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Feed additives strategies to control methanogenesis in ruminants, Review

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ABSTRACT

Methane gas produced by livestock animals contributes significantly to greenhouse gas emissions and is a major environmental issue. However, the use of feed additives for controlling ruminal methanogenesis has emerged as a promising solution for reducing these emissions. Numerous studies have demonstrated that the use of feed additives such as biological feed additives (enzymes, probiotics, algae), phytogenic plants and their extract (essential oils, saponins, tannins, flavonoids) and chemical feed additives (ionophores, nano minerals, chemical inhibitors, organic acids, lipids and oils, propolis) can effectively reduce ruminal methanogenesis while improving animal performance and health. Incorporating feed additives into livestock diets not only shows great potential for reducing methane emissions but also has positive impacts on animal health and productivity. Furthermore, feed additives are a relatively low-cost and practical solution for farmers looking to decrease their environmental impact without compromising animal welfare or productivity. In summary, the use of feed additives for controlling ruminal methanogenesis in livestock animals holds great promise for reducing greenhouse gas emissions, improving animal health and productivity, and promoting sustainable agricultural practices.

Keywords: methane production, methanogenesis, feed additives, ruminants, environmental, and animal welfare.

INTRODUCTION

Global warming and methane emissions have significant impacts on our planet and society. The release of greenhouse gases, such as methane, carbon dioxide, and nitrous oxide, into the atmosphere contributes to global warming and climate change (Jackson et al., 2020; Wei et al., 2021). This increase in global temperatures has detrimental effects on the environment, including rising sea levels, melting ice caps, and extreme weather events (Mamlu'atur et al., 2018). Furthermore, global warming affects various aspects of human society, including agriculture, health, and the economy (Wani et al., 2017). Agriculture is heavily impacted by global warming as changing temperatures and rainfall patterns affect crop yields and livestock production (Onugo and Onyeneke, 2022). Health also suffers as a result of global warming, as increased temperatures can lead to heat stress, which poses risks to vulnerable populations, such as the elderly and those with pre-existing health conditions (Kestens et al., 2011). In addition, global warming disrupts ecosystems and biodiversity, leading to the loss of plant and animal species (Crickenberger and Moran, 2013). To exacerbate the issue, methane, a potent greenhouse gas, plays a significant role in global warming (Tellier et al., 2018).

Methanogenesis is the biological process by which methane gas is produced (Aguilar-Marin et al., 2020). This process occurs naturally in anoxic environments like swamps, wetlands, and the digestive systems of ruminant animals (Giuburunca et al., 2015). Methanogenesis is a complex process that involves the conversion of organic compounds into methane gas by methanogens, which are microorganisms that thrive in these environments (Yang et al., 2021). Methanogenesis is a critical aspect of the global carbon cycle, as it contributes significantly to atmospheric methane concentrations (Pantha et al., 2014). Methanogenesis is also being researched as a potential source of renewable energy, as it can be harnessed to produce biogas from organic waste material (Luo et al., 2021). Furthermore, methanogenesis has been studied in the context of microbiology to understand the metabolic pathways and microbial communities involved (Vargas et al., 2020). Understanding methanogenesis has deep implications for global climate change and stakeholders in agriculture, bioremediation of wastewater streams, and landfill management (Conaway et al., 2018). Carbohydrate fermentation produces hydrogen, and if this end product is not removed, it can inhibit the metabolism of rumen microorganisms (Sharp et al., 1998). The methanogen bacteria allow the microorganisms involved in fermentation to function optimally and support the complete oxidation of substrates (Sharp et al., 1998). Methane formation in ruminants, the major methanogens bacteria in the digestive system of ruminants utilize H+ and CO2. Also, methanosarcina is a genus of euryarchaeote archaea that grows slowly on H+ and CO2, thus maintaining a niche by utilizing methylamine and methanol to produce methane (Hungate et al., 1970; Patterson and Hespell, 1979). Volatile fatty acids are not usually used as substrates for the methanogenesis process, as their conversion into hydrogen and carbon dioxide is a long process, which is inhibited by rumen turnover (Hobson and Stewart, 1997). Therefore, methanogenesis often uses the hydrogen and carbon dioxide generated by carbohydrate fermentation, where volatile fatty acids are formed (Hungate et al., 1970).

