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REVIEW

USING NUTRITIONAL STRATEGIES TO MITIGATE RUMINAL METHANE EMISSIONS FROM RUMINANTS

Jian SUN, Guangyong ZHAO, Meng M. LI (✉)

State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing 100193, China.

KEYWORDS

nutritional strategy, mitigation, microbe, methane, ruminant

HIGHLIGHTS

- Microbial fermentation in the rumen is a main source of methane emissions.
- Nutritional strategies can effectively mitigate methane emissions by manipulating biochemical reactions in the methanogenesis pathways.
- Mitigation practices must be evaluated in an integrated animal production system instead of as isolated components.

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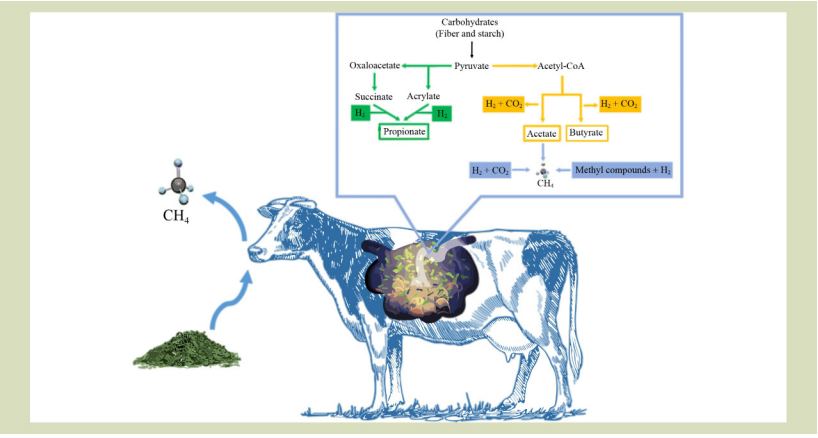
Correspondence: limeng2021@cau.edu.cn

GRAPHICAL ABSTRACT



ABSTRACT

Within the agricultural sector, animal production contributes to 14.5% of global anthropogenic greenhouse gas emissions and produces around 37% of global CH_4 emissions, mainly due to ruminal fermentation in ruminants. Over 90% of CH_4 is synthesized by methanogens in the rumen during carbohydrate fermentation. According to different substrates, methanogenesis pathways can be divided into four categories: (1) hydrogenotrophic pathway; (2) acetoclastic pathway; (3) methyl dismutation pathway; and (4) methyl-reducing pathway. Based on the principle of biochemical reactions in the methanogenesis pathways, this paper reviews the latest publications on CH_4 decreases in ruminants and described three nutritional strategies in terms of dietary nutrient manipulation (feeding management, feed composition, forage quality and lipids), microbial manipulation (ionophore, defaunation, methanogen inhibitors and probiotics), and chemical manipulation (nitrate, organic acids, plant secondary metabolites and phlorotannins, or halides in seaweeds). For each mitigation strategy, the review discusses effectiveness for decreasing CH_4 emissions, application prescription, and feed safety based on results from *in vitro* and *in vivo* studies. This review summarizes different nutritional strategies to mitigate CH_4 emissions and proposed comprehensive approaches for future feeding interventions and applications in the livestock industry.

1 INTRODUCTION

Methane concentrations have increased rapidly and have doubled in the atmosphere compared to preindustrial levels. Agriculture is one of the main sources of CH_4 emissions. Within the agricultural sector, animal production contributes to 14.5% of global anthropogenic greenhouse gas (GHG) emissions and produces around 37% of global emissions of CH_4[1]. Microbial fermentation in the rumen produces 6% of global anthropogenic GHG emissions representing around 40% of total livestock emissions[2]. The atmospheric lifetime of CH_4 is 8–11 years, which is much less than that of CO_2. However, CH_4 is more than 25 times as potent as CO_2 at trapping atmospheric heat. Therefore, decreasing CH_4 emissions from ruminants will be more significant in controlling GHG in the livestock production system[3].

With increasing awareness of environmental protection in the international community, extensive studies on decreasing CH_4 emissions in ruminants have been conducted over recent years[4], especially focusing on rumen microbial interactions, CH_4 production pathways, new additives, and practical pasture management[5,6]. Nutritional strategies, such as inhibiting substrate levels, regulating ruminal microbial compositions, and manipulating nutrient metabolic pathways, have been investigated to decrease methanogenesis. However, different strategies under *in vivo* and *in vitro* conditions might be inconsistent regarding prescriptions or potentials. For example, the defaunation treatment significantly deceases CH_4 production in the rumen, but long-term use in the pasture is not as efficient as short-term use[7]. Thus, CH_4 mitigation strategies must be evaluated from different perspectives regarding application scenarios, potentials, and usage concentrations or doses. In practice, some nutritional strategies can lower diet palatability, alter ruminal pH and induce ruminal acidosis. Meanwhile, inappropriate or non-judicious use of some additives may accumulate toxic and harmful residues in animal products. It is necessary to develop a suitable strategy without affecting the performance of animal production and food safety. In this review, we systemically describe the mechanisms of CH_4 production and discuss nutritional strategies to mitigate CH_4 emissions.

