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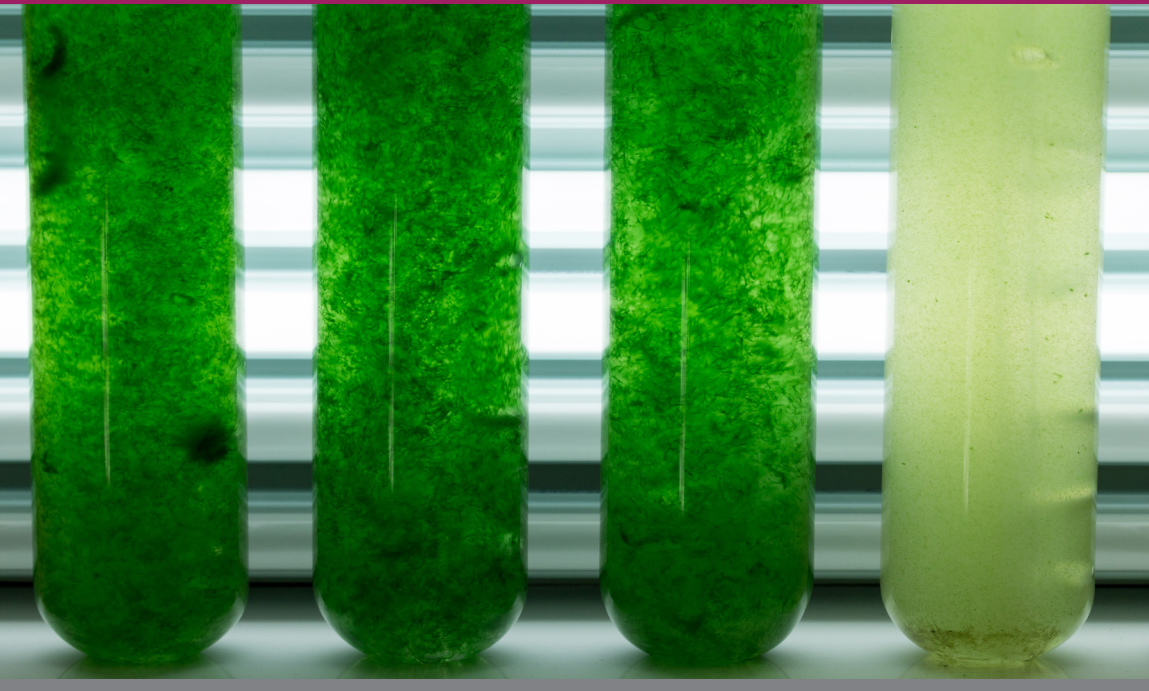

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# Nutritional and anti-methanogenic potentials of macroalgae for ruminants

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## 1 Introduction

The global human population is rising rapidly and has been projected to be ~10 billion by 2050 (Holt-Giménez, 2019). This implies that by that time, an increase of 60–70% in overall food and ~78% in meat production is required (Estrada et al., 2011; Alexandratos and Bruinsma, 2012; Holt-Giménez, 2019). The livestock sector supplies ~28% of the global protein consumption, but in developed countries this contribution may be as high as 48% (Estrada et al., 2011). Thus, the livestock sector will continue to play a crucial role as a source of high-quality protein for human consumption in the future (Åby et al., 2014). However, ruminant livestock species such as cattle, goats and sheep are responsible for ~17% of the total anthropogenic enteric methane

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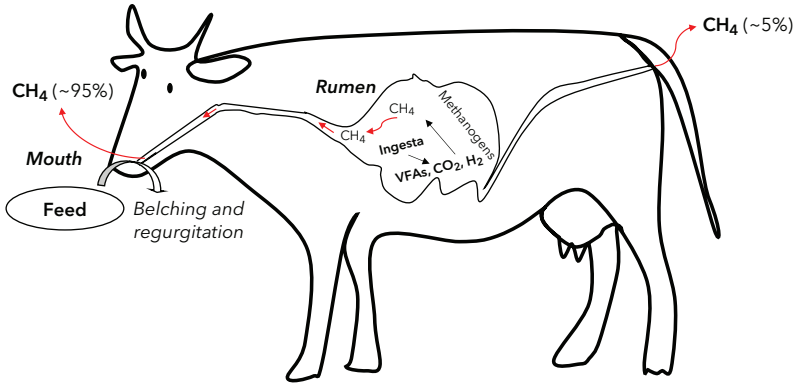


**Figure 1** A general outline of methane production and emissions from ruminant animals. Enteric fermentation of ingested feeds occurs in the rumen, where the majority of $CH_4$ is released from the mouth by eructation. $CH_4$, methane; VFAs, volatile fatty acids; $CO_2$, carbon dioxide; $H_2$, hydrogen.

($CH_4$) emissions, via fermentation of feeds in their forestomach (Fig. 1) (Knapp et al., 2014). Ruminants possess a unique digestive system comprised of a four-chambered stomach: rumen, reticulum, omasum and abomasum. The rumen is the residence of a large number of microorganisms, including bacteria, fungi, protozoa and archaea, and these microorganisms play a vital role in feed degradation and energy supply to the host animals (Bergman, 1990; Maia et al., 2016). Feed components, particularly carbohydrates, get partially or completely fermented in the rumen and produce volatile fatty acids (VFAs) such as acetate, propionate, butyrate, and also carbon dioxide ($CO_2$) and hydrogen ($H_2$) (Van Nevel and Demeyer, 1996) (Fig. 2). Volatile fatty acids are an important energy source for ruminants, while $CO_2$ and $H_2$ may later be reduced to $CH_4$ by the action of methanogenic archaea before getting eructed from animals into the environment (Bergman, 1990).

Methane is one of the major contributors of global warming and has a 28 times higher global warming potential than another greenhouse gas, $CO_2$ (Grossi et al., 2019). The $CH_4$ emission from the rumen represents a loss of up to 15% of gross energy (GE) from the feed, which could otherwise be utilized for animal growth and production (Van Nevel and Demeyer, 1996), and is, therefore, unfavorable for the animal. Enteric methanogenesis is thus both an environmental and nutritional concern, and any interruption in this process could provide nutritional benefits to the animals and result in the release of the less potent greenhouse gases $CO_2$ and $H_2$, compared to the highly potent $CH_4$ (Patra et al., 2017; Grossi et al., 2019). Hence, development of appropriate $CH_4$ abatement strategies is important to attain sustainable ruminant production systems in the future (Grossi et al., 2019).

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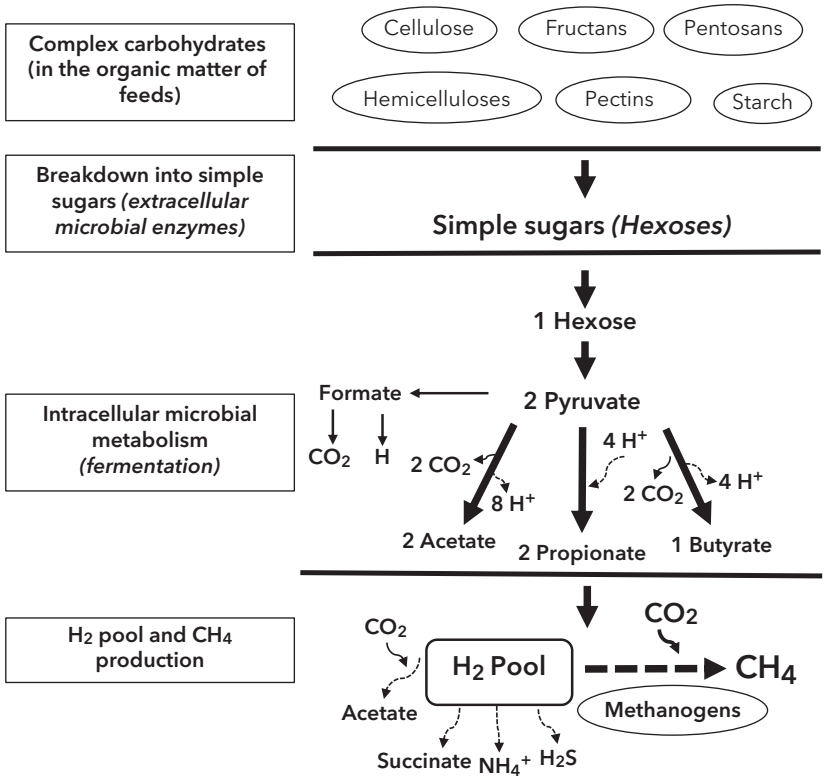


**Figure 2** Major pathways involving degradation of carbohydrates, and production of volatile fatty acids and methane by microbial fermentation in the rumen. Adapted with some modifications from (McDonald et al., 2011; Haque et al., 2014; Kohn and Boston, 2000; Ungerfeld, 2013). $CH_4$, methane; $CO_2$, carbon dioxide; $NH_4$, ammonium; $H_2S$, hydrogen sulfide. Dotted lines represent pathways involving metabolic hydrogen (H) or dihydrogen ($H_2$).

