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Opportunities to reduce methane emissions (CH_4) in the digestive processes of ruminant animals. Review

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Abstract: The article covers an extremely important problem concerning the pollution of the environment with greenhouse gases, considered one of the causes of global warming of the Earth. The greenhouse gas (GHG) methane is continuously released as a by-product of enteric fermentation, which is largely produced by the digestive system of ruminants. Methane reduction from ruminants is not a new area of research. However, the number of scientific publications in this field has increased rapidly over the past two decades due to the emphasis placed on the effects of greenhouse gas emissions on climate change. The review aims to explore different options and strategies for reducing methane emissions in the digestive process of ruminants. In conclusion, the authors believe that the studies done so far are substantial but insufficient to draw definitive conclusions. Further studies are needed to develop a coherent system and strategy to reduce methane emissions from ruminants.

Keywords: greenhouse gas emissions; enteric fermentation; mitigation strategies; ruminants

INTRODUCTION

The role of livestock in the global nitrogen (N) and carbon (C) cycles underlies climate change. Animal husbandry and especially ruminants are one of the main sources of greenhouse gases. Methane production is continuously released in the digestive system of ruminants, which breaks down food in the rumen (first stomach) of cows, buffalo, sheep, and goats (EPA, 2022). Unlike carbon dioxide (CO_2), which remains in the atmosphere for hundreds years, methane has a short lifetime in the atmosphere of 10 - 12 years (Stavert et al., 2022). The breathing of ruminants is a source of carbon dioxide in inhaled air is 0.035%, and in exhaled air 4-5%. Carbon dioxide, the most commonly emitted anthropogenic greenhouse gas, accounts for 79% of emissions each year. Other greenhouse gases contributing to the ongoing climate crisis include nitrous oxide (6% of emissions) and fluorinated gases, including hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulfur hexafluoride (SF_6), which collectively account for the remaining 2% of global greenhouse gas emissions (EPA, 2022).

The ability of greenhouse gases to trap heat in the atmosphere is described as *Global Warming Potential* (GWP). Greenhouse gases with low global warming potential values such as carbon dioxide (1 GWP per 100 years), are less efficient at trapping atmospheric radiation and have a lower energy absorption capacity and as such show a smaller magnitude of impact on climate change. Comparatively, greenhouse gases with higher GWP values such as methane (25-36 GWP per 100 years), are much more efficient at trapping atmospheric radiation and have a greater capacity to absorb energy and as such show a greater impact on climate change in the long term (EPA, 2022). In other words, one ton of methane emitted today will have approximately 80 times the global warming impact of a ton of carbon dioxide emitted over the next 20 years *Environmental Defense Fund* (EDF, 2022). Therefore, although carbon dioxide makes up the majority of annual global greenhouse gas emissions, methane emissions have a much greater potential to accelerate the rate of global warming and ecological dysfunction (Denchak, 2018).

In recognition of the dangers and opportunities of methane mentioned above (including that it is an intensive gas), the 26th United Nations Conference of the Parties on Climate Change (COP26) through the *Global Methane Pledge* set itself the goal of reducing emissions of methane in agriculture by 30% by 2030 (Meinshausen et al., 2022).

Determining the factors that influence metabolism in the digestive system of ruminants and formulating process performance indicators is essential. Elucidation of the relationship between the biochemistry and microbiology of methanogenesis and the influence of factors on the efficiency of the processes should be the subject of extensive scientific research.