2 RUMINAL MICROBIOTA ASSOCIATED WITH METHANOGENESIS

Ruminal microbiome is extremely complex, being comprised of bacteria (10^{10}–10^{11} cfu·mL^{-1}, > 200 species), protozoans (10^4–10^6 cfu·mL^{-1}, 25 genera), fungi (10^3–10^5 cfu·mL^{-1}, 6 genera) and methanogens (10^6 cfu·mL^{-1}). Ruminal microbes are highly specialized in degrading dietary nutrients and generating energy and microbial protein to the host for maintenance, growth and lactation. Different microorganisms can form encampments or symbiotic relationships, which perform the carbon cycling and electron transport processes.

Henderson et al.[8] demonstrated that *Prevotella*, *Butyrivibrio* and *Ruminococcus*, and the unclassified Lachnospiraceae, Ruminococcaceae, Bacteroidales, and Clostridiales were the most abundant bacterial groups in the rumen, representing 67.1% of all bacterial sequence data. Most ruminal bacteria have amylolytic, cellulolytic, xylanolytic and proteolytic activities. These bacteria ferment carbohydrates to produce volatile fatty acids (VFA), CO_2, and H_2. During the process of VFA production, CH_4 is synthesized to ensure the fermentation is thermodynamically favorable. In the anaerobic fermentation chain, CH_4 emissions are affected by bacteria species with different functions. Among them, *Ruminococcus, Lachnospira, Catabacter* can generate more hydrogen than other bacteria[9]. In low CH_4 emitting ruminants, propionate-producing bacteria and succinic acid-producing bacteria are the most active bacteria, which can be used as alternatives to CH_4 producing bacteria for hydrogen-consuming. Danielsson et al.[10] reported that *Succinivibrio*, as the most abundant Proteobacteria genera, can decrease CH_4 emissions in the rumen.

Protozoans account for almost half of the biomass in the rumen, and they can be divided into flagellate and ciliate protozoans. Rumen ciliates are the most abundant protozoans, being composited of holotrich ciliates and Entodiniomorphid ciliates. Holotrich ciliates primarily digest soluble carbohydrates, while Entodiniomorphid ciliates ingest and utilize particulate materials. Protozoans are also metabolically associated with bacteria and fungi, which consume oxygen and increase the abundance of anaerobic bacteria. In addition, some protozoans contain a lot of hydrogenases and can coexist with methanogens. As methanogenic syntrophic partners, protozoans can provide not only H_2 but also other substrates (such as NH_3, formate, acetate and ethanol) for methanogens during ruminal fermentation[11]. Solomon et al.[12] demonstrated that different protozoa communities exerted a differential impact on the composition of the prokaryotic community and CH_4 production.

Ruminal fungi comprise about 8%–10% of microbial biomass. Most fungi in the rumen are anaerobic and have a significant

role in fiber degradation by secreting highly active cellulases and hemicellulases. Fungi can be associated with CH_4 emissions through hydrogen production, and some fungi can also convert methionine to CH_4[13]. Li et al.[14] found that *Piromyces* sp. F1 can stabilize pH and increase fibrinolytic enzyme activity when cultured with methanogens. Cheng et al.[15] demonstrated that genera *Cecomyces* and *Neocallimastix* could increase the growth rate of methanogens by providing growth factors.

As one of the earliest life forms of life on Earth, methanogens participate in the last step of the anaerobic fermentation chain to produce CH_4[16]. Methanogens can circulate carbon by using CO_2, formic acid, acetic acid, ethanol and methyl compounds (methanol, methylamines, and methyl sulfides) in the rumen with electrons provided by soluble H_2 or formic acid shuttles[17]. Methanogens produce CH_4 using methyl-coenzyme M reductases (MCR) which can be used as molecular markers for methanogenesis.

Although the proportions and relative abundances of methanogens in the rumen vary from ruminants, their composition is highly overlapping. Methanogens are classified into seven orders: Methanomicrobiales, Methanococcales, Methanopyrales, Methanobacteriales, Methanosarcinales, Methanocellales and Methanomassiliicoccales. *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* had the highest abundance species in the rumen accounting for 61.6% of the methanogens[8]. In an anaerobic and low hydrogen environment, the newly discovered Methanomassiliicoccales becomes the second largest methanogen order in the rumen. Methanomassiliicoccales grow well at 37 °C and are a dominant due to their high energy acquisition efficiency and high salt tolerance[18].

Methanogens have a wide range of substrates. Although methanogens are conservative in their evolution, the carbon metabolism pathways show a diversity trend under different environmental conditions. Methanomassiliicoccales archaeon RumEn M2 strain in the bovine rumen can produce CH_4 using trimethylamine alone, while methanogenic archaeon isolate ISO 4-H5 can produce CH_4 using methanol, monomethylamine, dimethylamine and trimethylamine[19].