Several $CH_4$ mitigation strategies have been suggested to cope with the $CH_4$ emissions from ruminants. These include (i) mitigation of $CH_4$ emissions via genetic selection (González-Recio et al., 2020), (ii) use of anti-methanogenic chemical compounds such as nitrate, chloroform and 3-nitroxy propanol (Patra et al., 2017) and (iii) dietary interventions using alternative feed ingredients and nutritional strategies (Knapp et al., 2014). Genetic selection can permanently reduce $CH_4$ production from an individual animal and can be inherited to the offspring (González-Recio et al., 2020). However, this approach could be technically demanding and time consuming, and convincing outcomes of genetic selection are yet to be obtained (Knapp et al., 2014; González-Recio et al., 2020). The application of anti-methanogenic chemical compounds is an effective strategy in reducing $CH_4$ emissions; however, their effects can be

transitory and such compounds may have adverse impacts on both animal performance and the environment (Patra et al., 2017). Dietary interventions can be a relatively simple and environmentally friendly approach and can lead to no or lower negative consequences to animal health and performance (Haque, 2018; Benchaar et al., 2001; Haque et al., 2014). Despite some advantages, only a modest reduction (5–40%) in $CH_4$ emissions by dietary interventions has been reported (Benchaar et al., 2001; Knapp et al., 2014). In this context, identification of alternative and novel feed materials that can substantially decrease enteric $CH_4$ production without compromising animal health and production would be important to develop novel $CH_4$ mitigating strategies in the future.

Marine macroalgae (also commonly known as seaweeds) have been identified as an alternative feed resource that can largely decrease enteric $CH_4$ production from ruminants (Machado et al., 2016; Maia et al., 2016). Macroalgae consist of 6000–10 000 diverse marine species distributed along the coastal regions worldwide, and they can be categorized into three types based on their pigmentation: brown, red and green (Tiwari and Troy, 2015; Makkar et al., 2016; Rajauria, 2015). Within the three categories of macroalgae, there are large species variations with respect to chemical composition (carbohydrates, proteins and minerals), and how digestible the organic components are in ruminant animals (Makkar et al., 2016; Dawczynski et al., 2007). Additionally, macroalgal species produce a wide range of bioactive components, such as halogenated compounds, polyphenols, complex polysaccharides and pigments (O'Sullivan et al., 2010; Charoensiddhi et al., 2017; Machado et al., 2016). Their bioactivities include antioxidative (Kannan et al., 2007; Ling et al., 2015), anti-microbial, immunomodulatory (Turner et al., 2002; Kim et al., 2018), anti-diabetic (Kang et al., 2016), anti-inflammatory, prebiotic (Cañedo-Castro et al., 2019; Reilly et al., 2008; O'Sullivan et al., 2010) and anti-methanogenic properties (Machado et al., 2014; Roque et al., 2019a). This broad range of chemical activities may enhance the future commercial application of macroalgae as multipurpose feed ingredients (Øverland et al., 2019).

Macroalgae have long been utilized as a feed ingredient for ruminant animals in different parts of the world. In many countries, including Iceland, Norway, France, Germany, Sweden, Finland, Scotland and the USA, macroalgae were used as an occasional or regular animal feed, particularly during extreme winter conditions, when the availability of other feed resources was limited (Makkar et al., 2016; Evans and Critchley, 2014; Chapman, 2012; Hansen et al., 2003; Applegate and Gray, 1995). However, there are very few published studies on the application of macroalgae as commercial and regular feed resources for ruminant animals. A brown macroalgae, *Ascophyllum nodosum*, has been reported to be used in small amounts as a feed additive for dairy cows in some organic farms in the USA (Erickson et al., 2012). North Ronaldsay (Orkney) sheep in Northern Scotland are purported to survive by grazing on different macroalgal species: *A. nodosum,*

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*Alaria esculenta, Fucus* spp., *Laminaria* spp., *Saccharina latissima* and *Palmaria palmata* (Hansen et al., 2003; Makkar et al., 2016). However, their commercial application for farm animals on a large scale is yet to be achieved.

Macroalgal species within all three (red, brown and green) categories have been identified to have $CH_4$ mitigating properties both *in vitro* and *in vivo* (Machado et al., 2014; Maia et al., 2016; Belanche et al., 2016b). However, when using some macroalgal species as feed, rumen fermentation patterns and total tract digestibility may be negatively affected due to high contents of ash and complex carbohydrates of low rumen degradability (Bikker et al., 2020). This can reduce the overall animal performance, particularly when such macroalgae are fed in large amounts (Bikker et al., 2020). Hence, the implications of the anti-methanogenic properties of macroalgae must be evaluated based on their overall impacts on feed intake, digestibility and animal performance. To be able to exploit macroalgae as potential feed resources, it is essential that species of commercial relevance be extensively characterized from both a biochemical (including anti-methanogenic compounds) and a nutritional point of view as presented in Fig. 3.

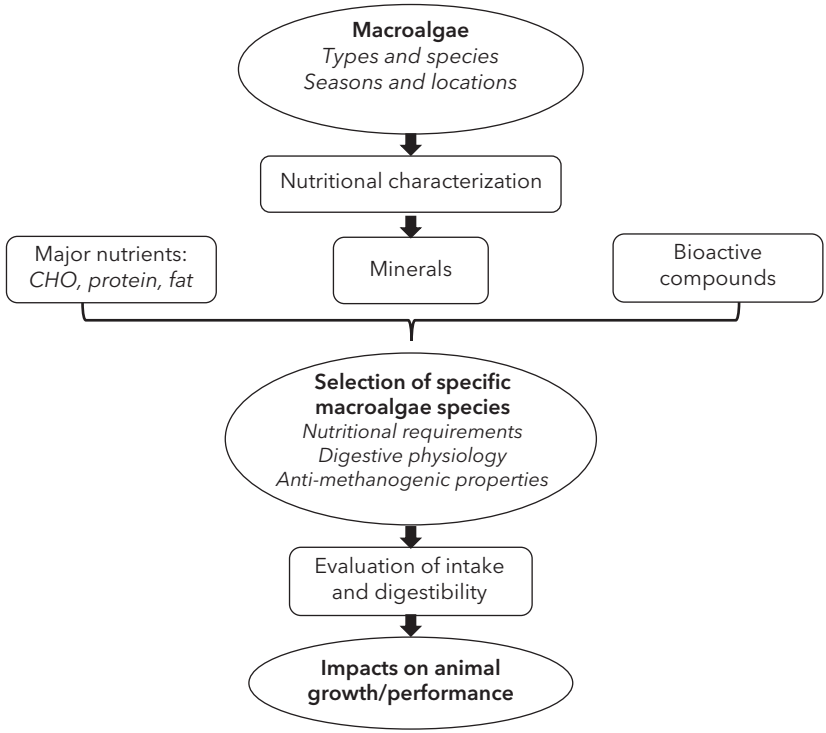


**Figure 3** A flowchart for the evaluation of macroalgae as a potential ruminant feed ingredient in future. CHO, carbohydrates.

This chapter aims to evaluate the role of macroalgae as a potential anti-methanogenic ruminant feed resource. Similarly, effects of different intrinsic (macroalgal species, types) and extrinsic (growing season, post-harvest processing) factors on nutritional value as well as concentration of bioactive compounds and anti-methanogenic properties will be discussed. This will enable us to evaluate whether macroalgae can be used as anti-methanogenic dietary additive without compromising overall animal production and performance.

## 2 Nutritional value of macroalgae

Fresh macroalgae biomass normally contains about 70–90% water and various macro- and micro-nutrient fractions (Kılınç et al., 2013; Biancarosa et al., 2017). In this section, protein, carbohydrate, mineral and lipid contents of various macroalgal species will be described, and their potential as ruminant feeds will be evaluated. Unless otherwise stated, the contents are reported as % of dry matter (DM) to allow comparisons.