Methanogenesis in ruminants

Methane production in the digestive system of ruminants occurs mainly in the rumen. Total methane (CH_4) production about 87% is produced in the rumen, while 13% is produced in the lower digestive tract (Murray et al., 1976). Rectal emissions are about 2-3% of total methane emissions in sheep and dairy cows (Muñoz et al., 2012), specifically, 11% is excreted through the anus, while 89% is excreted through the lungs (Murray et al., 1976). The rumen is an anaerobic fermenter in which food components (carbohydrates, proteins and to a lesser extent fats) are broken down by the rumen microbial community (bacterial, protozoan and fungal species) and transformed mainly into volatile fatty acids (acetate, propionate and butyrate) (Mitsumori and Sun, 2008). A smaller amount of formate, ethanol, lactate, succinate and branched chain volatile fatty acids are formed, ammonia, carbon dioxide and hydrogen gas are also produced (Janssen, 2010). The fermentation pathways in the rumen have been intensively studied and to date it is known that carbon dioxide (CO_2) and hydrogen (H_2) are the main precursors of methane (CH_4) and that hydrogen (H_2) originates mainly from the breakdown of carbohydrates. Hydrogen gas (H_2) must be eliminated to maintain hydrogenase activity and avoid negative feedback on microbial degradation of organic matter (Wallace and Chesson, 2008). The removal of hydrogen (H_2) can be by methanogenesis (reaction 1) and acetogenesis (reaction 2) as described in the following pathways:

[reaction 1] $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
[reaction 2] $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$

Another way to sequester hydrogen (H_2) in the rumen is to stimulate propionic acid (reaction 4) producing organisms, while acetate (reaction 3) and butyrate (reaction 5) productions are hydrogen (H_2) producers (Hegarty and Gerdes, 1998).

[reaction 3] $C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_4O_2 + 2CO_2 + 4H_2$
[reaction 4] $C_6H_{12}O_6 + 2H2 \rightarrow 2C_3H_6O_2 + 2H_2O$
[reaction 5] $C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2CO_2 + 2H_2$

The hydrogen (H_2) and carbon dioxide (CO_2) formed are further used as energy sources by the methanogenic *Archaea* to produce methane (Janssen, 2010). To date, about 40 species of archaea have been identified and they are divided into 3 subgroups: *Halophiles, Thermophiles* and *Methanogens*. The latter produce methane as a by-product of their metabolism and they can be classified into 2 different physiological pathways of methane production: hydrogenotrophic methanogens and hydrogen-requiring methylotrophic methanogens (Wedlock et al., 1987). The former converts one or four moles of hydrogen (H_2) to one mole of methane (CH_4), while the latter metabolizes one mole of hydrogen (H_2) plus methanol (and other methyl compounds) to one mole of methane (CH_4). Formate is also a precursor of methane (Reaction 5) and this mode of formation accounts for about 15-20% of the total methane production in the rumen (Mitsumori and Sun, 2008). Some species of Archaea use (H_2)/ (CO_2) and formate to make methane. Therefore, methane is a physiological end product of microbial fermentation of carbohydrates in the rumen, and it is a major metabolic pathway for removal of hydrogen (H_2) in the rumen. Regardless of the rich range of hydrolytic enzymes involved in the digestive process, there is a lot of undigested food residue in the feces and the formation of a large amount of methane in the rumen and large intestine. A major approach to overcome this problem is the addition of feed additives to the feed of ruminants, which leads to the optimization of fermentation processes in the rumen and the reduction of greenhouse gas emissions into the atmosphere.

Nutritional strategies to reduce methane emissions

The potential of food strategies to reduce methane emissions is a large overview (Boadi et al., 2004; Benchaar et al., 2011; Bayat and Shingfield, 2012; Hristov et al., 2013a; Hristov et al., 2013b; Knapp et al., 2014; Kumar et al., 2014) and the two main areas of intervention arising from these reviews are represented by ration changes and the use of feed additives.

Dietary manipulations

Changing the nutritional balance, increasing or decreasing the concentration of one food chemical component, will decrease or increase the concentration of another of them. With this strategy, potential effects on methane emissions are often a consequence of changes in other constituents (Hristov et al., 2013a), leading to a combined and confusing interpretation. In this thesis, nutrient balance modification is evaluated for its effectiveness in reducing methane emissions from ruminants.

Decreasing the roughage: concentrate ratio, due to increasing the inclusion of concentrate in the ration, is one of the most researched feeding strategies. This type of nutritional manipulation reduces rumen pH and the acetate: propionate ratio, therefore also reducing the amount of methane produced per unit dry matter consumption DMI (Beauchemin et al., 2008). However, the proportion of concentrations required to cause this effect must be above 35 to 40%, while at smaller concentrations or moderate changes do not appear to affect methane emissions (Hristov et al., 2013a). It should be emphasized that high levels of concentrate feed are not desirable due to health consequences.