3 METHANOGENESIS PATHWAYS

The majority of CH_4 is synthesized by methanogens in the rumen, and only a small fraction is produced in the hindgut. Although the total number of methanogens is not significantly associated with CH_4 emissions, the abundances of dominant methanogenic flora are highly associated with CH_4 emissions, suggesting that the composition and proportion of methanogens have a significant impact on CH_4 production[10]. As shown in Fig. 1, the CH_4 production pathways are: (1) hydrogenotrophic pathway; (2) acetoclastic pathway; (3) methyl dismutation pathway; and (4) methyl-reducing pathway[20]. The CH_4 production pathways usually utilize the decomposition of low carbon organics. Although CH_4-producing pathways in methanogens are complex, almost all CH_4-producing reactions require minerals (cobalt, iron and nickel) as cofactors. Hydrogen is required in most of the methanogenesis pathways. The majority of hydrogen used by methanogens is dissolved hydrogen and gaseous hydrogen only accounts for 2.7% of hydrogen used in the methanogenesis pathways[21].

Methane can be produced by Methanobacteriaceae, Methanococcales, Methanopyrales, Methanosarcinales and Methanomicrobiales via the hydrogenotrophic pathway in the rumen. In this pathway, methanogens reduce CO_2 to CH_4 using hydrogen as an electron donor via a seven-step enzymatic reductive reaction. Briefly, CO_2 is activated by a unique cofactor methanofuran, and transferred to formylmethanofuran. Then, formylmethanofuran is successively reduced to tetrahydromethanopterin, 5,10-methenyltetrahydromethanopterin, 5,10-methylenetetrahydromethanopterin and N^5-methyltetrahydromethanopterin. Finally, the methyl group is transferred to the reduced methyl-CoM to generate CH_4. This cycle of reaction is also called the Wolf cycle. The acetoclastic pathway is only found in Methanosarcinales, but can produce up to 60% of global CH_4[22]. This pathway converts methyl from acetate to CH_4. Methanogens in methyl dismutation pathways can use methanol, methylamines and methanethiol as substrates to produce CH_4. *Methanosarcina*, *Methanosphaera* and *Methanomassiliicoccus* are the main methyl dismutation methanogens. Methylated compounds is directly converted to CH_4, and the electrons are derived from the partial oxidation of methyl compounds to generate CO_2. The methyl-reducing pathway is identified in Methanomassiliicoccales, which reduces C_1-methylated compounds using hydrogen due to the absence of related CO_2 reductase, dehydrogenase, coenzyme M methyltransferase and other enzymes in the classical hydrogenotrophic pathway. The main components of this pathway are substrate-selective methyltransferase systems. The Gulin-like protein transmits methyl to coenzyme M, which is further reduced to form heterodisulfide (CoB-S-S-CoM) and CH_4 by activated coenzyme B[23].