The use of feed additives in ruminant diets has emerged as an effective strategy for mitigating methane production in these animals (Rosa and Gabrielli, 2023). By altering the composition of the diet, such as increasing the proportion of concentrate, feed additives improve feed efficiency and reduce methane emissions per unit of feed intake and per unit of animal product (Nunes et al., 2022). The inclusion of various feed additives in ruminant diets has shown promising results in reducing methane emissions (Rosa and Gabrielli, 2023). These additives can range from yeast, ionophores, and probiotics to Aspergillus oryzae, which can alter rumen fermentation and decrease methane production (Mohammad and Alsahaf, 2022). Additionally, dietary enzymes such as cellulases and hemicellulases are effective in reducing methane production by modifying the acetate-to-propionate ratio in the rumen (Wang and Xue, 2015). Furthermore, the use of prebiotics such as chitosan, yeast products, galactooligosaccharides, and inulin has also been found to decrease methane production in ruminants (Yuan et al., 2023). Some feed additives play a crucial role in mitigating methane production in ruminants. By inhibiting methanogens in the rumen, these additives act as methane-reducing agents and help reduce enteric methane emissions (Battumur et al., 2019). This approach not only reduces the environmental impact of ruminant farming but also improves the efficiency of feed utilization and promotes animal growth and production (Santos et al., 2022). So, the aims of this state of art were to investigate the impacts of using feed additives on methanogenesis process and methane emissions in ruminants.

EFFECT OF BIOLOGICAL FEED ADDITIVES ON METHANOGENESIS AND METHANE EMISSION

Enzymes

Enzymes supplementations have been gaining popularity in recent years as a potential solution for reducing methane emissions from livestock (Prasad et al., 2022). Enzymes play a crucial role in the digestion process of ruminants such as cattle, buffaloes, goats, and sheep, which are important sources of meat and dairy products (Wang et al., 2019; Khattab et al., 2024). However, the same enzymes that help in digestion also contribute to the emission of methane gas, which is a potent greenhouse gas and a significant contributor to climate change (Abdeltawab et al., 2022). According to a research study, adding fibrolytic enzymes to the diet of livestock can help break down cellulose and other plant fibers in their rumens more efficiently, which could lead to a decrease in methane emissions by up to 20 percent (Li et al., 2021). This could be an important step in mitigating the impact of livestock on climate change, as methane emissions from livestock are a significant contributor to greenhouse gases (Sutaryo et al., 2019). Furthermore, using enzyme additives may also improve feed efficiency and animal health by increasing nutrient availability for the animals to absorb (Prihartini et al., 2021). However, it is important to note that enzyme additives should not be considered a standalone solution for reducing methane emissions (Prasad et al., 2022).

Other measures such as improving animal diets, manure management, and breeding for lower-emission livestock should also be taken into account (Li et al., 2022). Tang et al. (2013) found that adding cellulase enzymes led to a quadratic decrease in methane generation per dry matter digestibility (DMD) in an *in vitro* study. Also, methane production was reduced by xylanase supplementation *in vitro* fermentation (He et al., 2015). While, McGinn et al. (2004) found that supplementation of proteolytic enzymes in the diet of beef cattle did not affect methane emission, methane (DMI), % of methane energy loss per gross energy intake, or ruminal fermentation pattern. Furthermore, dry matter digestibility was improved by 8%. Methane production and methane production per dry matter intake increased with a higher level of fibrolytic enzyme (1 ml/dry matter) in a total mixed ration with no influence on bacteria communities, methanogens and protozoa, or acetate, propionate, and butyrate concentrations in dairy cattle (Chung et al., 2012). Likewise, other studies found no influence on the effects of cellulase and xylanase enzyme addition on methane production and methanogen activity in growing goats (Lu et al., 2016), or exogenous enzymes derived from Aspergillus niger and Aspergillus oryzae concentrations of ruminal acetate, propionate, and butyrate, or methane production in dairy cattle (Oh et al., 2019).

Probiotics

The use of probiotics as feed additives in ruminants has been shown to have implications for methanogenesis, the process by which methane gas is produced in the rumen of ruminant animals (Amin and Mao, 2021). Study has shown that certain strains of probiotics can help reduce methane emissions in ruminants. For example, probiotic bacteria (Lactic acid bacteria) and yeast (Saccharomyces cerevisiae) can help divert hydrogen atoms away from methane-producing methanogens and towards acidogenesis bacteria to produce propionate in the rumen (Suharja et al., 2012). While, Bintsis (2018) and Doyle et al. (2019) noted that the use of lactic acid bacteria (LAB) such as Lactobacillus, Leuconostoc, Lactococcus, and Pediococcus to reduce CH4 emissions in ruminants is limited. *in vitro* studies, lactic acid bacteria can reduce methane production effectively.

