it beneficial for consumption as food and use in making folk medicine (Hong et al. 2007; Titlyanov et al. 2012).

4 Ranking of Seaweed Species

4.1 Methodology

A database was constructed based on information from current literature on selected Vietnamese seaweed species (Wong and Cheung 2001; Zubia et al. 2008; Matanjun et al. 2009; Ermakova et al. 2011; Msuya et al. 2012; Junaidi 2013; Cuong et al. 2016; Roy and P 2017; Nurjanah et al. 2018; Bui et al. 2019; Rasyid et al. 2019; Hung et al. 2019, 2021; Lumbessy et al. 2019; Suryaningrum and Samsudin 2020; Rodrigues et al. 2021; Widiawati and Hikmawan 2021). It should be noted that this is a tentative list based on current information available on chemical composition of the local seaweed species.

4.1.1 Species determination

Of the 739 species of local macroalgae (consisting of 412, 180 and 147 species of red, green and brown seaweeds), 82 economically important seaweed species that are harvested year-round and used mainly for food or medicine were first selected (Van Nguyen et al. 2013; Van Nguyen 2015). *Asparagopsis taxiformis* was added to the seaweed list since it is an important specie previously identified for mitigation of methane emissions. The current literature pool for Vietnamese seaweeds is insufficient and there was no available information on several local species. Thus, a final list consisting of 24 seaweed species and 2 undifferentiated species (*Sargassum* and *Gracilaria*) was used for the ranking.

4.1.2 Criteria for ranking

Although the ideal amounts of the seaweed-derived bioactive compounds needed to successfully reduce livestock enteric emissions remain unknown, we have knowledge of compounds that potentially play critical role in enteric methane reduction (Further elaborations in Section 2.1). All bioactive compounds used as criteria for ranking are present in (specific) seaweeds. Seaweed species were scored between 0 to 12 based on the presence of specific bioactive compounds.

The first category consists of bioactive compounds where *in vitro* and/ or *in vivo* experiments have been reported to reduce methane emissions (Vissers et al. 2018; Roque et al. 2019, 2021). These are bromoform/ halocarbons and phlorotannin. In the database: the absence, low or high quantities of bromoform in a seaweed specie was scored as 0, 3 or 12 respectively while the absence or presence of phlorotannins was scored as 0 or 6 respectively. The difference in ultimate values between bromoform and phlorotannins was due to the effectivity of bromoform over phlorotannins in reducing livestock methane emissions (Vissers et al. 2018; Roque et al. 2021). The second category of bioactive compounds are those that can still reduce methane emissions but experiments have not yet been performed with the inclusion of seaweeds in livestock feed. These compounds are monosaturated (MUFAs), polysaturated fatty acids (PUFAs), nitrate and sulphate. All seaweeds contain fatty acids, nitrate and sulphate, hence seaweeds were scored between 0 to 4, and 0 to 2 based on their concentrations of fatty acids, and nitrate/ sulphate respectively. The third (last) category consists of bioactive compounds that theoretically have a positive effect on reducing enteric methane emissions. These compounds are saponins, alkaloids and sulphated carbohydrates (carrageenan, agar, fucoidan and ulvan). Scores for saponin and alkaloids were based on absence or presence as 0 or 1 respectively. All seaweeds contain some type of sulphated carbohydrates therefore, the seaweeds were scored between 0 to 2 based on their concentrations of the carbohydrates.

Since data on local seaweed cultivation costs and productivity is scarce and unreliable, it was not used as a criterion for selection of potential candidates.