Another approach is to select higher quality forages (low in fiber and high in soluble carbohydrates), as low quality forages have a higher proportion of methane released per kg digestible organic matter OMI (Boadi and Wittenberg, 2002). In addition, methane reduction is associated with greater forage digestibility and maturity (Hristov et al., 2013a).

Increasing dietary fat content is another strategy that has been suggested as promising for reducing methane emissions from ruminants (Eugène et al., 2008, Rasmussen and Harrison, 2011). Specifically, it has been estimated that methane emissions can be reduced by 4–5% (g/kg DMI) for every 1% increase in dietary fat content (Grainger and Beauchemin, 2011). However, fat inclusion above 6–7% of dry matter DMI (Hristov et al., 2013) in dairy cows consumption can cause a negative reduction in feed intake and fiber digestibility (Jenkins, 1997).

Feed additives

Feed additives used to reduce methane production are generally classified into different categories and based on their respective mechanisms of action.

Inhibitors are chemical compounds that directly affect Archaea in the rumen, with potential anti-methanogenic activity (Moate et al., 2014). Methane inhibition alters the microbial community, hydrogen gas (H_2) production and rumen fermentation in ruminants (Martinez-Fernandez, 2016). In this category, the most successful compounds are halogenated methane analogs ie bromochloromethane, 2-bromoethane sulfonate (Mitsumori et al., 2012), chloroform (Knight et al., 2011) and cyclodextrin. They can be used alone or in combination with each other to stabilize the effect to obtain a more pronounced reduction of enteric methane (Kumar et al., 2014). These compounds lead to large reductions (from 25% to 95%) of methane production according to *in vivo* studies with sheep, goats and cows (Hristov et al., 2013a; Martinez-Fernandez et al., 2013).

Bromochloromethane (BCM) and chloroform, for example, are potent inhibitors of methane formation in ruminants. Bromochloromethane achieved reductions in (CH_4) emissions of 57.84% and 91% (on a dry matter basis) on farms with increasing dosage (Tomkins & Hunter, 2003). Methane (CH_4) production was found to decrease by up to 26% (on a dry matter basis) because methanogens were often attached to the surface or in endosymbiosis in the rumen with ciliated protozoa (Mcallister & Newbold, 2008).

Some of these compounds are potent methane inhibitors, both *in vitro* (Klein et al., 1988; Romero-Peréz et al., 2015) and *in vivo* study (Knight et al., 2011; Abecia et al., 2012; Mitsumori et al., 2012), but the long-term effect is uncertain, suggesting a type of ecosystem adaptation in the rumen (Hristov et al., 2013b). Furthermore, the use of these compounds as a feed additive is not well received by the public because of the possible risk to animal and human health (ie, chloroform is a known carcinogenic molecule and bromochloromethane is a recently banned ozone-depleting molecule).

Electron receptors are compounds acting as alternative scavengers of hydrogen gas in the rumen (Hristov et al., 2013b). Dicarboxylic acids (such as fumarate and malate), nitrates and sulfates are the most studied reducing compounds belonging to this category (Ungerfeld et al., 2007; van Zijderveld et al., 2010; Hulsof et al., 2012; Pal et al., 2014). Malate and fumarate are precursors for propionate production in the rumen, consuming H_2 hydrogen gas in the process (McAllister and Newbold, 2008). Nitrates and sulfates may replace ammonia-forming carbon dioxide (CO_2) as alternative H_2 in the rumen (McAllister and Newbold, 2008). Recent studies in sheep (Nolan et al., 2010; van Zijderveld et al., 2010) and cows (van Zijderveld et al., 2011; Hulshof et al., 2012) have shown promising results with nitrate supplementation leading to a reduction of methane production up to 50%, especially when combined with forage to the basic ration (Troy et al., 2015). Nitrate should be supplemented with caution as it can be toxic above certain doses leading to methemoglobinemia and carcinogenesis (Sinderal and Milkowski, 2012). Reviews by Lee and Beauchemin (2014) and Yang et al., (2016) discuss in detail the role of nitrates in metabolism, animal productivity, methane emissions from fermentation, their toxicity and how they can be safely used in practice.