In the metabolic pathway of ruminants, production of acetate and butyrate releases pure hydrogen, while propionate formation creates a competitive pathway for H+ use in the rumen (Palangi et al., 2022). According to Miller-Webster et al. (2002), brewer's yeast culture improved microbial activity that converted hydrogen ions to propionate and diminished methane production by 25% in a continuous culture system. This shift in microbial activity results in an increased production of propionate, volatile fatty acids (VFAs) that serves as an energy source for the animal, rather than being converted into methane gas (Su et al., 2022; Ziauddin, 2022). Furthermore, live yeast has also been found to improve overall rumen health and fermentation, leading to increased nutrient utilization efficiency and improved animal performance (Garcia Diaz et al., 2018). Additionally, they can improve gut health and digestion, enhance nutrient absorption, and boost the immune system (Garcia Diaz et al., 2018). *In vivo* studies have found that there is no influence of Saccharomyces cerevisiae on methane emission and/or methane production per dry matter intake in beef or dairy cattle (Muñoz et al., 2017; Bayat et al., 2015; Oh et al., 2019). In contrast according to Mwenya et al. (2004) addition of yeast culture to goat or sheep diets at a level of 4 g/day resulted in a 6.85% and 10.19% reduction in methane production and methane production per dry matter intake, respectively. While Shibata and Terada (2010) indicated that the molar proportions of volatile fatty acids change as a result of the use of live microorganisms as probiotics for ruminants in such a way that the proportion of acetate decreases, while the proportion of propionate increases. Frumholtz et al. (1989) observed a 50% reduction in methane production due to a reduction in the number of protozoa population with the addition of Saccharomyces cerevisiae, also Mutsvangwa et al. (1992) found a 10% reduction in methane release with the addition of Aspergillus oryzae in an *in vitro* study.

Algae

The most promising and effective seaweed species for reducing enteric CH4 are Asparagopsis (red macroalgae) and Dictyot (brown macroalgae), which have the potential to reduce enteric CH4 production by 98.9% and 92.2%, respectively (Machado et al., 2014). Therefore, more comprehensive information on the antimetagoneic properties of Asparagopsis (red macroalgae) and Dictyot (brown macroalgae). Various research studies have shown the potential of algae as an alternative source of renewable energy (Broch et al., 2013). However, it is important to consider the impact of algae additives on methanogenesis in anaerobic digestion processes, as these additives can significantly affect methane production. Incorporating algal products into an anaerobic digestive system requires careful consideration and testing to ensure that the methanogenesis process is not adversely affected, although it is beneficial in terms of energy production. According to studies by Anele et al. (2016) and Brooke et al. (2020), as well as reviews by McCauley et al. (2020) and Makkar et al. (2016), micro and macroalgae have been used successfully as feed additives. Also, Red algae decreasing methanogenesis was initially identified by Machado et al. (2014). According to Glasson et al. (2022), bromoform ($CHBr_3$) is the algae's most potent active ingredient for preventing methanogenesis. It is important to comprehend how that substance affects both the chemistry of the atmosphere and animals.

EFFECT OF PHYTOGENIC PLANTS AND THEIR EXTRACT AS FEED ADDITIVES ON METHANOGENESIS AND METHANE EMISSION

Herbal plants have long been recognized for their medicinal properties and potential benefits in various aspects of human and animal health (Parvez et al., 2019). Recently, medicinal plants and their extracts have been successfully used as an alternative to chemical compounds (antibiotics) as feed additives in the livestock production system (Ugbogu et al., 2019). Through the presence of secondary metabolites, such as essential oils, saponins, alkaloids, flavonoids, organosulfides, and terpenes, herbal plants possess potent properties that can improve ruminal microbial ecosystems and nitrogen metabolism, reduce methane emissions, and improve overall well-being for animals (Khattab et al., 2016, 2017; Patra et al., 2020; Lambo et al., 2024). Similarly, the secondary metabolites present in herbal plants have also shown potential for addressing the issue of methanogenesis in ruminants (Ponte et al., 2022). These plant-derived substances have been found to possess inhibitive effects on ruminal methanogenesis, which has gained considerable attention in recent studies.