*Protein*: Red macroalgae species generally contain greater levels of crude protein (CP) than brown and green species. Some red species belonging to the genera *Palmaria, Pyropia* and *Porphyra* have been reported to contain 20–50% CP (Tibbetts et al., 2016; Fernández-Segovia et al., 2018; Marsham et al., 2007; Jung et al., 2016). The green macroalgae *Acrosiphonia* spp. and *Ulva* spp. also contain high levels of CP (appr. 31% and 25%, respectively) (Biancarosa et al., 2017; Peña-Rodríguez et al., 2011; Tayyab et al., 2016), whereas CP levels in most of the brown macroalgae are <15% (Dawczynski et al., 2007; Biancarosa et al., 2017). Thus, red and green macroalgae are the most relevant to consider as protein sources for animals.

Macroalgae proteins are reported to have a high quality due to their high proportion of essential amino acids (EAA) (Angell et al., 2016; Mišurcová, 2012). The red species *P. palmata, Porphyra* spp. and *Vertebrata lanosa*, the brown species *A. nodosum* and *Undaria pinnatifida* and the green species *Enteromorpha intestinalis* (*Ulva* sp.) have a higher EAA index and are thus considered to be superior compared to cereals from a nutritional point of view (Mæhre et al., 2014; Dawczynski et al., 2007; Gaillard et al., 2018). The EAA proportion in macroalgae can account for 45.7% of the total amino acids, which is similar to that of the conventional protein feed resource soybean meal (46%) and greater than fishmeal (43.4%) (Angell et al., 2016; Dawczynski et al., 2007; Biancarosa et al., 2017). Although the requirements for EAA would vary based on specific animal parameters (e.g. age, growth stage, production purpose), the EAA content of selected macroalgal species are reported to be able to fulfill the human and animal requirements (Mæhre et al., 2014). Therefore, selected macroalgae, particularly red and green species, could be considered

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as alternative sources of quality feed protein but their biomass yield and technologies for large-scale cultivation must be taken into account. This is, however, beyond the scope of the present chapter.

The significance of alternative proteins in ruminant nutrition depends on their digestibility. Studies regarding *in vivo* digestibility of macroalgae proteins are relatively scarce; however, *in vitro* protein digestibility (IVPD) has been explored for a number of species. The IVPD of the red macroalgae *Chondrus crispus, P. palmata, Sarcodiotheca gaudichaudii* and *Meristotheca papulosa* have been found to be ~85% of the total CP content, whereas IVPD for the brown species: *A. esculenta, A. nodosum, Fucus vesiculosus* and *S. latissima* are reported to be slightly lower (~80%) (Tibbetts et al., 2016). In ruminants, a significant part of the feed CP is degraded via microbial action in the rumen and subsequently utilized in microbial protein synthesis, including synthesis of EAA (Hvelplund and Weisbjerg, 2000). The amount of protein that passes un-degraded by the microbes to the small intestine is called rumen escape protein (REP). The bioavailability and amino acid composition of this fraction becomes particularly important, when feed protein degradability and hence microbial protein supply from the rumen is low (Hvelplund and Weisbjerg, 2000). An in situ study illustrated that 50–70% of the CP from *A. esculenta, L. digitata* and *P. palmata* is degraded in the rumen within 24 h, while for other species including *M. stellatus, Ulva* and *Pelvetia canaliculata* rumen CP degradability was substantially lower (<35%) (Tayyab et al., 2016). Hence, for many of the above mentioned macroalgal species, a large proportion (30–51% of total CP) of protein supply to the small intestine will be REP, and the intestinal digestibility of the REP becomes important for the potential amino acid supply to the animal (Tayyab et al., 2016). In the same study, degradation of CP in the small intestine was negligible for *A. esculenta* and *P. canaliculata*, while this value was similar or greater than the rumen degradability in others (*Porphyra*, *Palmaria*, *Ulva*, *Acrosiphonia*, *Mastocarpus*) (Tayyab et al., 2016). In addition, in situ total tract amino acid degradability of *Porphyra* sp. and *P. palmata*, and green macroalgae *Cladophora rupestris* and *Ulva* sp. has been found to be the highest among macroalgal species (Gaillard et al., 2018). These studies suggest that green and red macroalgae species are interesting new potential sources of rumen degradable and intestinal digestible protein for ruminants.

*Carbohydrates*: Carbohydrates are generally the most abundant organic compounds in macroalgae and may account for 25–75% of their DM (Jiménez-Escrig and Sánchez-Muniz, 2000; Rioux and Turgeon, 2015). They comprise both soluble and non-soluble carbohydrates and their relative amounts and composition vary depending upon macroalgae type and species (O'Sullivan et al., 2010). The major carbohydrates in macroalgae are unknown in terrestrial plants, and include alginate, fucoidan, mannitol and laminarin in brown; agar, carrageenan and porphyran in red; and ulvan and xylans in green species

(Cherry et al., 2019). Despite being indigestible in monogastric animals, macroalgae polysaccharides, particularly from brown species, have attracted research interest as prebiotics due to their beneficial gut impacts (O'Sullivan et al., 2010). These polysaccharides can partially or completely be fermented in the hindgut by the action of specific gut commensal bacteria producing short-chain fatty acids, and can thereby contribute to inhibit the growth of gut pathogens, such as *Clostridium* spp., *Escherichia coli* and *Salmonella* spp. (Braden et al., 2004; Seong et al., 2019). The prebiotic effect of macroalgae polysaccharides has mostly been studied in non-ruminant animals, including weanling piglets and humans (Reilly et al., 2008; Smith et al., 2011), and information about prebiotic effects for ruminant animals, containing relatively complex digestive systems, is limited. However, Tasco-14, an *A. nodosum*-based commercial additive, has been found to be effective in reducing the fecal shedding of *Escherichia coli* (O157:H7) and *Salmonella* spp. in feedlot cattle and lambs when supplemented in the diet at 2% DM basis (Braden et al., 2004; Bach et al., 2008). Further studies are needed to identify whether such reduced fecal shedding is due to the action of polysaccharides in the hind gut of cattle.

The nutritional value of macroalgae polysaccharides for ruminants depends on whether they can be degraded by the microbial population in the forestomach. Studies on the rumen microbes isolated from macroalgae-fed Ronaldsay sheep have revealed that polysaccharides from brown species, that is, alginate, fucoidan and laminarin, can variably and only partly be degraded by selective rumen microorganisms (Orpin et al., 1985; Williams et al., 2013). Only nine, out of 65, cultured isolates of rumen microorganisms were able to degrade >90% of the laminarin and 70–80% of alginates, but <20% of the fucoidans (Williams et al., 2013). The rumen microorganisms involved in the degradation of macroalgae carbohydrates include *Prevotella* spp., *Clostridium butyricum, Streptococcus bovis, Selenomonas ruminantium, Butyrivibrio fibrisolvens* and *Dasytricha ruminantium* (Orpin et al., 1985; Williams et al., 2013). However, as only a limited number of rumen microbes were included in the studies due to problems associated with microbial cultivation in artificial media, results from these *in vitro* fermentations may not be representative of the whole *in vivo* scenario of rumen degradability of macroalgal polysaccharides. Hence, further *in vivo* studies evaluating the digestibility of these polysaccharides are important to establish their nutritional value.

*Minerals*: Although mineral contents of macroalgae are affected by both intrinsic (macroalgae types and species) and environmental factors (culture conditions, seasons etc.), they are generally an excellent source of both macro and trace minerals. They are capable of accumulating a large quantity of minerals from seawater, and hence, the levels of various minerals including iodine, sodium, potassium, iron, chlorine and calcium in macroalgae are found to be 10–20 times higher than the levels found in terrestrial plants and fresh

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water algae (Pereira, 2011; Gómez-Ordónez et al., 2010; Makkar et al., 2016; Mišurcová et al., 2010). The capacity of macroalgae to concentrate minerals has been linked to their mineral-rich growing environment and the content of unique cell wall polysaccharides such as alginic acid, salts of alginate, agar and carrageenan that can absorb different inorganic ions from the seawater (Mišurcová, 2012). The ash content in macroalgal species can vary between 20% and 72% of DM (Cabrita et al., 2016; Rupérez, 2002; D'Armas et al., 2019). In general, brown and green species contain higher amounts of minerals than red species (Pereira, 2011; Cabrita et al., 2016; Fernández-Segovia et al., 2018). Due to the abundance of minerals in macroalgae, they are considered natural mineral sources for both livestock and humans; for example, they can be used for the prevention of iodine deficiency (Baňoch et al., 2010).