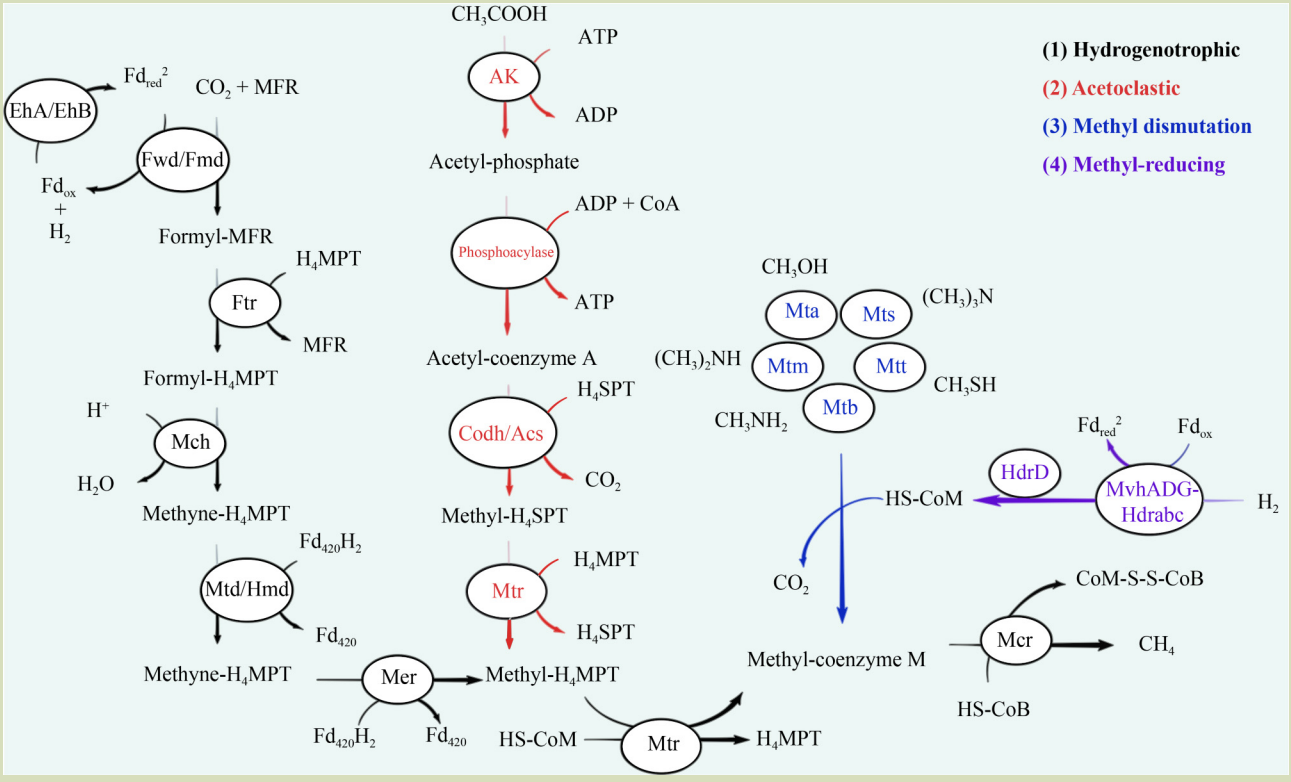


Fig. 1 Methanogenic pathways in methanogens. EhA/EhB, energy-converting [NiFe]-hydrogenases; Fd_{ox}/Fd_{red}^2, oxidized ferredoxin/reduced ferredoxin; Fwd/Fmd, tungsten-dependent formylmethanofuran dehydrogenase/molybdenum-dependent formylmethanofuran dehydrogenase; Ftr, formyltransferase; Mch, methenyl-H_4MPT + cyclohydrolase; Mtd/Hmd, Fd_{420}-dependent methylenetetrahydromethanopterin dehydrogenase/[Fe]-hydrogenase; Mer, Fd_{420}-dependent methylene-H_4MPT reductase; Codh, carbon monoxide dehydrogenase; Acs, acetyl-CoA synthase; Mtr, membrane-associated methyltransferase complex; Mta/Mtm/Mtb/Mtt/Mts, methyltransferase; MvhADG-Hdrabc, heterodisulfide-reductase/[NiFe]-hydrogenase complex; HdrD, heterodisulfide-reductase D; and Mcr, methyl-coenzyme M reductase.

4 MITIGATING CH_4 EMISSIONS BY DIETARY NUTRIENT MANIPULATION

4.1 Feeding management, feed composition, and forage quality

To increase energy conservation and decrease GHG emissions, nutritional strategies, such as changing feeding management and feed composition, modifying microbial community in the rumen, and adding chemical additives into diets, have been widely investigated in ruminants (Fig. 2). Using precision feeding or feed restriction can decrease CH_4 emissions[24]. Compared with corn silage or wheat grazing, programmed high-concentrate diets can lower DMI and improve feed conversion efficiency[25]. Galyean et al.[25] demonstrated that conducting programmed feeding management could decrease CH_4 emissions by 30% in feedlot cattle. Changing grazing management practices (such as stocking rate or rotational stocking) can affect CH_4 emissions in grassland systems[26].

Changing dietary nutrient compositions, especially the content of non-fiber carbohydrate (NFC) and neutral detergent fiber (NDF), has been proven to be an effective strategy to decrease methanogens abundance and CH_4 emissions by manipulating H_2 production, dry matter intake (DMI), rumen nutrient outflows, and nutrient digestibility. Hydrogen is a coproduct of carbohydrate fermentation when generating acetate and butyrate, while it can be used for propionate synthesis. Therefore, the acetate to propionate ratio (C_2/C_3) is an important indicator to evaluate CH_4 production. When C_2/C_3 is close to 0.5, the H_2 produced by the acetate pathway could be maximally utilized for propionate synthesis, leading to decreased CH_4 emissions. In theory, NDF fermentation can generate more acetate and less propionate than NFC. Thus, changing nutrient compositions, such as increasing starch contents or decreasing the roughage to concentrate ratio, could promote propionate yield and decrease CH_4 emissions. Compared with low starch diets, high starch diets can decrease CH_4 emissions by 35%[27]. Although high starch diets can break the mutualistic symbiosis relationship between protozoa