Essential Oils

Numerous studies have investigated the use of herbal plant compounds and extracts as possible mitigation strategies for reducing methane production in ruminants (Matloup et al., 2017; Kumari et al., 2020; Khattab et al., 2020; Abd El Tawab et al., 2020; Selim et al., 2021). However, it is important to note that there have been confounding effects observed among different studies (Chao et al.,2000; Kalaitsidis et al., 2021; Moosavi-Zadeh et al., 2023). This could be attributed to the complexity and purity of the research objects, as well as variations in dosage and formulation (Alves et al., 2019). Additionally, the bioactive compounds in herbal plants may interact with other components of the ruminant's diet, potentially influencing their effectiveness in mitigating methanogenesis (Patra and Yu, 2015). Nonetheless, the potential of herbal plants to mitigate methane production in ruminants and improve their productive functions is promising (Jiménez-Ocampo et al., 2019). Herbal plants have emerged as a potential solution to the issue of methanogenesis in ruminants (Jiang et al., 2022). Pérez-Barbería et al. (2020) found that feeding heather and European blueberry (Vaccinium myrtillus L.)

resulted in decreased methane production in sheep and red deer. According to Khattab et al. (2020) adding thyme or celery powder to ruminants' diets could enhance nutrients digestibility, ruminal fermentation, and reduce gas emissions. Also, there is a decrease in $CH4$ and $CO2$ emissions from goats that were fed diets supplemented with Moringa oleifera extract and Saccharomyces cerevisiae (Pedraza-Hernandez et al., 2019). Several medicinal plants, such as lemongrass, galangal, rosemary, and cinnamomum were investigated for modifying ruminal fermentation, methane mitigation, nutrient digestibility, and decreasing ruminal bio-hydrogenation (Khattab et al., 2016, 2017). Furthermore, Sinz et al. (2019) reported a decrease in methane production by the combination of acacia, grape seed, and green tea in an *in vitro* study. Moreover, adding bamboo grass (Tiliacora triandra, Diels) pellets to Thai native beef cattle diets could lead to a reduction in methane production (Wann et al., 2019). Essential oils from white thyme and oregano have the ability to modulate rumen fermentation and mitigate rumen methanogenesis without negative effects on feed digestibility in beef cattle (Benetel et al., 2022).

The use of essential oils (EOs) in livestock feed as a means to mitigate rumen methanogenesis is an important area of research given the significant contribution of livestock methane emissions to global greenhouse gas (GHG) emissions (Afdal et al., 2021). Additionally, Studies have shown that the incorporation of EOs in livestock diets can be effective in reducing rumen methanogenesis, which not only reduces methane emissions from livestock but also improves animal health and productivity (Kataria, 2016; Firmino et al., 2021). Interestingly, specific classes of active compounds naturally present in essential oils, such as thymol, eugenol, limonene, p-Cymene, γ-terpinene, linalool, carvacrol and terpinolene have been reported to possess the ability to inhibit the growth and activity of methanogens (Murrell & Jetten, 2009; Pandey et al., 2015; Matloup et al., 2017; Khattab et al., 2020). Thymol exhibits broad-spectrum antimicrobial activity, effectively inhibiting both gram-positive and negative bacteria through its role as a membrane permeabilizing agent (Walsh et al., 2003; Abd El Tawab et al., 2020). Also, limonene exhibits extensive antimicrobial activity against gram-negative bacteria (Dorman and Deans, 2000; Khattab et al., 2020). Eugenol, a phenolic compound found in the essential oils of plants like cloves, which have been shown to exhibit antimicrobial and inhibitory properties against methanogenic archaea (Patra et al., 2017). Carvacrol, a phytochemical derived from various aromatic plant species, has been reported to exhibit antimicrobial properties that can selectively target and disrupt the activity of methanogens, the microorganisms responsible for the production of methane (Patra & Saxena, 2010). Methane production was not affected by the addition of with the essential oil blend Agolin Ruminant to dairy–beef steers (Miller et al.,2023).