The use of nitrate as an additive has been largely overlooked because of the potential from toxic effects of intermediate products (nitrites) that are formed during nitrate reduction in the rumen. Recently, nitrite toxicity has been more thoroughly studied and it has been learned that its production from nitrate in the rumen can be prevented by dietary management (Leng, 2008).

McAllister and Newbold, 2008 reviewed studies that showed between 0 and 75% reduction of (CH_4) with the addition of fumaric acid. However, the relatively high doses of dicarboxylic acids required are too expensive.

Cultures of *Cerevisiae Saccharomyces* (yeast species) potentially stimulate rumen microbial acetone synthesis, consuming H_2 to form acetate (Chaucheyras et al., 1995), thereby potentially reducing methane production. Probiotic species (*Saccharomyces cerevisiae* and *Aspergillus oryzae*) have been found to reduce methane emissions (Boadi et al., 2004). Also, in vitro studies have shown that *A. oryzae* and *S. cerevisiae* are able to reduce methane emissions by 50% and 10%, respectively) (Mutsvangwa et al., 1992).

The authors conclude that more research is needed on a large number of yeast strains to isolate those that are useful for production and have methane (CH_4) reduction potential.

Ionophores are organic molecules, often antibiotics, that transport ions across the lipid bilayer of the cell membrane (Pressman, 1976). Monensin is one of the commonly used ionophores in ruminant nutrition, although ionophores are banned in the European Union (Hristov et al., 2013b). It shifts the acetate to propionate ratio in the rumen towards propionate, there by reducing methane production (Eckard et al., 2010). Guan et al., (2006) with monensin supplementation found a 27% to 30% reduction in methane production over 2 to 4 weeks in the rumen in ruminants for meat and an 8% to 9% reduction in methane when used in dairy cows (Appuhamy et al., 2013).

In addition, it also reduces the number of protozoa in the rumen (Beauchemin et al., 2008) and has long-term resistance (Odongo et al., 2007). The effect of monensin depends on dose, food consumption and ration composition (Hristov et al., 2013b). The effect of monensin on methane production appears to be dose dependent, with lower doses (10-15 PPM) in dairy cows showing no effect on methane production (Grainger et al., 2008; Waghorn et al., 2008), but at higher doses (24-35 PPM) (Sauer et al., 1998; McGinn et al., 2004; Van Vugt et al., 2005) resulted in a reduction of methane production by up to 10% (g/kg DM).

Plant bioactive compounds are various plant secondary compounds, in particular tannins, saponins, essential oils and their active constituents (Hristov et al., 2013b). Many of these compounds have been investigated for their potential to reduce methanogenesis (Hu et al., 2005; Calsamiglia et al., 2007; Hart et al., 2008; Hristov et al., 2008; Macheboeuf et al., 2008; Rochfort et al. al., 2008; Spanghero et al., 2008; Holtshausen et al., 2009; Mao et al., 2010; Patra and Saxena, 2010; Soliva et al., 2011) and the results of these studies are very promising.

In a review by Hristov et al., (2013), tannins were reported to show good potential for reducing CH_4 emissions, by up to 20%. By adding condensed tannins (CT) to feed, methane production was reduced by 13 to 16% on a dry matter DM basis (Waghorn et al., 2002; Woodward et al., 2004; Carulla et al., 2005; Grainger et al., 2009), mostly through a direct toxic effect on methanogens. However, high concentrations of condensed tannins (greater than 55 g CT/kg dry matter DM) can reduce voluntary feed intake and digestibility (Min et al., 2003; Veauchemin et al., 2008; Grainger et al., 2009).

The general mode of action of saponins is their interaction with cholesterol present in the protozoan membrane, causing membrane disintegration, cell lysis and death (Cheeke, 2000). Plant saponins have methane-reducing potential, and some sources of saponins are more effective than others in reducing methane production due to their antiprotozoal properties (Beauchemin et al., 2008).

Table 1. Effect of essential oils from various plant sources on methane emission.