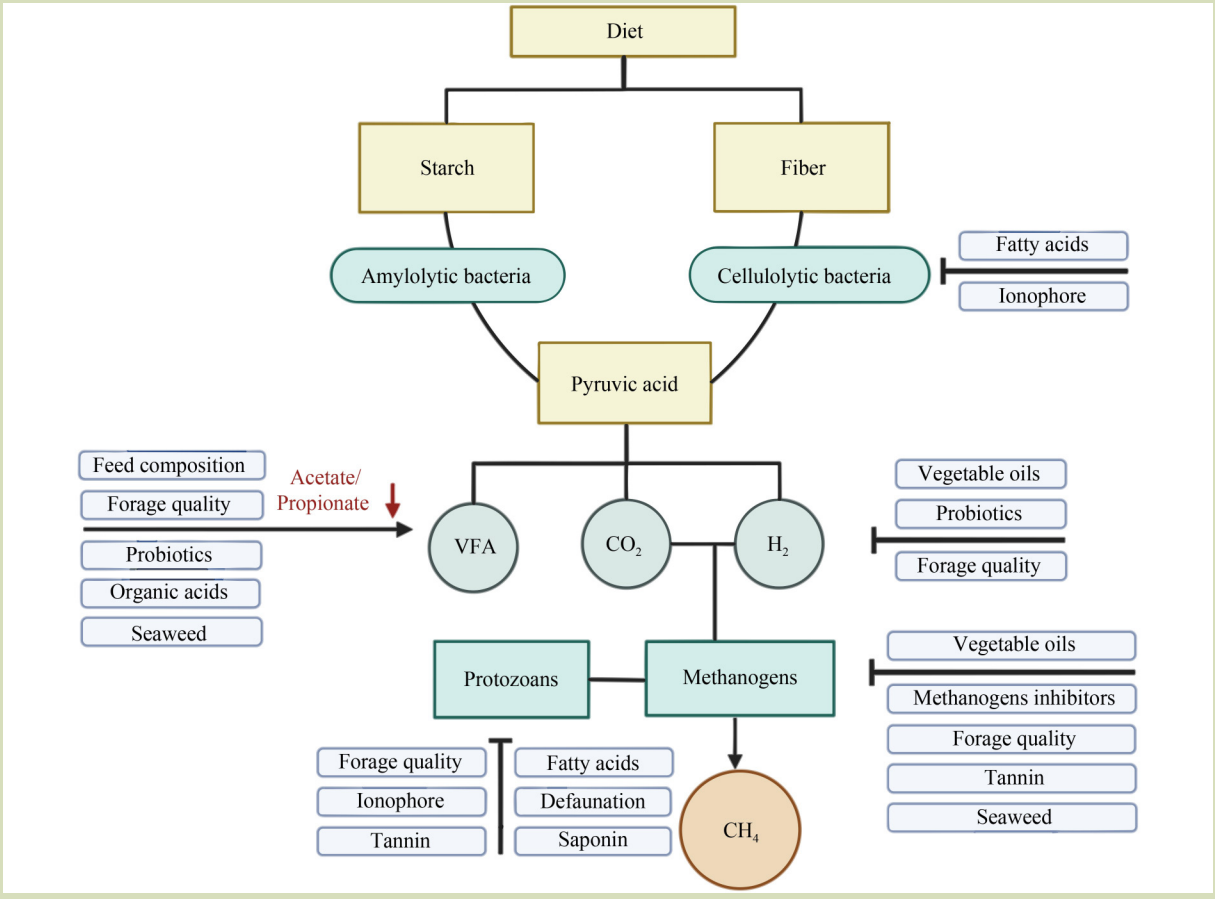


Fig. 2 Nutritional strategies in decreasing CH_4 emissions based on different mechanisms in carbohydrate fermentation and methanogenesis pathways.

and methanogens to inhibit methanogenesis, acidosis risk must be evaluated when high NFC diets are fed to ruminants.

Methane emissions could be mitigated by modifying forage harvest time, forage species, and forage conservation methods. High quality forages typically have a lower content of lignified-NDF, which can enhance ruminal fermentation and digestibility of organic matter, leading to an increase of DMI. Because the contents of soluble polysaccharide are high in low maturity forages, feeding forage with low maturity can increase forage digestibility and inhibit CH_4 biosynthesis[28]. In addition, forage processing methods, such as ammoniated grass, grass crushing or pelleting, and silage, can also improve the digestibility of forage in the rumen. Martin et al.[29] showed that corn silage could eliminate more CH_4 than alfalfa silage mainly because of its higher starch and less NDF content.

4.2 Lipid supplementation

Beauchemin et al.[4] summarized that a reasonable dietary lipid concentration that less than 6% can decrease 24-h CH_4 emissions by up to 20% with improved feed efficiency. Lipids contain polyunsaturated fatty acids and medium-chain fatty acids, which can influence ruminal fermentation and decrease CH_4 production in different metabolic pathways. Long chain saturated fatty acids have a negligible effect on decreasing CH_4 emissions. However, polyunsaturated fatty acids can be used as reductive hydrogen receptors, which are able to inhibit hydrogen dependent pathways of methanogenesis competitively. Medium-chain fatty acids have toxic effects on methanogens and protozoa, and thus they can break CH_4 producing reactions. The supplementation of medium- and long-chain fatty acids in diets can be manipulated by adding vegetable oils, such as garlic oil, corn oil, canola oil, peppermint oil, eucalyptus oil and commercial blended products. Patra et al.[30] demonstrated that the medium-chain fatty acids in palm kernel oil and coconut oil could inhibit methanogenesis by poisoning protozoa. Zhang et al.[31] reported that unsaturated fatty acids in corn oil might lower dissolved hydrogen in the rumen by methanogens via hydrogenation.

Therefore, choosing a proper composition and proportion of fatty acids will potentially enhance their inhibitory effect on CH_4 production. However, it is important to note that dietary lipid supplementation may decrease feed efficiency and the digestibility of fiber. Judy et al.[32] reported that adding medium and long chain unsaturated fatty acids in high-fiber diets lowered DMI, NDF digestibility, and feed efficiency. Considering the adverse effect of adding lipids on feed efficiency and feed cost, this strategy must be carefully evaluated in CH_4 mitigation before being applied.

5 MITIGATING CH_4 EMISSIONS BY MODIFYING MICROBIAL COMMUNITY

5.1 Ionophores

Ionophores have been used for over 40 years to protect animal health and improve feed efficiency. More recently, significant effects of ionophores on the ruminal microbiota and CH_4 emissions have been reported. The most commonly used ionophores in the livestock industry are monensin, salinomycin, lasalocid and laidlomycin. In theory, ionophores can anchor to cell membranes in gram-positive bacteria and protozoans and remove the difference in H^+ concentration between the interior and exterior of the cell membranes, leading to cellular apoptosis. Such a result would benefit gram-negative bacteria in the rumen, thus improving propionate fermentation and decreasing hydrogen production[33]. Ionophores are typically added to high-concentrate diets to alleviate acidosis, causing a 5% decline in total CH_4 production. In high-fiber diets, monensin inhibits the activity of cellulose-decomposing bacteria and decreases CH_4 emissions[34]. Ionophores can lower the abundance of gram-positive bacteria, such as cocci bacteria, and then effectively inhibit acetate and CH_4 production[35]. However, studies have shown that the mitigation effect of ionophores on CH_4 emissions fades gradually[36,37]. Due to the problems of antibiotic residues and microbial adaptation, the application of ionophores is inhibited in some countries. Thus, healthy and harmless alternatives need to be investigated.