Several studies have highlighted the efficacy of EOs such as thymol, eucalyptus, and clove in this regard, demonstrating their potential to reduce methane emissions in ruminants while simultaneously improving animal welfare and performance (Khattab et al., 2020; Yu et al., 2020). As such, further investigation and implementation of the use of EOs as a means to mitigate rumen methanogenesis should be considered in efforts towards reducing the environmental impact of livestock production and decreasing global GHG emissions (Calsamiglia et al., 2007). In addition, the use of EOs as a natural feed additive is advantageous in that it does not have any adverse effects on animal health or product quality (Sankar et al., 2022). Furthermore, to fully understand the potential of EOs as a feed additive for ruminants, more information is required on the major components of essential oils that have a significant impact on rumen fermentation and ecology (Kalaitsidis et al., 2021). Moreover, the active components of EOs have stronger antimicrobial activity against microorganisms such as bacteria, fungi, and protozoa (Chao et al.,2000; Abd El Tawab et al., 2019). Also, it had more deactivation of some microbial enzymes (El-Zaher et al., 2020). The essential oils of several medicinal plants, such as cinnamomum, garlic, coriander, rosemary, galangal, thyme, celery, and lemongrass, have been suggested as rumen fermentation modifiers by inhibition of methanogenesis, decreased ruminal bio-hydrogenation, and deamination, resulting in lower ammonia nitrogen, methane emission, and acetate concentrations and higher concentrations of propionate and butyrate (Matloup et al., 2017; Abd El Tawab et al., 2020; Khattab et al., 2016, 2017, 2020). Selim et al. (2021) reported that supplementation of basil or marjoram oils to diets had a negative effect on total gas production, ammonia, and SCFA in an *in vitro* study. A reduction in methane emissions from dairy cows using essential oils as a feed additive in diets (Hart et al. 2019). Moreover, Canbolat et al. (2011) found a significant decrease in methane and CO2 production with an increase in the levels of orange (Citrus sinensis), mint (Mentha piperita), and thyme (Thymus vulgaris) essential oils in diets. The inhibitory effects of EOs may be due to their toxicity, which decreases ruminal fermentation, microorganism population, and methanogenesis.

Saponins

Saponins (triterpenoid glycosides) are a group of natural compounds found in various plants that have potent detergent properties due to their amphipathic nature (Deshpande et al., 2016). The saponins present in the tropical plants reduce the methane, ammonia productions and eliminate or suppress the rumen protozoa (Kamra et al., 2000; Guo et al., 2008; Newbold et al., 2015; Yanza et al., 2024). The use of saponin-rich plants in the diet of ruminants has been shown to have a positive effect on the reduction of methanogenesis, which is the production of methane gas by rumen microbiota during enteric fermentation (Takahashi, 2014; Qu et al., 2023) and helps to improve the productivity and health of ruminants (Wanapat et al., 2021; Balamurugan et al., 2022). Furthermore, this alternative approach to mitigating methane emissions has gained attention as it does not involve any detrimental effects on animal well-being and performance in comparison to conventional methods such as antibiotics or low-quality diets (Yuliana et al., 2019). Wang et al. (2009) noted a reduction in methane emissions as a result of adding Yucca schidigera or Sapindus saponaria fruits (Hess et al., 2004) to sheep diets. Other studies on sheep found a nonsignificant reduction in methane emission by adding saponin-rich extracts of alfalfa (Medicago sativa L.) root (Klita et al., 1996), tea saponin (Liu et al., 2019), Yucca schidigera, and Quillaja saponaria (Sliwiński et al., 2002; pen et al., 2007). Correspondingly, the addition of whole plant Quillaja Saponaria powder (Holtshausen et al., 2009) and Yucca schidigera powder had insignificant effects on dairy cattle (Van Zijderveld et al., 2011). However, Mao et al. (2010) noted a significant reduction in methane production per dry matter digestibility in the group fed tea saponin compared with the control. It may be due to the effect of saponin reduction on protozoa or methanogenic archaea counts (Patra and Saxena 2009). Also, defaunation (removal of protozoa) reduces the population of methanogens, resulting in lower methane emissions (Jayanegara et al., 2014). Wina et al. (2005) observed an increased propionate proportion and decreased levels of acetate, butyrate, and the acetate/propionate ratio by adding saponin to diets, which led to a low ruminal protozoa count. Protozoa in the rumen are extremely sensitive to saponins, which have shown potential as antiprotozoal agents to increase microbial supply to the host and decrease the number of methanogens in the rumen and methane emissions (Guo et al., 2008; Newbold et al., 2015).