Plant source	Effect on methane emissions	Reference
Garlic	91% Reduction in CH_4 production (*in vitro*)	[Soliva et al., 2011]
	73% Reduction in CH_4 production (*in vitro*)	[Busquet et al., 2005]
	Improved feed digestibility in dairy cows	[Yang et al., 2007]
Thyme	30% Reduction in CH_4 production (*in vitro*)	[Günal et a.l, 2017]
	21% Reduction in CH_4 production in cows	[LaabouriI et al., 2017]
	Increased propionate production in Holstein calves	[Vakili et al., 2013]
Rosemary	Over 20% reduction in CH_4 production (*in vitro*)	[Roy et al., 2014]
	9% Reduction in CH_4 production (*in vitro*)	[Cobellis et al., 2015]
Oregano	87% Reduction in CH_4 production (*in vitro*)	[Patra et al., 2012]
	11% Reduction in CH_4 production (*in vitro*)	[Zhou et al., 2020]
Clove	34% Reduction in CH_4 production (*in vitro*)	[Benchaar, 2020]
Eucalyptus	No effect on CH_4 production in dairy cows	[Sallam et al., 2009]
	Up to 85% reduction in CH4 production (*in vitro*)	[Wang et al., 2018]
	No effect on CH_4 production in sheep	[Yadeghari et al., 2015]
Lavender	Up to 60% reduction in CH_4 production (*in vitro*)	[Ozkan et al. ,2015]
	54% Reduction in CH_4 production (*in vitro*)	[Beyzi, 2020]
Peppermint	Over 30% reduction in CH_4 production (*in vitro*)	[Guyader et al., 2017]
	52% Reduction in CH_4 production (*in vitro*)	[Woodward et al., 2001]

Essential oils with antimicrobial properties against bacteria and fungi act on the control of rumen fermentation, gas and VFA production (Boadi et al., 2004). Their effectiveness in vitro studies of some plant extracts with known antimicrobial activity, able to reduce the production of CH_4 (allyl-sulfide, cinnamaldehyde, eugenol) is investigated.

A good potential is that garlic and citrus fruit extracts (15 $g.d^{-1}.animal^{-1}$) have been shown to reduce methane production in large Angus × Hereford ruminants (Roque et al., 2019).

A recent *in vitro* study (Beck et al., 2018) shows that the addition of whole cotton seed to the ration of grazing ruminants is an efficient solution to reduce the intensity of methane emissions from rumen fermentation.

Further research is needed to determine the optimal sources, oil types, and dosage percentages required to reduce ruminant methane production.

Nitrooxy compounds

Chemically synthesized inhibitors that inhibit methanogenesis and thereby prevent methane production in the rumen is an important area of research (Beauchemin et al., 2020). The chemical 3-nitrooxypropanol (3-NOP) is a precise additive targeting the active site of the nickel enzyme methyl CoM reductase (MCR), catalyzing the final step in methanogenesi (Duin et al., 2016).

Tri-nitrooxypropanol (3-NOP) is a recently developed compound that has specific anti-methanogenic effects and can reduce rumen methane production by 25 to 45% in seven studies without affecting animal productivity (Romero-Perez et al., 2014; Hristov et al., 2015; Vyas et al., 2016; Vyas et al., 2018). In addition, McGinn et al., (2019) indicated that there was a large reduction in CH_4 methane emissions of about 70% (±18%) when tri-nitrooxypropanol (3-NOP) was added to the ration. Tri-nitrooxypropanol achieved a 24% reduction in methane emissions *in vivo* experiments with sheep (Martinez-Fernandez et al., 2013), but more pronounced reductions were observed in cows (7% to 60%) (Haisan et al., 2014; Reynolds et al., 2014). Tri-nitrooxypropanol has been found to reduce the amount of methane (CH_4) released in the rumen by up to 30% in dairy cows (Lopes et al., 2016; Melgar et al., 2020a, 2021). By inhibiting hydrogen uptake and methane formation with 3-NOP, an increase in hydrogen gas (H_2) evolution has been shown in *in vivo* (van Gastelen et al., 2020; Melgar et al., 2020a, 2021) *and in vitro* studies (Guyader et al., 2017). When 3-NOP is included in the diet, it is broken down in the rumen to form nitrite, nitrate and 1,3-propanediol (Duin et al., 2016).