5.2 Defaunation

Defaunation mainly acts on protozoans that coexist with methanogens inhibiting methanogenesis by ceasing hydrogen supply. Defaunation treatments include using sodium dodecyl sulfate, polyoxyethylene nonylphenol ether (NP-9) and other chemicals to remove protozoans. The actual gas emission can be lowered by 7.96 $g \cdot d^{-1}$ for a short period[38]. The inhibitory effect of defaunation is significantly associated with diet nutrient compositions. Given the relative abundance of protozoans in the rumen on grain-based diets is 10–100 times greater than that on forage-based diets, defaunation on grain-based diets was superior. In high-concentrate diets, starch fermentation produces the main source of hydrogen. The defaunation can effectively break protozoan activity and lower acetate production and CH_4 emissions[7]. However, defaunation has side effects, which cannot accurately eliminate those protozoans adhering to methanogens[24].

5.3 Methanogen inhibitor

Methanogens are the main microorganisms in methanogenesis. The growth of methanogens can be inhibited by manipulating its enzymatic reaction process. The main methanogens inhibitors include quinones, bromochloromethane, chloral hydrate, trichloroacetamide, nitroethane, 2,4-dihydroxypteridine, 2-bromoethanesulfonicacid and 3-nitroxypropyl alcohol (3-NOP). These compounds are typically used as substrates or analogs of enzymatic factors to inhibit the enzymatic reaction in the methanogenesis pathways. Polyhalogen compounds can inhibit CH_4 emissions by more than 20%[39]. The main mechanism is that polyhalogen compounds inhibit the generation of methyl-coenzyme M. Taking bromochloromethane as an example, bromochloromethane can maintain its activity in the rumen for a long time after being wrapped by α-cyclodextrin. Bromochloromethane can effectively interact with VB_{12} to inhibit the production of methyl-coenzyme M by inhibiting cobalamin methyltransferase. However, due to the high volatility and strong toxicity, the use of polyhalogens in animal production is limited. As an analog of tetrahydromethanopterin, 2,4-dihydroxypteridine mainly affected the first step reaction of tetrahydromethanopterin generation, inhibiting the transfer of methyl group. In addition, membrane-associated methyltransferase complex (Mtr) is a key factor for CH_4 biosynthesis in methanogens. Adding its substrate analogs can effectively decrease CH_4 without affecting organic matter degradation and VFA concentrations. Similarly, 2-bromoethanesulfonicacid is a bromide of coenzyme F, which can inhibit the activity of methyltransferase and block the carbon cycle[40]. Distinct from other inhibitors, a previous study revealed that 9, 10-anthraquinone can have an uncoupling effect on ATP synthesis and effectively inhibit CH_4 synthesis by affecting methyl-coenzyme M[41].

3-NOP is a small organic compound that functions as an analog of methyl-coenzyme M reductase (MCR). In the hydrogenotrophic pathways, MCR catalyzes the final step of

CH_4 biosynthesis. It catalyzes the reaction of methyl-coenzyme M (CH_3-S-CoM) and coenzyme B (HS-CoB) to generate CH_4 and the corresponding heterodisulfide CoM-S-S-CoB. This reaction proceeds under a strictly anaerobic environment and in the presence of F_{430}, and it is only active when nickel is in the Ni^+ state. 3-NOP has two functional groups, including a primary alcohol and a nitrate ester. Nitro ester can precisely localize the active site of the MCR, oxidize nickel and inhibit the formation of CH_4. Generally, the effect of 3-NOP on decreasing CH_4 production in dairy cows is slightly greater than that in beef cattle, possibly due to the lower concentration of methyl-coenzyme M in dairy cows[42]. For a similar reason, the effect of 3-NOP in low-fiber diets is greater than that in high-fiber diets[43]. Within a range of 0.75–4.50 mg·kg^{-1} BW[44], the CH_4 mitigation effect is linearly increased with increased 3-NOP supplementation[45]. Jayanegara et al.[46] reported that 3-NOP addition could directly affect methanogens and increase the proportion of propionate. The degradation of 3-NOP is rapid, so no studies have shown it has toxic residues or microbial adaptation. It has been proven to be safe in a large number of animal trials and approved for use in some countries around the world[47,48].

5.4 Probiotics

Probiotics are live microorganisms that can improve host health and have been studied as alternatives to antibiotics in recent years. Given that probiotics have a significant effect on stabilizing rumen pH and improving ruminal fermentation and feed digestibility, the impact of probiotics on CH_4 mitigation has also been gradually reported. Probiotics used in ruminant production include lactic acid bacteria, acetic acid bacteria, propionic acid bacteria and yeasts, which can effectively colonize in the rumen or stay in the rumen for a short period to alter the metabolic efficiency and enzyme activity.