Tannins

Tannins are naturally occurring polyphenolic compounds found in various plants, including forage consumed by ruminants such as cattle, buffalo, camels, sheep, goats, and deer (Teixeira et al., 2014; Gemeda and Hassen, 2015). Tannins can be complexed with proteins, protecting them from degradation in the rumen and releasing them later in the intestine for better utilization by the animal (Zhao et al., 2019). Research has shown that tannins can have a positive impact on ruminant nutrition by improving protein utilization and reducing methane emissions (Makmur et al., 2022; Brutti et al., 2023).

Carulla et al. (2005) found that the reduction in fiber degradation caused by condensed tannin's suppression of methanogens reduces the hydrogen produced by acetate production. Patra et al. (2017) reported reduced methane emissions by goats fed tannins containing forage. Reducing methane emissions is an important goal in sustainable agriculture, and tannins offer a natural solution to this issue (Furtado et al., 2023).

Flavonoids

Flavonoids are a class of plant compounds that have drawn significant interest in recent years due to their potential effects on ruminant methanogenesis and rumen microbial populations (Yao et al., 2021). Numerous studies have demonstrated that supplementing ruminant diets with flavonoid-rich plants or extracts can manipulate rumen ecology and lead to various beneficial outcomes (Morales and Ungerfeld, 2015; Cherdthong et al., 2019; Olagaray et al., 2019). These outcomes include enhanced milk production, protection against ruminal acidosis, decreased methane emissions, and lower populations of protozoa and methanogenic bacteria attached to their surfaces in both *in vitro* and *in vivo* studies (Cherdthong et al., 2019; Zhang et al., 2022). Also, Kim et al. (2015) noted that adding flavonoids to diets reduces methane emissions by 39-48% in the *in vitro* study. Moreover, flavonoids have the potential to alter microbial populations within the rumen, which in turn can affect ruminal pH, dry matter intake, and nutrients digestibility (Booyens et al., 2013).

EFFECT OF CHEMICALS FEED ADDITIVES ON METHANOGENESIS AND METHANE EMISSION

Ionophore

Since the mid-1970s, ionophores such as monensin have been widely used as livestock feed additives all over the world. Ionophores are a type of feed additive used in the livestock industry to improve animal health and production (Besi et al., 2018). Specifically, ionophores are antibiotics that selectively target gram-positive bacteria and ciliate protozoa normally found in the rumen of cattle and alter their metabolism to improve feed efficiency and prevent disease (Rivaroli et al., 2017). Monensin, one of the most well-known ionophores used in calves, reduces methane production by 7% while having no negative effect on milk yield (Odongo et al., 2007). It has beneficial effects on rumen fermentation, including propionate promotion, ammonia reduction, and methane reduction, as well as preventing bloat, lactic acidosis, and coccidiosis, which are attributed to the selectivity of the ruminal bacteria monensin (Stahl et al., 1988). However, the use of ionophores is a controversial topic due to concerns over the potential development of antimicrobial resistance and their possible impact on human health (Granstad et al., 2020). Recently, a study has suggested that ionophores like monensin can also reduce methane emissions from cattle (da Silva Zornitta et al., 2021). Furthermore, the administration of monensin in ruminants inhibits methanogens and results in a reduction in protozoal population by disrupting the transfer of hydrogen ions from the protozoa cell membrane (Tokura et al., 1997). Guan et al. (2006) reported that supplementation of ionophores to ruminant diets decreased ruminal methane emission and ruminal ciliate protozoal populations. Also, enteric methane production was decreased by monensin addition (0.6 mg/kg BW) in growing heifers (Gupta et al., 2019).