Minerals are important for normal functioning of different hormones and enzymes in the body (Trumbo et al., 2001; Mæhre et al., 2014). However, due to the high mineral contents such as sodium, chlorine, calcium, iron and iodine in many species (*Codium* spp.*, Himanthalia elongata, Laminaria* spp.*, Saccharina* spp., *Bifurcaria bifurcata* and *Ulva* spp.), an excess intake of macroalgae-based diets may result in mineral toxicity, particularly in monogastric species as they are at higher risk due to a generally lower tolerance towards excess mineral uptake than in ruminants (Bikker et al., 2020; Cabrita et al., 2016). Excessive uptake of iodine from macroalgae-based ruminant feeds can be excreted in milk or accumulated in body tissues, leading to undesirably high levels of iodine in animal products that can have adverse consequences for human health (van der Reijden et al., 2017). The maximum recommended level of iodine is 2 mg/kg feed for dairy ruminants in the European Union (EU) (Additives and Feed, 2013), due to concerns of toxic levels in ruminant products destined for human consumption. Therefore, an abundant mineral content limits the inclusion of macroalgae on a larger scale in ruminant diets, unless special precautions are undertaken while formulating diets (Bikker et al., 2020).

Macroalgae are also able to concentrate heavy metals such as arsenic, mercury and cadmium from seawater, which are known to have a range of adverse health impacts, such as cancer and renal dysfunctions (McLaughlin et al., 1999). Particularly the contents of inorganic versus organic arsenic must be considered due to the greater toxicity of the inorganic form, although the predominant form of arsenic in macroalgae is normally organic (~ 90%) (Díaz et al., 2012; Mæhre et al., 2014; Biancarosa et al., 2018). The levels of these heavy elements in 21 macroalgal species from the Norwegian coast were found to be far below the maximum tolerable levels set in the EU region (Biancarosa et al., 2018).

*Lipids*: Macroalgae generally contain a low level of lipids (<5%) (Makkar et al., 2016; Øverland et al., 2019). Lipids from macroalgae are considered beneficial for human health due to their bioactive properties (Mæhre et al.,

2014). However, it can be insignificant with respect to the supply of (essential) fatty acids in ruminant feeds due to their very low lipid content, and most of it is utilized by rumen microbes (Bikker et al., 2020).

## 3 Digestibility of macroalgae as a feed or feed ingredients

A broad range (20–97% organic matter, OM) of ruminal and post-ruminal degradability of macroalgae has been reported earlier (Table 1). The rumen degradability of brown macroalgae *A. nodosum, F. serratus,* and *F. vesiculosus* has been observed to be low (<33% of OM) when they were used as a sole ruminant diet (Greenwood et al., 1983). Moderate *in vitro* rumen DM degradability (40–65%) has been recorded for other brown (*M. pyrifera, A. esculenta, L. digitata, P. canaliculata*) as well as red (*M. stellatus, P. palmata, Porphyra* sp.) and green macroalgae (*Ulva lactuca* and *Acrosiphonia* sp.) (Gojon-Báez et al., 1998; Ventura and Castañón, 1998; Molina-Alcaide et al., 2017). However, *in vitro* rumen OM degradability for selected brown (*A. esculenta, L. digitata, L. hyperborea, Sargassum* spp., *S. latissima*) and red (*P. palmata*) species was found to be higher (80–89%) (Hansen et al., 2003; Makkar et al., 2016; Marín et al., 2009). Since the later studies were performed using rumen fluid and microbial inoculum obtained from macroalgae-eating Ronaldsay sheep (Orkney), the greater degradability could be the function of potential adaptation of rumen microbes to those particular macroalgae (Hansen et al., 2003). Hence, a gradual increase in the digestibility of macroalgae may be observed over time and therefore, animals may require an adaptation period to achieve an acceptable digestibility level. However, it is not known whether exposure to macroalgae-based diets at a young age would lead to a better feed digestibility in adults.

Feeding macroalgae at larger doses for a long duration can result in adverse health consequences such as bone and kidney dysfunctions in animals, probably due to mineral overload (Britt and Baker, 1990). Thus, long-term use of macroalgae as sole feed may not be safe, unless excess minerals and potentially toxic heavy metals are removed prior to feeding. Rinsing of macroalgae biomass with fresh cold or hot water (e.g. 40°C) for a short duration (30 min) could be effective in removing excess mineral salts from macroalgae (Magnusson et al., 2016). These types of processing may also enhance palatability of macroalgae and nutrient digestibility, though a loss of soluble nutrients can be expected (Magnusson et al., 2016). Thus, proper post-harvest processing of macroalgae biomass prior to animal feeding may minimize the risks of adverse health impacts on animals and can improve nutrient utilization.

Macroalgae can affect animal feed intake and rumen degradability of the feed, depending upon the inclusion level and the macroalgal species (Choi

**Table 1** Effects of macroalgae on rumen fermentation and animal performance

| Macroalgae species | Study type | Animal/rumen fluid donor | Dose | Impacts on ruminant nutrition | References |
|---|---|---|---|---|---|
| Red macroalgae: | | | | | |
| *Asparagopsis armata* | *In vivo* | Dairy cow | 0.5–1% OM | Reduces $CH_4$ but also reduces feed intake (−38%), lower milk yield (−11.6%) at 1% OM inclusion | (Roque et al., 2019b) |
| *Asparagopsis taxiformis* | *In vitro/ In vivo* | Steers/sheep | 0–16.7% OM | Effectively reduces $CH_4$, enables ADWG at low inclusion, lowers total VFA (≥2% OM inclusion) and OMD at ≥10% inclusion | (Machado et al., 2016; Li et al., 2018; Kinley et al., 2020) |
| *Gracilaria vermiculophyla* | *In vitro* | Cow | 25% DM | Reduces $CH_4$, no adverse effects on rumen fermentation | (Maia et al., 2016) |
| *Gigartina* sp. | *In vitro* | Cow | 25% DM | Reduces $CH_4$, no adverse effects on rumen fermentation | (Maia et al., 2016) |
| *Mastocarpus stellatus* | *In vitro* | Sheep/dairy cow | 20% DM | Lowers VFA production and has low in situ DM and crude protein degradability | (Molina-Alcaide et al., 2017) |
| *Palmaria palmata* | *In vitro/ in situ* | Sheep/dairy cow | 20% DM | High DMD, supplies high level of digestible protein, high amino acid index | (Tayyab et al., 2016; de la Moneda et al., 2019; Gaillard et al., 2018) |
| *Porphyra* spp. | *In vitro/ in situ* | Sheep/dairy cow | 20% DM | High DMD, provides high digestible and rumen by-pass protein (~50% of total protein content) | (Tayyab et al., 2016; de la Moneda et al., 2019; Gaillard et al., 2018) |
| Brown macroalgae: | | | | | |
| *Alaria esculenta* | *In vitro/ In situ* | Dairy cow, goat | 20% DM | High apparent DMD and protein digestibility | (de la Moneda et al., 2019; Tayyab et al., 2016) |

(*Continued*)

**Table 1** Effects of macroalgae on rumen fermentation and animal performance (*Continued*)

| Macroalgae species | Study type | Animal/rumen fluid donor | Dose | Impacts on ruminant nutrition | References |
|---|---|---|---|---|---|
| *Ascophyllum nodosum* | *In vitro/ in vivo* | Cattle, lambs, steers | 2–5% DM | Alters rumen and gut microbiome, and reduces OMD, Total VFA and $CH_4$ production | (Wang et al., 2009a; Belanche et al., 2016b) |
| *Fucus serratus* | *In vitro* | Sheep | ? | Low rumen degradability (15%) | (Greenwood et al., 1983; Makkar et al., 2016) |
| *Fucus vesiculosus* | *In vitro* | Sheep | ? | Low rumen degradability (26%) | (Makkar et al., 2016; Greenwood et al., 1983) |
| *Laminaria digitata* | *In vitro* | Cow/sheep | 5–20% DM | ~80% *in vitro* OM digestibility, high rumen degradability, enables microbial protein synthesis, provides greater AA supply in intestine | (Belanche et al., 2016a; Hansen et al., 2003; de la Moneda et al., 2019) |
| *Laminaria hyperborea* | *In vivo* | Sheep | ? | High rumen degradability, high in vitro OM digestibility (~80%) | (Hansen et al., 2003) |
| *Laminaria ochroleuca* | *In vitro* | Cow | 25% DM | No adverse effects on fermentation parameters (e.g. Total VFA, $CH_4$) | (Maia et al., 2016) |
| *Macrocystis pyrifera* | *In situ* | Bull | ? | ~ 85% degradability of DM (96 hr) and high by-pass protein | (Gojon-Báez et al., 1998) |
| *Pelvetia canaliculata* | *In vitro* | Goat | Sole feed /20% DM | Low DMD, lowers VFA volume | (de la Moneda et al., 2019; Molina-Alcaide et al., 2017) |
| *Saccharina latissima* | *In vitro* | Cow/ goat | 20–25% DM | Improves DMD and OMD of feed, no effects on VFA production and rumen fermentation | (de la Moneda et al., 2019; Maia et al., 2019) |
| *Sargassum* spp. | *In situ/in vivo* | Bull/sheep | 10–30% DM | No effect on feed intake and digestibility, ~55–79% DMD and high protein digestibility (>85%) | (Gojon-Báez et al., 1998; Marín et al., 2009) |