Factors that have been observed to have a negative effect on the rate of methane (CH_4) reduction are the inclusion of too low a dose of 3-NOP intake (Dijkstra et al., 2018; McGinn et al., 2019) or a high content of fiber (Dijkstra et al., 2018). Feed ration affects the amount of methyl-coenzyme M in ruminants. Diets high in fiber are thought to increase methyl-coenzyme M concentration, therefore requiring an increased amount of 3-NOP to increase the reduction potential of supplementation (Dijkstra et al., 2018). Offering the supplement evenly throughout the day produces better results than occasional feeding (Reynolds et al., 2014; Van Wesemael et al., 2019).

Experiments that have tested (3-NOP) have not reported any side effects on animal health due to its application over a period of 3 to 5 weeks. Another study (Hristov et al., 2015) extended its duration of application to 14 weeks, achieving an average 30% reduction in methane (CH_4) with no observed toxic effects.

The European Food Safety Authority (EFSA) carried out a comprehensive scientific evaluation of the safety of the use of the Bovaer® additive with the active substance 3-NOP in dairy production. Experts at the Group on Additives and Products or Substances Used in Animal Feed (FEEDAP) concluded that Bovaer® has the potential to reduce methane emissions in the rumen of ruminants. The additive is safe for use in dairy, other ruminant production and poses no risk to human, animal or environmental health. EFSA concluded that feeding the additive at 60 mg/kg dry matter (DM) feed had no effect on performance (EFSA et al., 2021). In February 2022, the marketing of 3-NOP was approved by European Union (EU) member states and the supplement is available on the market with in months. The supplement is the first of its kind on the EU market (European Commission, 2022a). Regarding the impact of feeding 3-NOP on milk production and milk composition, recent studies have shown inconsistent results. When including 3-NOP supplementation in the feed ratio as recommended by EFSA at a daily intake of 60 mg/kg dry matter (DM) feed (EFSA et al., 2021), studies have shown both an increase (Lopes et al., 2016; Melgar et al., 2020b, 2021) and that it had no effect (Melgar et al., 2020a) on milk fat yield and concentration.

The compound (3-NOP) is expected to be an effective and harmless dietary strategy to reduce methane production in ruminants.

Microalgae

Studies using microalgae as methane reducing agents showed that methane production was reduced by 99% with the addition of 2% *Asparagopsis microalgae* in *in vitro* study (Machado et al., 2014). The use of *Chlorella vulgaris algae* improves rumen bacterial growth as well as increases total VFA and increases milk production in dairy cows (Anele et al., 2016; Kholif et al., 2017; Tsiplakou et al., 2017). The algal strain has also been identified as a promising candidate for reducing methane emissions (Bohutskyi et al., 2014; Tsiplakou et al., 2017; Wild et al., 2019). Additionally, *Oedogonium*, a member of the *Filamentous microalgae*, reduces rumen methane production (Machado et al., 2014). *Cystoseira trinodis* and *Dictyota bartayresii* members of brown algae can inhibit methane production *in vitro* study. In addition, Sucu (2019) reported that careful selection and combination of substrate and algae (*Chlorella vulgaris* and *C. variabilis*) effectively manipulate rumen fermentation and can inhibit methane production.

Macroalgae

Several peer-reviewed studies have investigated the anti-methanogenic properties of various species of macroalgae, both *in vivo* and *in vitro* expreriments. Each species of macroalgae contains a unique combination of bioactive compounds in varying degrees of anti-methanogenic efficacy and long-term efficacy (Kinley et al., 2016). Researchers are particularly excited about the red macroalgal species, *Asparagopsis Taxiformis* (*A. taxiformis*) and its promising potential for use as an anti-methanogenic supplement for ruminants to reduce methane production at the source. When supplemented in ruminant diets as a feed additive at low levels of inclusion (<1–2% dry matter consumption (DMI) of the ruminant ration), the potent bioactive compounds in *A. taxiformis* have consistently shown that both safe and and effectively inhibits methanogenesis during intestinal fermentation, resulting in up to a 90% reduction in methane emissions from test animals without adverse risk or consequences to animal health, productivity, digestive tract and fermentation efficiency (Kinley et al., 2016; Li et al., 2016; Machado et al., 2016; Mernit, 2018; Chagas et al., 2019; Roque and Salwen et al., 2019; Brooke et al., 2019; Vijn et al., 2020; Kinley et al., 2020; Min et al., 2021; Roque et al., 2021). Furthermore, *A. taxiformis* is completely safe for human consumption. Macroalgae - dietary supplements have also been widely sold in stores as health foods for years, touted for their significant nutritional benefits and other powerful health-promoting properties (Biris-Dorhoi et al., 2020).