Nano minerals

Nanoparticles have been steadily gaining popularity in various fields of science and technology due to their unique properties (Zhang et al., 2018). Recent research has shown that the use of nanoparticles can also have an impact on methanogenesis, which is the process by which microorganisms produce methane gas (Wang et al., 2017; Gelaye, 2023). Functional nanoparticles with higher specific surface areas, and enhanced absorption capabilities are another efficient method for reducing enteric methane emissions. These substances have been found to improve the bioavailability of feed. The primary characteristic of interaction with biological systems is the capacity of nanoparticles to pass through cell membranes. In this way, biological processes such as immune system interaction, uptake, absorption, distribution, and metabolism are promoted (Abdelsalam et al., 2016). This discovery is valuable as a potential means of mitigating methane emissions. Studies have demonstrated that certain nanoparticles, such as silver, copper oxide, and iron oxide nanoparticles, can inhibit or enhance methanogenesis depending on the concentration used (Ali et al., 2015). Additionally, it was demonstrated by Fujinawa et al. (2019) that carbon nanoparticles particularly inhibit methanogens in an anaerobic environment. Similar to this, Jiang et al. (2022) showed that under anaerobic conditions, granular activated carbon had an inhibitory impact on methane. Moreover, according to Wang et al. (2019), the addition of magnesium oxide decreased the amount of *in vitro* gas generation, and the molar ratio of acetate while raising the molar ratio of propionate. Additionally, as shown by Kazemi and Vatandoost (2019), magnesium oxide increases the degradability of organic matter (OM) by reducing methane output. On the other hand, changes in ruminal digestion and fermentation are induced by zinc intake by the microbial community in ruminants (Salem et al., 2011). Zinc oxide nanoparticles were added to the diet to effectively increase energy intake and the growth of rumen bacteria *in vitro* (Chen et al., 2011). But according to Hernández-Sánchez et al. (2019), adding various dosages of elemental copper could reduce methane generation. Moreover, Maorong et al. (2008) noted that supplementation of copper increases rumen microbiota growth and essential fatty acid concentrations (omega-3 and omega-6).

Chemical inhibitors of methane emission

Several methane inhibitors are added to the diets of ruminants to reduce methane emissions and prevent energy losses, which have positive economic and environmental effects (Palangi and Lackner, 2022). Bromomethane is one of these agents that was discovered to stop the final step of methane synthesis by interacting with Coenzyme M. (Zhenming et al., 2011). There are significant effects on methane production with no negative effects on animal productivity with the addition of 3-nitrooxypropanol (3-NOP) to the diet, which is an inhibitor of the enzyme methyl coenzyme M reductase (MCR) (Hristov et al., 2015; Kim et al., 2020). Nitrite, nitrate, and 2-bromoethane sulfonic acid have similarly been shown to reduce methane emissions in invitro and invivo studies (Patra and Yu, 2014; Troy et al., 2015). Nitrite and nitrate are substituted hydrogen sinks that pull hydrogen ions away from methanogenesis (Yang et al., 2016), while Balch et al. (1979) observed that methyl coenzyme M reductase is inhibited by 2-bromoethanesulfonic acid. Also, Nkemka et al. (2019) and Kim et al. (2020) found a significant methane reduction with 3-nitrooxypropanol addition. According to Rebelo et al. (2019) animals fed non-protein nitrogen (NPN) showed decreased daily methane emissions compared to diets high in soybean meal. Natel et al. (2019) showed that substituting encapsulated nitrate products with soybean meal prevented the formation of methane by reducing the ruminal methanogens community. Similarly, Alvarez-Hess et al. (2019) reported decreased *in vitro* methane production with the addition of nitrate, fat, and 3-nitrooxypropanol.

Organic acids

The organic acids are a comprehensive report that provides invaluable insights into the role of acetic acid, formic acid, and fumaric acid in the process of methanogenesis and its impact on ruminants' methane production (Partanen and Jalava, 2008). These organic acids, specifically acetic acid, formic acid, and fumaric acid, play a crucial role in modulating methanogenesis in ruminant animals (Kara et al., 2017). A research study has shown that these organic acids have the potential to decrease methane production by redirecting the flow of hydrogen from methane formation to other reductive reactions such as propionic acid synthesis (Prathap et al., 2021). Furthermore, dicarboxylic organic acids like fumaric acid and malic acid have demonstrated their ability to inhibit methanogenesis both *in vitro* and *in vivo* studies (das Neves et al., 2022). These organic acids act as alternative hydrogen sinks in the rumen, reducing the production of methane gas (Palangi and Lackner, 2022). However, it is important to note that the quantity of fumaric acid fed to ruminants needs to be restricted due to its impact on ruminal pH. If the pH in the rumen falls too low, it can negatively affect fiber breakdown and feed intake, potentially leading to acidosis (Hernández et al., 2014). In addition to their role in reducing methane production, organic acids have been used as rumen modifiers in ruminant diets to stabilize ruminal pH (Hernández et al., 2014).