| | | | | | |
|---|---|---|---|---|---|
| *Undaria pinnatifida* | *In vitro/ In situ* | Cow | 10% DM | Improves feed digestibility and rumen environment, VFA production, provides quality and digestible protein | (Choi et al., 2019) |
| Green macroalgae: *Acrosiphonia* sp. | *In situ* | Dairy cow | 20% DM | Provides high rumen digestible (~46%) and rumen by-pass protein (~31%) | (Tayyab et al., 2016; Molina-Alcaide et al., 2017) |
| *Chaetomorpha* sp. | *In vivo* | Sheep | 20–40% DM | No adverse effects on feed digestibility and growth performance up to 30% inclusion | (Rjiba-Ktita et al., 2019) |
| *Cladophora patentiramea* | *In vitro* | Steers | 16% (OM) | Reduces $CH_4$, feed degradability and VFA volume | (Machado et al., 2014) |
| *Cladophora rupestris* | *In situ* | Dairy cow | - | High protein content, 75% total tract AA degradability | (Gaillard et al., 2018) |
| *Ruppia* sp. | *In vivo* | Sheep | 20–40% DM | Improves feed intake, no adverse effects on feed digestibility and growth performance up to 30% inclusion | (Rjiba-Ktita et al., 2019) |
| *Ulva rigida* | *In vivo/ in vitro* | Sheep/ cow | 25% DM | Increases DMD of the feed, no effect on VFA and $CH_4$ production | (Cabrita et al., 2017; Maia et al., 2019) |
| *Ulva* sp. | *In vivo/ in vitro* | Sheep | 20–40% DM | High rumen degradable and by-pass protein, no effects in feed intake and degradability for 30% DM inclusion | (Tayyab et al., 2016; Rjiba-Ktita et al., 2019) |

DM, dry matter; OM, organic matter; DMD, dry matter degradability; OMD, organic matter degradability; AA, amino acids; ADWG, average daily weight gain; VFA, volatile fatty acids.

et al., 2019; Maia et al., 2019; Rjiba-Ktita et al., 2019). For instance, a low inclusion level of the anti-methanogenic red macroalga *Asparagopsis* taxiformis resulted in an improved average daily weight gain and OM degradability of the feed in cattle when included at ≤5% of OM, but the opposite effects were observed as the dose was increased to 10% OM of the total ration (Machado et al., 2016; Kinley et al., 2020). Hence, an inclusion level of <5% OM appears to be the cut off value for *A. taxiformis* in terms of maintaining the feed digestibility and fermentation parameters, such as VFA production (Machado et al., 2016; Roque et al., 2019a). Another anti-methanogenic red macroalga *Asparagopsis armata*, however, reduced feed intake, weight gain and milk yield at a relatively low inclusion level (≤1% OM) in the feed of dairy cattle (Roque et al., 2019b). This indicates that *Asparagopsis* spp. can be included in the ruminant feed at a low inclusion to achieve beneficial impacts in overall animal performance.

Other, different, red, brown and green species have also shown similar trends as *Asparagopsis* spp.; however, they can possibly be included at higher doses. Increased *in vitro* DM degradability and VFA production were observed with the edible brown macroalgae, *Undaria pinnatifida*, when it was incorporated up to 10% DM in the feed (Choi et al., 2019). Similarly, stable feed digestibility and animal performance were achieved with other brown (*A. esculenta, L. digitata, S. latissima*), red (*G. vermiculophyla, M. stellatus, P. palmata, Porphyra* sp.) and green (*Cladophora* sp. and *Ulva* spp.) macroalgae up to 20–25% of DM inclusion (de la Moneda et al., 2019; Maia et al., 2019). A few other green macroalgae (*Chaetomorpha* sp., *Ruppia* sp., and *Ulva* sp.) produced no significant negative effects on feed intake and digestibility in Barbarine sheep at up to 30% DM inclusion, but feed digestibility was reduced while the inclusion was increased to 40% (Rjiba-Ktita et al., 2019). This suggests that these macroalgal species can be incorporated to 10–30% in the ruminant rations, though more *in vivo* studies are needed to establish a beneficial inclusion level of a broad range of macroalgal species.

Macroalgae are generally low energy containing feeds due to low contents of lipid and starch, a large proportion of complex polysaccharides and relatively large content of ash (Bikker et al., 2020; Angell et al., 2016; Øverland et al., 2019). The GE contents of macroalgae, including *U. lactuca, Ulva rigida, G. vermiculophyla* and *S. latissima*, have been reported to be less (14–15.2 MJ/kg DM) than conventional ruminant feeds such as corn silage, hay silage and commercial concentrates (17.4–18.9 MJ/kg DM) (Maia et al., 2019; Ventura and Castañón, 1998). However, some brown macroalgae including *A. esculenta, A. nodosum* and *F. vesiculosus* have higher GE and digestible energy levels than the terrestrial forages such as winter rye and lichen (Applegate and Gray, 1995). Thus, although the majority of macroalgae lead to a lower energy supply compared to conventional feeds, there is a scope for their future use

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as feed additives in the ruminant's rations due to their high mineral contents and promising anti-methanogenic potentials as described in the following sections.

## 4 Anti-methanogenic properties of macroalgae

In addition to the aforementioned macro- and micro-nutrients, macroalgae are also rich sources of a wide range of bioactive components (such as pigments, tocopherols and various secondary metabolites) (Gupta and Abu-Ghannam, 2011). Macroalgae are gaining interest as anti-methanogenic feed ingredients in ruminants due to their richness of bioactive compounds, particularly halogenated and polyphenolic secondary metabolites that are able to inhibit $CH_4$ formation during the fermentation of feed in the forestomach (Roque et al., 2019a; Wang et al., 2008). In the following sections, the anti-methanogenic potentials of different macroalgal species will be discussed.

***Red macroalgae***: The potential of macroalgae to suppress enteric $CH_4$ formation in ruminants has been evaluated using both *in vitro* and *in vivo* studies (Table 2). The most convincing anti-methanogenic properties have been found among the red macroalgae, particularly *Asparagopsis* spp. (Machado et al., 2016; Roque et al., 2019b; Kinley et al., 2020). It was reported that a 40–98% reduction of $CH_4$ emission in steers could be achieved by adding as little as 0.1–0.2% (OM basis) *A. taxiformis* to a high grain diet (Kinley et al., 2020). Similarly, a 72-day feeding trial in sheep using the same macroalgae in a mixed ration (3% of the OM of the diet) containing a high proportion of fiber, resulted in an overall 80% reduction of enteric $CH_4$ production (Li et al., 2018). This is consistent with several *in vitro* fermentation studies, where addition of 0.5–5% OM of this macroalgae along with different substrates resulted in an ~74–99% decline in $CH_4$ formation over a 72-h incubation period (Roque et al., 2019a; Brooke et al., 2018; Machado et al., 2016). Another red macroalgae species belonging to the same genus, *A. armata* has also been shown to suppress the $CH_4$ production by ~67%, when fed to dairy cattle at 1% of OM (Roque et al., 2019b). Thus, *Asparagopsis* spp. can be an effective feed additive which can reduce enteric $CH_4$ production dramatically at a minimal inclusion in the ruminant diet.