4.2 Results: Overview of Potential Candidates

	Type	**Seaweed specie**	**Bromoform**	**Phlorotannins**	**MUFAs/ PUFAs**	**Nitrate/ Sulphate**	**Saponins/ alkaloids**	**Sulphated carbohydrates**	**Total score**	**Rank**
1	R	*Asparagopsis taxiformis*	+++	-	+	-	-	+	20	1
	B	**Sargassum spp*	-	+++	++	+	-	+++	19	2
2	B	*Sargassum polycystum*	-	+++	++	+	-	+++	19	2
3	G	*Ulva lactuca*	+	-	++	+	+	++	18	4
4	G	*Ulva intestinalis*	+	-	++	+	+	++	18	4
5	R	*Eucheuma denticulatum*	-	-	+++	-	-	++	13	6
	R	**Gracilaria spp*	-	-	+	+	+	+++	12	7
6	R	*Kappaphycus alvarezzi*	-	-	+	+	+	+++	12	7
7	B	*Sargassum mcclurei*	-	++	+	-	-	+	12	7
8	G	*Caulerpa lentillifera*	-	-	++	-	+	++	12	7
9	R	*Gracilaria salicornia*	-	-	+	-	-	++	11	11
10	G	*Ulva reticulata*	-	-	+	-	+	+	11	11
11	R	*Kappaphycus striatus*	-	-	+	-	-	++	11	11
12	R	*Hydropuntia eucheumatoides*	-	-	+	-	-	++	11	11
13	R	*Laurencia obtusa*	-	-	+	+	-	+	10	15
14	G	*Caulerpa racemosa*	-	-	+	-	+	+	10	15
15	R	*Gracilaria tenuistipitata*	-	-	+	-	-	++	8	17
16	B	*Sargassum henslowianum*	-	+	+	-	-	+	7	18
17	R	*Gracilaria arcuata*	-	-	-	-	-	++	7	18
18	B	*Sargassum oligocystum*	-	+	-	-	-	++	6	20
19	R	*Eucheuma arnoldii*	-	-	-	+	-	++	6	20
20	R	*Laurencia similis*	-	-	+	-	-	+	5	22
21	R	*Kappaphycus cottonii*	-	-	-	+	-	+	4	23
22	R	*Hydropuntia edulis*	-	-	-	-	-	+++	3	24
23	G	*Ulva papenfussii*	-	-	-	-	-	++	2	25
24	R	*Kappaphycus inermis*	-	-	-	-	-	++	2	25

Table 1: Ranking of local seaweed species. The seaweed type, specie, levels of bioactive compounds (bromoform, phlorotannins, MUFAs, PUFAs, nitrate, sulphate, saponins, alkaloids and sulphated carbohydrates i.e., carrageenan, agar, fucoidan and ulvan), total score and rank are indicated in their respective columns. **Key**: R, B and G indicate red, brown and green seaweeds respectively; * denote species within the same genera that were undifferentiated in literature; while -, +, ++ and +++ represents absent/ negligible-, present/ low-, moderate- or high concentrations of specific bioactive compounds respectively, relative to the typical quantities measured in seaweeds.

Unsurprisingly, *A. taxiformis* was determined as the seaweed specie with the best potential to reduce enteric emissions from our table, supporting what is already known from literature (Roque et al. 2019, 2021). *Sargassum* appears 3 times in the top 10 with the undifferentiated specie and *S. polycystum* ranked in second place while *S. mcclurei* came in 7th place. Although (all) these specific species still need further investigation, previous research already shows that inclusion of another *Sargassum* specie *S. flavicans* (originating from Australia), reduced methane emissions *in vivo* by 34% (Machado et al. 2014; Abbott et al. 2020). Both *Ulva* seaweeds: *U. lactuca* and *U. intestinalis* are ranked in the 4th position. Certain *Ulva spp* from Australia reduced methane emissions by 45 to 50% in cattle (Machado et al. 2014; Abbott et al. 2020). *E. dentriculatum* appears in the 6th position while *K. alvarezzi*, *C. lentillifera* and an undifferentiated *Gracilaria spp* all come in the 7th place. An undifferentiated *Gracilaria spp* originating from Indonesia and included in sheep feed (at 2% of dry matter) reduced methane emissions *in vitro* by 49% (Prayitno et al. 2019; Abbott et al. 2020). Overall, the reported literature supports potential of the selected seaweeds in reducing livestock methane emissions and therefore the usefulness for further experimental validation of the high-ranking seaweed species.

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