Yeasts can consume oxygen in the rumen to improve the anaerobic environment. However, given that yeasts are unable to colonize the rumen, they can only maintain metabolic activity for around 30 h. *Saccharomyces cerevisiae* and *Candida untilis* are some of the most common harmless and healthy yeasts. They had strong tolerance in the rumen environment and could maintain metabolic activity under pH 3. Yeasts can not only compete with lactic acid-producing bacteria in hexose and pentose decomposition but also promote the utilization of hydrogen produced by acetic acid producing bacteria. Thus, they can lower the hydrogen utilization efficiency in methanogens. However, the effect of yeasts on decreasing CH_4 production is inconsistent between beef cattle and dairy cows. Some results in meta-analyses showed that yeast addition had no significant impact on ruminal CH_4 emissions[49,50].

Non-lactic acid bacteria, such as ruminal acetogens, succinic acid producing bacteria and sulfate-reducing bacteria, also have the potential to compete with methanogens for hydrogen utilization. *Ruminococcus flavefaciens* is the dominant fibrinolytic bacteria in the rumen. Hassan et al.[51] found that adding *Ruminococcus flavefaciens* into diets could improve nutrient digestibility and ruminal fermentation and decrease CH_4 emission in sheep. *Propionibacterium* P63 and *Lactobacillus rhamnosus* 32 in low-starch diets improved rumen pH and decreased CH_4 production, while they had no significant effect on VFA concentrations[52]. Villar et al.[53] reported that *Enterococcus* producing fumarate reductase changed ruminal microbiota structure, increased propionic acid fermentation proportion and decreased CH_4 emissions *in vitro*. When sulfate was added to diets, sulfate-reducing bacteria could promote the competition between sulfate and methanogens for hydrogen utilization and decrease the accumulation of hydrogen sulfide in the rumen[54]. When high sulfate levels are added *in vitro*, sulfate-reducing bacteria (such as *Fusobacterium*) can decrease 72-h CH_4 emission by 62%[55].

6 MITIGATING CH_4 EMISSIONS BY CHEMICAL MANIPULATION

6.1 Nitrate

Nitrate has a greater susceptibility to hydrogen reactions than CO_2. Thus, it can decrease CH_4 emissions by directly using hydrogen against methanogens or indirectly by its intermediate nitrite. Beauchemin et al.[4] reported that dietary nitrate supplementation decreased CH_4 production by 10%–22% while having no significant effect on NDF digestion. Long-term use of nitrate over several months can stably decrease CH_4 emissions by 12% in beef cattle and 16% in dairy cows[56]. Studies found that nitrate at 15 g·kg^{-1} DM decreased DMI by 8%[57,58], likely due to its bitter taste and decreased palatability. In this scenario, CH_4-producing capacity is more likely caused by decreased DMI instead of the inhibition effect of nitrate. However, Ortiz-Chura et al.[59] demonstrated that decreasing CH_4 production was independent of DMI changes, and nitrate lowered the relative abundances of methanogens and increased propionate fermentation.

Nitrite is a metabolite of nitrate, which can inhibit the oxygen transport ability of hemoglobin and has a carcinogenic effect on the body. Thus, nitrate supplementation is prohibited in some countries. To decrease nitrite production and increase

the mitigation effect, nitrates could be supplemented with lipids or other abatement strategies. Nitrate gave a greater decrease in CH_4 production when combined with oils[60], suggesting that the potential of nitrate and other hydrogen receptors can continue to be exploited.

6.2 Organic acids

Organic acids, such as fumaric and malic acid, act as propionate precursors, which can decrease CH_4 emissions by competing with hydrogen. When different diets were fermented, increasing the concentration of malic acid significantly decreased ruminal CH_4 production[54]. Fumaric acid can decrease CH_4 production by up to 42%, which is greater than malic acid[61,62]. However, organic acids reported in *in vitro* studies are less effective than *in vivo* studies, which may be because the reaction rate of hydrogen utilization is lower than hydrogen formation.

6.3 Plant secondary metabolites

Using plant secondary metabolites as feed additives to mitigate CH_4 emissions is continuously increasing. Approximately 25 of 500 plant extracts assessed proved to be additives with the potential to influence ruminal fermentation. Among the plant secondary metabolites, studies have focused on tannins, flavonoids, saponins and organic sulfur compounds in vegetable oils. Compared with other mitigation methods, plant secondary metabolites showed moderate performance in decreasing net CH_4 production and CH_4 emissions. However, because plant secondary metabolites are rich in resources, some species have stable long-term effects for decreasing CH_4 emissions[63].

Tannins are polyphenols in natural plants, including condensed tannins (CT) and hydrolyzed tannins (HT). Tannins are primarily accumulated in plants in temperate or tropical regions[64] and have been found in pods of sweet acacia, pine bark, chestnut, and pomegranate. Condensed tannins have a molecular weight up to 28,000 and are mostly detected in legumes, shrubs and trees. The main functional units in CT are catechins. Condensed tannins can bind to proteins and bacterial membranes. Therefore, they can lower the hydrogen-producing capacity of *Fibrobacter* and directly decrease the abundance of methanogens or protozoans[65]. Increasing the dose and the molecular weight of CT was effective in lowering the abundance of *Methanobacillus* and CH_4 emissions in sheep[66]. Similar results were reported in beef cattle and goats[67,68]. Adding a low dose of tannin (10–20 $g \cdot kg^{-1}$) in high protein diets can decrease CH_4 emissions by up to 50%[69].