The anti-methanogenic property of red macroalgae is not limited to the genus *Asparagopsis*. Two other red species *Gigartina* sp. and *Gracilaria vermiculophyla* have also demonstrated anti-methanogenic attributes in *in vitro* fermentations, but a greater amount of the macroalgae was added (16–18% OM), and the magnitude of reduction was substrate dependent (Maia et al., 2016). For example, a 60% reduction in $CH_4$ production was observed when *G. vermiculophyla* was supplemented to either meadow hay or corn silage, whereas *Gigartina* sp. reduced $CH_4$ production by 44%, but only when added

**Table 2** Macroalgae species and their anti-methanogenic potential

| Macroalgae species | Study type | Inclusion dose (%) | $CH_4$ ↓ (%) | Main active compound | Reference |
|---|---|---|---|---|---|
| Red macroalgae: | | | | | |
| *Asparagopsis armata* | *In vivo* | 1% OM | 67.2 | Bromoform | (Roque et al., 2019b) |
| *Asparagopsis taxiformis* | *In vivo/in vitro* | 0.1–16% DM/OM | 74–99 | Bromoform | (Kinley et al., 2020; Machado et al., 2016; Roque et al., 2019a) |
| *Gracilaria vermiculophyla* | *In vitro* | 25% DM | 63 | ? | (Maia et al., 2016) |
| *Gigartina* sp. | *In vitro* | 25% DM | 44 | ? | (Maia et al., 2016) |
| Brown macroalgae: | | | | | |
| *Ascophyllum nodosum* | *In vitro* | 2% OM | 15 | Phlorotannin, polysaccharide (?) | (Belanche et al., 2016b) |
| *Cystoseira trinodis* | *In vitro* | 20% OM | 80 | Terpenes, phlorotannin (?) | (Dubois et al., 2013) |
| *Dictyota bartayresii* | *In vitro* | 2% OM | 92 | Phlorotannin, isoprenoids | (Machado et al., 2014) |
| *Zonaria farlowii* | *In vitro* | 2% OM | 11 | Phenolic lipids | (Brooke et al., 2018) |
| Green macroalgae: | | | | | |
| *Cladophora patentiramea* | *In vitro* | 16% OM | 66.3 | ? | (Machado et al., 2014) |
| *Oedogonium* sp. | *In vitro* | 75% OM | 30.3 | ? | (Machado et al., 2016) |
| *Ulva* sp. | *In vitro* | 25% DM | 55 | ? | (Maia et al., 2016) |

DM, dry matter; OM; organic matter; ↓, decrease in methane ($CH_4$) production; ?, unknown.

to meadow hay and not corn silage (Maia et al., 2016). However, no significant anti-methanogenic properties were detected for three other red macroalgae species studied, *M. stellatus, P. palmata* and *Porphyra* sp., when they constituted 8.4–20% fresh matter in the concentrate portion of the goat diet in an *in vitro* fermentation study (de la Moneda et al., 2019). Thus, some, but not all, red macroalgae species have strong anti-methanogenic properties, but their quantitative impact on $CH_4$ emission may depend on both the composition of the ruminant diet and the type and concentration of bioactive components present in the macroalgae and this requires further investigations in the future.

***Brown macroalgae***: Certain brown macroalgae species also have $CH_4$ mitigating potential, but the documentation stems primarily from *in vitro* studies. Two species *Dictyota bartayresii* and *Cystoseira trinodis* were able to reduce *in vitro* $CH_4$ production by 90% and 80%, respectively, when 16% of OM was added to Rhodes grass (Machado et al., 2014; Dubois et al., 2013). Other brown species, such as *A. nodosum* and *Zonaria farlowii*, have been shown to reduce $CH_4$ *in vitro* by 11–15% at inclusions of 2% and 5%, respectively (Brooke et al., 2018; Belanche et al., 2016b). However, no anti-methanogenic properties have been detected with 20–25% DM inclusion in feed in other *in vitro* trials, when using *L. digitata*, *L. ochroleuca*, *P. canliculata* and *S. latissima*, (Maia et al., 2016; de la Moneda et al., 2019). This suggests that only specific brown macroalgae species possess anti-methanogenic properties, and these are less powerful than those of the red species. However, further studies are needed to estimate their most effective dietary inclusion rates, and to confirm whether such outcomes are also evident *in vivo*.

***Green macroalgae***: Methane reduction properties have been observed in a few green macroalgae species. *Cladophora patentiramea* and the fresh water green algae *Odogonium* sp. have been shown to reduce $CH_4$ production by 66% and 30% *in vitro*, when added at 16% OM to decorticated cottonseed meal *in vitro* (Machado et al., 2014). With a similar inclusion rate in corn silage, another unspecified green macroalga from the genus *Ulva* illustrated a 55% suppression on enteric $CH_4$ production *in vitro* (Maia et al., 2016). However, the same authors later revealed that 25% DM addition of *Ulva rigida* to a mixed ration *in vitro* did not reduce $CH_4$ production (Maia et al., 2019). Thus, compared to brown and particularly red species, green macroalgae seem to have limited anti-methanogenic potential, which would require high levels of inclusion in the feed.

The anti-methanogenic macroalgae have also been found to affect other rumen fermentation parameters. With a concomitant reduction in the $CH_4$ production, they will decrease total VFA amount, feed intake and degradability when included in large amounts in the feed (Machado et al., 2014; Roque et al., 2019b). These effects were clearly observed with various macroalgal species such as *Asparagopsis* spp. (red), *C. trinodis* (brown) and *D. bartayresii*

(brown) either *in vitro* or *in vivo* models, when macroalgae supplementation was gradually increased (Machado et al., 2014; Li et al., 2018; Roque et al., 2019b). Thus, it is important to include macroalgae at an optimum level so that possible negative impacts on rumen fermentation and animal performance are minimized.

### 4.1 Anti-methanogenic factors in macroalgae and potential mechanisms

Mechanistic insights into the anti-methanogenic properties of macroalgae are needed to identify the most efficient and safe ways of using them as feed additives to reduce enteric methane formation. Enteric $CH_4$ emissions can be reduced by macroalgae through two different mechanisms: (a) a direct inhibition of the methanogenic archaea themselves or rate-limiting steps in their methane formation or (b) through alteration of the rumen environment by reducing substrate availability or altering the rumen microbiota composition to disfavor the methanogens (Fig. 4).

#### 4.1.1 Direct impacts: inhibition of methanogens and the methanogenic pathways

Macroalgae produce a number of secondary metabolites that protect them from a complex and possibly stressful seawater environment and help them to cope with various microbial infections (Li et al., 2017) and such metabolites may

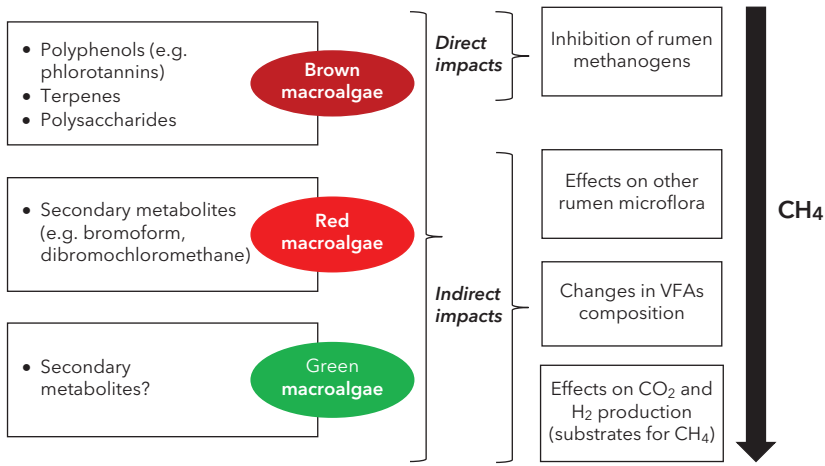


**Figure 4** Potential anti-methanogenic factors of macroalgae and their mode of action in minimizing methane production in ruminants. $CH_4$, methane; VFAs, volatile fatty acids; $CO_2$, carbon dioxide; $H_2$, hydrogen.

also to a large extent account for the anti-methanogenic properties of some species. These compounds and their mode of action are described hereunder.