Australian study sought to identify the optimal feed dose required to effectively and consistently reduce methane production to minimize adverse risk to ruminant health and fermentation efficiency. The study consistently confirmed that the addition of *A. taxiformis* at low levels of inclusion (<1-2% DMI dry matter consumption of the ruminant ration), could reduce methane production by up to 99%, without risk to animal health, the productivity or efficiency of rumen fermentation (Machado et al., 2016). Another Australian study used *in vitro* fermentation with rumen inoculum to investigate anti-methanogenic effects of *A. taxiformis* under laboratory conditions. Again, the study results indicated that feed supplementation of *A. taxiformis* could safely inhibit methanogenesis in ruminants without adverse effects on animal health, welfare or metabolic productivity (Kinley et al., 2016). A Swedish study conducted in 2019 year further substantiated these findings and confirmed that the potent bioactive compounds found in *A. taxiformis*, dibromochloromethane and bromoform, do not cause adverse effects on animal health and are safe for ruminant consumption and effective inhibition of methanogenesis during enteric fermentation (Chagas et al., 2019).

Multiple *in vivo* reviewed studies have also investigated antimethanogenics effects of feed additives of *A. taxiformis* on methane emissions from ruminants. Australian study found that significant and sustained reductions in methane production using dietary supplements of *A. taxiformis* were optimized in conjunction with a high fiber ration (Li et al., 2016). Another Australian study conducted in 2020 year investigated the direct effects of *A. taxiformis* dietary supplements for beef cattle. Research has confirmed that feed additives effectively inhibit methane production without negative impacts on daily ruminant feed intake or healthy rumen function. Additionally, after data collection ceased, when researchers prepared and tasted the beef carcass they reported no noticeable changes in meat quality or flavor (Kinley et al., 2020). Finally, a 2021 year study on beef animals in California found that daily supplementation of *A. taxiformis* continuously, safely and effectively reduced methane emissions over the entire duration (21-weeks) of the study (Roque et al., 2021). Results from *in vitro* study data further support the efficacy and safety of *A. taxiformis* anti-methanogenic feed additives for ruminants to reduce methane production without harm to ruminant health. Roque et al., 2021 reduces net methane emissions by up to 80% using macroalgae, but has a short-term effect.

Since then, small pilot studies have expanded the *in vivo* and *in vitro* studies. It is particularly important to note that the innovative startup CleanTech *Symbrosia* is a pioneer and conducted the world's first commercial trial of *A. taxiformis* in 2020 year to investigate the large-scale anti-methanogenic effects of *A. taxiformis* ruminant feed supplements on native Hawaiian animals. Results from pilot studies show over 75% reduction in methane production, with no adverse effects on ruminant health in the short term. The researchers also found no significant increase in bromoform residues when testing both ruminants and meat and dairy products. In addition, feed additives were not found to significantly affect volatile fatty acid concentrations in the short term, which are traditional biomarkers of animal productivity and rumen fermentation efficiency. Biomarkers indicate that animal health is not compromised by short-term use of *A. Taxiformis* feed additives for ruminants ***(Symbrosia*, *2021)***.

It should be noted that most of these options are in the early stages of investigation and further significant research is needed over an extended period of time to bring into practice biological control options that will be effective across a range of production systems and regions.

CONCLUSIONS

The known facts related to the synthesis and release of methane during the digestive process of ruminants are reviewed. Improving feed quality can both increase animal productivity and reduce methane production, but also improve efficiency by reducing of methane emissions per unit of animal product. In conclusion, we must summarize that the data presented so far only show that the studies carried out so far are not sufficient for definite conclusions. Further studies are needed to develop a coherent system and strategy to reduce methane emissions from ruminants.

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