Hydrolyzed tannins have a smaller molecular weight to CT, and their metabolites are generally divided into gallic and ellagic acids, which directly affect ruminal microorganisms after hydrolysis. Studies showed that tannins and gallic acid could selectively inhibit CH_4 related bacteria[70,71]. Given that gallic acid can bind to surface proteins in methanogens and form phenol-hydroxyl compounds, HT exhibit a more potent effect in decreasing CH_4 emissions than CT[72].

Fruit peel pellets as alternatives to antibiotics or chemicals impact ruminal pH, VFA production, and CH_4 emissions. Condensed tannins in these pellets could have a major role in decreasing CH_4 emissions. In various studies, rambutan fruit peel and dragon fruit peel was found to increase rumen pH, decrease protozoal population and improve nutrient permeability, and provide mitigation of CH_4 production[73–75]. When using composited fruit peel pellet (contains mangosteen peel, rambutan peel, banana flower powder and cassava starch), the propionate concentrations were enhanced with the increased composited fruit peel pellet, while C_2/C_3 ratio and CH_4 production were decreased[76].

Flavonoids, saponins and organic sulfur compounds in vegetable oils are promising plant secondary metabolites to mitigate CH_4 emissions. Oregano oil was effective in lowering the abundance and proportion of methanogens, dry matter degradation and CH_4 emission[77]. Saponins can decrease the proportion of gram-positive bacteria and promote the competitive use of hydrogen for propionate-producing bacteria[78]. Protozoan abundance linearly decreased with increased saponin concentrations from 0 to 0.5 $g \cdot L^{-1}$; consequently, CH_4 production decreased up to 29%[79].

6.4 Seaweeds

Seaweeds are complex multicellular organisms that live in both freshwater and ocean, which can decrease CH_4 emissions by 30%–69%. Seaweeds can be classified as red algae (Rhodophyceae), green algae (Chlorophyceae), and brown algae (Phaeophyceae) based on the pigments involved in photosynthesis. Phlorotannins in brown algae are polyphenols[80], also known as meso-phenol tannins. Adding *Ascophyllum nodosum* into diets altered the structure of ruminal flora and inhibited ruminal fermentation, liking due to the active effect of phlorotannins[81]. *Asparagopsis taxiformis* is one of representative species of red algae, which has the

greatest capacity for CH_4 mitigation relative to other seaweeds and has been well investigated *in vitro*. A series of halogenated compounds in red algae can effectively inhibit the activity of Mtr in the methanogenesis pathway by binding to VB_{12}. Both *in vitro* and *in vivo* studies reported that Rhodophyceae and associated halides can decrease emissions, and they are stable in high-roughage and high-concentrate diets[82]. Min et al.[80] reported that total gas, butyrate and glutaric acid production increased with increasing *Asparagopsis* concentration (0%, 2% and 4%) *in vitro*, while CH_4 emissions, C_2/C_3, and the respective yields were significantly lowered. More than 0.3 g (per 100 kg bodyweight) of tribromomethane in *Asparagopsis* may lead to poor palatability and decreased DMI[83]. Although, in most cases, seaweeds have no significant effect on milk yield and milk composition, the accumulation of iodine and bromine in milk might affect milk quality.

7 CONCLUSIONS

In summary, using nutritional strategies to regulate CH_4 emissions is becoming increasingly possible. These strategies are developed based on mechanisms that decrease H_2 production, promoting propionic acid fermentation, lower protozoa abundance and inhibit methanogen activity. Optimizing nutrient supply to animals according to their requirements can contribute to decreasing CH_4 emissions and allow for more efficient animal production. It is important to mention that CH_4 production cannot be decreased to a sufficient degree through dietary adjustments, as there are conflicts with animal production efficiency, rumen environmental health, and economic benefits. Therefore, mitigation practices must be evaluated in an integrated animal production system instead of as isolated components. Also, some strategies might have impacts on microbial adaptation, chemical residues in tissue, and the spread of antibiotic resistant genes and microbes. These research gaps need future exploration. Although the effect of chemical materials is highly efficient, the main issue lies in the difference between the *in vitro* studies and the actual process *in vivo*. The complex digestion process *in vivo* is generally inconsistent with the results obtained by *in vitro* fermentation. Some chemicals have great potential to decrease CH_4 emissions, which require further investigation in animal studies before they can be used as reliable tools. Consequently, dietary supplementation with 3-NOP, probiotics, organic acids or plant secondary metabolites, such as tannins and seaweed polyphenols, is recommended to decrease CH_4 emissions. Also, the combined use of probiotics and appropriate supplements can optimize the properties of probiotics. This also needs continue research to explore the effect of a typical feed resources, such as fruit peels and plant extracts, on CH_4 production and emissions. Overall, combined nutritional strategies and continuous technological innovations are greatly needed to accommodate the wide variation in the livestock production systems.

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Compliance with ethics guidelines

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