***Halogenated compounds***: Halogenated compounds are the aliphatic compounds containing one or two carbon atoms that are covalently linked with one or more halogen atoms (fluorine, bromine, chlorine or iodine). These compounds, such as bromoform, chloroform and bromochloromethane, irrespective of their source (synthetic or macroalgae), have shown a strong inhibitory action both *in vitro* and *in vivo* against rumen and other methanogens, significantly lowering their abundance in the rumen even at a low concentration (Paul et al., 2006; Roque et al., 2019a; Machado et al., 2018; Denman et al., 2015). Red macroalgae (e.g. *Asparagopsis* spp.) produce a high level of various brominated and chlorinated halocarbons, including bromoform, dibromochloromethane, chloroform, bromochloroacetic acid and dibromoacetic acid (Machado et al., 2018; Paul et al., 2006). They are structural analogs of $CH_4$ and other methanogenic intermediates and possess a higher affinity to enzymes, including corrinoid/porphinoid, which catalyzes the cobamide-dependent methyl transfer in methanogenesis (Wood et al., 1968; Yu and Smith, 2000; Roque et al., 2019b). Thus, the halogenated compounds can competitively inhibit the binding of intermediates or methane substrates into the corrinoid/porphinoid enzyme (Yu and Smith, 2000). Moreover, they are also structurally similar to CoM (a cofactor produced specifically by methanogens) which supplies the methyl group to methyl coenzyme-M reductase enzyme during the terminal reductive reaction of methanogenesis (Liu et al., 2011; Roque et al., 2019b; Li et al., 2018). Therefore, anti-methanogenic compounds from red macroalgae seem to exert their effects on $CH_4$ production directly by either of the two mechanisms: (a) minimizing the abundance of rumen methanogens through their anti-microbial activity or (b) interrupting their functional components such as enzymes, catalyzing the different steps of methane biosynthesis.

The anti-methanogenic property of synthetic halocarbons, such as chloroform, is dependent on the degree of chlorination, and this property can decrease over time due to the sequential reductive dechlorination during methane inhibition (Yu and Smith, 2000). In addition, methanogens have also been shown to develop resistance to synthetic anti-methanogenic compounds such as bromochloromethane when repeatedly exposed, potentially due to the adaptation of methanogens to those compounds (Patra et al., 2017). Although the rate of dechlorination and possibility of developing resistance to anti-methanogenic compounds derived from red macroalgae is unknown, two animal trials in steers and sheep have shown persistent $CH_4$ mitigating effect of *A. taxiformis* for 3 months (Li et al., 2018; Kinley et al., 2020). This indicates that anti-methanogenic compounds from these macroalgae might have more stable and effective $CH_4$ mitigation potential than synthetic halocarbons.

The excess intake of bromoform can be hazardous to human and animal health and therefore a maximum uptake level of 0.08 mg/L has been set for drinking water in the USA (EPA, 2012). In addition, synthetic aliphatic halocarbons are reported to cause ozone depletion and thus have environmental concerns (Patra et al., 2017; Roque et al., 2019b). Therefore, the possible toxicity of halogenated compounds from red macroalgae should be investigated to understand their effect on both animal health and environment.

*Polyphenols*: Polyphenols are a group of phenolic compounds and the concentration of these can account for up to 15% of DM in brown macroalgae (Wang et al., 2009a). The predominant form of polyphenols in brown macroalgae is phlorotannins (PT) and their anti-methanogenic properties have been described in *in vitro* studies (Hierholtzer et al., 2013; Wang et al., 2008). Though the effects of PT specific to rumen methanogenic archaea are not clear, a suppressive effect of condensed tannins (structural analogs of PT) on rumen archaea has been reported. For example, condensed tannins extracted from the terrestrial forage *Leucaena leucocephala* have exhibited a linear reduction of total rumen methanogens belonging to the orders *Methanobacteriales* and *Methanomicrobiales* with increasing doses (Tan et al., 2011). Due to the limited information available on the impacts of PT on rumen methanogens, it is too soon to evaluate whether there is a practical perspective for the use of PT in ruminants. However, because of the chemical and structural resemblances of PT and terrestrial tannins, antimicrobial activity of PT against rumen methanogens can be anticipated (Wang et al., 2008).

The mechanisms of action of PT on rumen methanogens are not known, but are described for other rumen microbes or methanogens isolated from wastewater treatment plant (Hierholtzer et al., 2013; Wang et al., 2009a). It has been revealed that PT can affect the integrity of microbial cell membrane and cell wall, via creating stress and ultimately causing cell lysis (Hierholtzer et al., 2013; Wang et al., 2009b). Other potential mechanisms of PT in relation to anti-microbial effects have been suggested to be via inactivation of extracellular enzymes and proteins necessary for growth and metabolism of microorganisms (Scalbert, 1991).

Species specific and time-dependent impacts of PT against various rumen microorganisms have been observed. For example, 500 $\mu gmL^{-1}$ PT isolated from *A. nodosum* resulted in the reduction of cellulolytic rumen bacteria *Fibrobacter succinogenes* by 78%, 83% and 65% in 6, 12 and 24 h, respectively in an *in vitro* batch culture (Wang et al., 2009a). The same level of PT caused a 42% decrease in *Ruminococcus albus* without affecting the population of *Ruminococcus flavefacien* during 24 h of cultivation. In contrast, it significantly increased the number of non-cellulolytic bacteria such as *Prevotella bryantii, Ruminobacter amylophilus* and *Selenomonas ruminantium* (Wang et al., 2009a). This suggests that even within an order, various bacterial strains may be

differentially affected by PT and that may also apply to the rumen methanogens. The underlying reason for this selective and differential anti-microbial property of PT is yet unknown. However, this could possibly be linked to the structure of PT, such as degree of polymerization (phloroglucinol units) and the number of reactive hydroxyl groups present (Wang et al., 2008; Hierholtzer et al., 2013). Furthermore, interspecies differences of macroalgae in the methane inhibition potential and the potency of PT from those macroalgae may also play some role in this selective action.

*Polysaccharides*: Macroalgae contain different kinds of polysaccharides, which are present either as structural components of the complex cell wall or as storage carbohydrates (O'Sullivan et al., 2010). Bactericidal and bacteriostatic effects of these polysaccharides have been documented against various hindgut microorganisms (Smith et al., 2011; Seong et al., 2019); however, specific information about their impacts on the rumen methanogens is yet to be evaluated. Polysaccharides from brown macroalgae (alginates, fucoidan and laminarin) can partially and selectively be fermented by specific bacteria in the rumen (such as *Prevotella* sp., *C. butyricum* and *Selenomonas* sp.) (Williams et al., 2013; Orpin et al., 1985). These polysaccharides have shown selective enrichment of beneficial gut bacteria, including *Bifidobacterium, Clostridium coccoides* and *Lactobacillus*, and a suppression of pathogenic gut microbes, including *E. coli, Salmonella* spp., *Enterococcus* and *Clostridium* spp., in monogastric animals (Charoensiddhi et al., 2017; Seong et al., 2019; Smith et al., 2011). Thus, macroalgae polysaccharides apparently have anti-microbial properties and whether such selective impacts are also evident with rumen microorganisms are not known.

*Isoprenoids and terpenes*: Macroalgae also produce various types of isoprenoids and terpenes, and over 200 different diterpenes have been identified in the single brown macroalgae of genus *Dictyota* (Chen et al., 2018). Isoprenoids have also been reported in other macroalgae, including *A. taxiformis* and *C. trinodis* (Brooke et al., 2018; Machado et al., 2014; Dubois et al., 2013). Although they have been suggested to contribute to the $CH_4$ mitigating properties, nothing is yet known about the mechanism underlying the anti-methanogenic effect of such compounds.

### *4.1.2 Indirect impacts: changes in the rumen environment affecting methanogenesis*

In addition to the direct influences, macroalgae macroconstituents and bioactive compounds can affect numerous microorganisms in the rumen leading to changes in fermentation parameters and the overall rumen environment. The factors affected include the relative abundance and activity of non-methanogenic microorganisms, VFA production and availability of substrates

for $CH_4$ production. The anti-methanogenic macroalgae species such as *A. taxiformis* and *A. nodosum* have been found to reduce the abundance of rumen microbes, including rumen protozoa (Roque et al., 2019a; Belanche et al., 2016b). A group of rumen methanogens (9–25% of total methanogens) live in a mutualistic relationship with protozoa and they generate a large amount of $H_2$ that is utilized by the methanogens to form $CH_4$ (Belanche et al., 2014; Newbold et al., 1995). Protozoans also get benefit from the methanogenic $H_2$ utilization because accumulation of $H_2$ in the rumen is inhibitory to their growth (Belanche et al., 2014). Thus, changes in the abundance and activity of the protozoa will result in $H_2$ deprivation in the rumen resulting in reduced methanogenesis (Morgavi et al., 2012).