Lipids and oils

The impact of oils and lipids additives on methane emissions in ruminants has been a topic of interest in the agricultural industry (Olivares-Palma et al., 2013). Supplementation of lipids to the diet has been shown to reduce methane emissions by inhibiting methanogens bacteria and protozoa (Beauchemin and McGinn, 2006). Also, Grainger and Beauchemin (2011) observed a reduction in enteric methane emissions with feeding fat and oils, but at high concentrations of free fat, more than 6% of dry matter can have a detrimental impact on the ruminal microorganisms (Patra, 2013). Dietary lipids have several impacts on the rumen environment, including biohydrogenation of unsaturated fatty acids, toxic effects on methanogens bacteria and protozoa, and a shift to propionate synthesis, which decreases enteric methane emissions (Beauchemin et al. 2008, 2009).

Several studies have shown that the inclusion of oils and lipids in the diets of ruminants can have a significant impact on reducing enteric methane emissions (Arango et al., 2020; Ibrahim et al., 2021). One study conducted by Jayanegara et al. (2017) found that the addition of oils and/or lipids to ruminant diets resulted in a decrease in methane emissions. A similar study by Beauchemin et al. (2008) and Eugene et al. (2008) observed a negative effect of dietary lipid content on reducing methane generation, suggesting that the source, type, and dosage of lipids included the majority of plant oil supplementation could reduce rumen protozoa population and inhibit rumen methanogenesis in ruminants due to the effects of medium-chain fatty acids, including capric and caprylic, lauric, and myristic acids, which reduced methane emission by 45%, 37%, and 3%, respectively (Dohme et al., 2000). Moreover, reduce enteric methane yield by 42% by supplementing cottonseed oil with diets (Nogueira et al. 2020). Furthermore, Bayat et al. (2015) noted a reduction in enteric methane output by 29.5% with no impact on milk production or composition with the addition of camelina oil (60 g/kg dry matter) as a source of polyunsaturated fatty acids to the diets.

Propolis

Propolis extract is a resinous substance that bees collect from the buds and bark of trees to protect their hives against pathogens and predators due to its antimicrobial and antioxidant properties (Popescu et al., 2021). Also, examines the potential benefits and drawbacks of using propolis extract, including its effects on animal health and productivity (Santos et al., 2016). The beneficial effects of propolis extract on animal performance are possibly due to changes that occur in ruminal fermentation by the selection of gram-positive bacteria, with changes in the molar ratio of volatile fatty acids (VFA's) and in the concentration of ammonia nitrogen (Costa et al., 2012; Filho et al., 2020; Deolindo et al., 2024). Moreover, propolis extract has antimicrobial properties that can manipulate the rumen microbial ecosystem, increase the rumen metabolism, reduce methane emissions, and improve animal health and production (Santos et al., 2022; Al-Homidan et al., 2022; Balamurugan et al., 2022). Research has shown that propolis extract holds promise as an alternative to conventional methane inhibitors in animal feed (Morsy et al., 2022). Additionally, propolis rich in polyphenols, especially flavonoids, have also been found to be a potential methane inhibitor that can decrease enteric methane production and ammonia nitrogen in ruminants (Kim et al., 2016; Ehtesham et al., 2018). Therefore, propolis extract and other plant extracts could be a promising solution for reducing the environmental impact of livestock farming while improving animal health and productivity (Kara et al., 2014).

CONCLUSION

In general, there are several different types of feed additives that had various positive effects on methane emissions and the methanogenesis bacteria in the ruminants, such as biological, chemical, and phytogenic and their extract. Feed additives have various modes of action on acting directly through the methane generation process or indirectly through interaction with the fermentation and digestion process. Biological feed additives can influence the breakdown of cellulose and other plant fibers in the rumen more efficiently and improved microbial activity that converted hydrogen ions to propionate and diminished methane production. Moreover, the active components of phytogenic and their extract have stronger antimicrobial activity against microorganisms' inhibition of methanogenesis, decreased ruminal bio-hydrogenation, and increased propionate proportion and decreased levels of acetate, butyrate, and the acetate/propionate ratio. Chemical inhibitors are agents that was discovered to stop the final step of methane synthesis by interacting with Coenzyme M. or substituted hydrogen sinks that pull hydrogen ions away from methanogenesis, and other reductive reactions such as propionic acid synthesis. Biohydrogenation of unsaturated fatty acids has toxic effects on methanogens bacteria and protozoa, and a shift to propionate synthesis, which decreases enteric methane emissions. Also, it contributed to solving some problems related to greenhouse gases, which play a significant role in global warming.

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