The incorporation of macroalgae with anti-methanogenic potential in ruminant feed changes VFA production profile. They can change patterns of rumen fermentation from acetate formation towards the formation of more propionate and thus reducing acetate:propionate ratio (Machado et al., 2014; de la Moneda et al., 2019; Belanche et al., 2016b). Acetate formation in the rumen results in the release of metabolic $H_2$, and this can be used by methanogens to produce $CH_4$ (Fig. 2). Therefore, reduced acetogenesis and increased propiogenesis are considered as factors indirectly decreasing methanogenesis (Roque et al., 2019a; Wolin and Miller, 1997). It has been noted that anti-methanogenic compounds such as PT and bromochloromethane increase the population of $H_2$-consuming bacteria, such as *Prevotella* spp., *F. succinogenes* and *Selenomonas* spp., in the rumen (Mitsumori et al., 2012; Denman et al., 2015; Wang et al., 2009a). The available $H_2$ can be re-channeled towards the formation of propionate, lactate and succinate by the action of $H_2$-consuming bacteria (Denman et al., 2015; Belanche et al., 2016a) which may also lead to $H_2$ deprivation. However, when the methanogenesis is highly inhibited (as with *A. taxiformis*), all the metabolic hydrogen produced cannot be captured by this re-channeling towards the formation of aforementioned fatty acids, and some will be eructed by animals (Martinez-Fernandez et al., 2016; Kinley et al., 2020). In fact, the actual causes and effects of macroalgae in changes of VFA, $H_2$ and populations of bacteria involved in the formation and consumption of these substances are yet to be clearly understood.

## 5 Processing and seasonal effects on anti-methanogenic properties of macroalgae

Post-harvest processing of macroalgae biomass, such as washing, drying and storage conditions may impact, not only the nutritional contents, but also the bioactive potential of harvested macroalgae (Kadam et al., 2015; Paull and Chen, 2008). The drying of *A. taxiformis* biomass at 45°C for 48 h led to a substantial reduction in the concentration of bromoform and eventual anti-methanogenic

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activity (Vucko et al., 2017). Similarly, oven drying at a higher temperature (80°C for 24 h) caused a significant reduction in the level of phytochemicals including polyphenols and flavonoids in *Kappaphycus alvarezzi* as compared to a lower temperature (40°C for 24 h) (Ling et al., 2015). However, the extractability of polyphenols and flavonoids could be greater when macroalgae biomass is semi-dried (e.g. 35–40°C for 2 h) as noted with the semi-dried *H. elongata* biomass (~40% increase) (Gupta et al., 2011). For other macroalgal species such as *F. vesiculosus* and *Porphyra* spp., drying methods (oven, sun or freeze drying) did not alter the amount of bioactive phytochemicals (Jiménez-Escrig et al., 2001). These results indicate that an appropriate drying/processing protocol for macroalgae biomass after harvesting may be beneficial to achieve increased levels of bioactive phytochemicals. Further studies are required to establish such optimal procedures, not in the least, in light of the high cost associated with the commercial production and transportation of macroalgae biomass.

The concentration of nutrients and bioactive components in macroalgae can vary across the seasons and geographical locations (Tayyab et al., 2016; Schiener et al., 2015). In macroalgae harvested in Norway, the level of protein and minerals have been found to be higher in spring than in autumn while polysaccharides (e.g. fucoidan and laminarin) are noted to be higher in summer (Kim, 2012; Rioux et al., 2009; Tayyab et al., 2016; Molina-Alcaide et al., 2017). On the other hand, in the same location, total polyphenol content in brown (*A. esculenta, L. digitata, P. canaliculata* and *S. latissima*), red (*P. palmata, M. stellatus* and *Porphyra* sp.) and green (*Acrosiphonia* sp., and *Cladophora rupestris*) macroalgae have been found to be around two-fold higher in autumn compared to the spring season (Molina-Alcaide et al., 2017; de la Moneda et al., 2019). In agreement with these findings, we have also found that Norwegian brown species (e.g. *A. esculenta*, *F. vesiculosus, P. canaliculata, H. elongata, L. digitata, S. latissima*) harvested in the autumn have higher polyphenol levels compared to those harvested in the spring (Deepak et. al. unpublished data). However, a study from Scotland which included some of the aforementioned macroalgae (*A. nodosum, A. esculenta, L. digitata, L. hypeborea* and *S. latissima*) recorded a higher total polyphenol content during the summer compared to the autumn (Schiener et al., 2015). These variations are associated with the growth stage of the macroalgae and season-specific environmental factors such as temperature, light intensity and nutrient content in the seawater (Mišurcová, 2012; Parys et al., 2009). It has been mentioned that at the beginning of the spring season, vegetative growth of the macroalgae is rapid and the level of polyphenols is low during the rapid growth stage (Parys et al., 2009). Furthermore, due to the geographical differences, variations in these environmental factors may exist within the same season. Therefore, a specific harvesting period needs to be established based on compounds

of interest and the spring season could be appropriate to harvest algae to achieve a maximum level of nutrients.

## 6 Future perspectives

Bioactive components of certain macroalgal species have the potential to be utilized as anti-methanogenic feed additives for ruminant animals to mitigate enteric $CH_4$ production. However, only a few species have been evaluated so far in this respect, and the most powerful anti-methanogenic compounds identified (halogenated carbons) are both ozone depleting and having documented adverse health impacts on humans (Roque et al., 2019b; Patra et al., 2017). It is therefore uncertain whether they can be approved (at least within the EU) as $CH_4$ mitigating instruments. Future studies should be directed towards the screening of a large number of macroalgal species to potentially identify efficient and safe compounds to be employed in climate-friendly feeding of ruminants. In this context, fractionation and/or extraction of targeted bioactive compounds (e.g. polyphenols, polysaccharides) and the characterization of their chemical and functional properties are important.

Utilization of macroalgae biomass as novel ruminant feeds on a larger scale is presently challenged by high costs associated with post-harvest processing as well as limited digestibility of several of the brown algae species that can most easily be cultivated. In addition, there are large variations between and within species in chemical composition and digestibility as well as contents of bioactive compounds depending on season, geographical location and post-harvest processing (Tiwari and Troy, 2015; Paull and Chen, 2008; Tayyab et al., 2016). This should encourage future research to develop cost-efficient techniques to increase the nutritional quality and anti-methanogenic potential of cultivable macroalgae by optimizing cultivation, harvest and post-harvest processing techniques.

## 7 Conclusions

The relevance of macroalgae as alternative and anti-methanogenic ruminant feeds depends upon their nutritional contents, digestibility, $CH_4$ mitigating potential, and influences on animal and environmental health. Red macroalgae such as *A. taxiformis and A. armata* seem to be promising anti-methanogenic feed ingredients and do not lead to significant adverse impacts on feed degradability with an inclusion rate of under 5% of OM. However, the impacts on other parameters of animal performances (e.g. feed intake, weight gain and milk yield) and rumen fermentation products, such as total VFA, should be carefully monitored. Moreover, due to a high concentration of halogenated compounds (e.g. bromoform) in those species, their potential adverse effects on human and environmental health must also be assessed. Brown macroalgae, such as

*D. bartayresii, C. trinodis* and *A. nodosum*, can be effective anti-methanogenic feed ingredients. Nevertheless, a high phlorotannin and polysaccharide content of these species can negatively impact the rumen degradability at high inclusion levels and thus optimal supplementation levels of these algae need to be carefully maintained. Green algae, including *C. patentiramea*, can also mitigate enteric $CH_4$ production, but the active anti-methanogenic compounds in green macroalgae are unknown.

Red macroalgae, such as *P. palmata, Porphyra* spp. and *Gracilaria* spp., and the green species *Acrosiphonia*, *C. rupestris*, *Ruppia* and *Ulva* can be used as a nutrient source due to their better nutritional composition and greater degradability compared to other species. Among brown macroalgae, *A. esculenta, Laminaria* spp., *S. latissima* and *U. pinnatifida* could be used as feed additives in up to 10–25% of DM in the ruminant feed provided that the excess mineral content is removed. Overall, macroalgal species could be an important component of future ruminant feed, but further *in vivo* studies are required to identify any potential adverse impacts on animal health and performance.

## 8 Where to look for further information

Further useful information about macroalgae and their applications can be obtained from the following resources:

- Sustainable use of seaweeds for food and non-food applications: Ed. Tiwari, Brijesh and Troy D. J. 2015. Seaweed sustainability–food and nonfood applications. Elsevier, https://doi.org/10.1016/C2013-0-12836-X.
- Seaweed biology – Novel insights into Ecophyisology, Ecology and Utilization. 2012. Springer. https://doi.org/10.1007/978-3-642-28451-9.
- Application of seaweeds as feed: "Seaweeds for livestock diets" Makkar et al 2016. https://www.sciencedirect.com/science/article/pii/S0377840115300274?via%3Dihub.

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