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Livestock Methane Emission: Microbial Ecology and Mitigation Strategies

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Additional information is available at the end of the chapter

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Abstract

Rumen microbiome plays a critical role in the development and nutrition of the host, and any alteration in the rumen microbiome has an important effect on the animal. Rumen microbial ecology is always dynamic in response to the diets and physiological conditions of the host. Ruminal microorganisms are mainly anaerobic and provide around 75% of the energy needed by the animal. The importance of microbial diversity in rumen has gained attention not only due to its significance on the productivity of the host, but also due to the emission of greenhouse gases (GHGs) and their environmental impact. Livestock is one of the most important sources of GHGs from agriculture, contributing more than 25% of global GHGs emissions. However, the variations in livestock emission in different regions of the world could be attributed to the changes in diversity and abundance of rumen microbial communities, which vary according to the type and age of animal, type of feeds, feeding strategies, climate, etc. This chapter deals on rumen microbial ecology, the role of microorganisms in enteric fermentation and the different mitigation strategies based on manipulation of rumen microbial diversity to reduce the methane emissions from livestock.

Keywords: methane, rumen, enteric fermentation, rumen ecology, mitigation strategies

1. Introduction

Global warming has been attributed to the increment of atmospheric concentration of greenhouse gases (GHGs). Since 1750, concentrations of CO₂, CH₄ and N₂O had increased by 40, 150 and 20%, respectively, until 2014, and the rate of increment of GHG per year from 2000 to 2010 was approximately 2.2% [1]. Of various anthropogenic activities contributing to global

warming, agriculture is an important source. This sector is responsible for 18% of the total anthropogenic GHG emissions annually [2]. Livestock represents the most important cause of GHG from agriculture contributing approximately to 80% of these emissions [3] and more than 25% of global GHG emissions [4].

Herrero et al. [5] estimated the total emissions from livestock were in the range of 5.6 – 7.5 GtCO₂-eq/year (5.6 to 7.5 × 10¹² kg CO₂-eq) between 1995 and 2005. They observed that the main sources were enteric CH₄ (~32.2%), N₂O emissions associated with feed production (~27.45%) and land use for animal feed and pasture (~24.42%). Havlík et al. [6] opined that ruminants represent more than 80% livestock emissions; particularly, beef and dairy sector contribute to about 60% [7]. Emissions from enteric fermentation contribute to 8% of total CH₄ emissions and are estimated to increase to 30% between 2000 and 2020 [8]. Enteric fermentation is the normal process of feed digestion in ruminants and is mediated by the microbial activity in the rumen and in the large intestines. Significant amount of methane is produced by methanogens residing within the rumen (87%) [9], which is released principally through eructation, approximately 10–15% is emitted by normal respiration and via flatus [10].

The continued growth of human population and consequent demand for food are potential drivers of GHG emissions. International climate negotiators have been focused to reduce GHG emissions by the improvement of engineering processes, energy efficiency and investments on alternative energy generation technologies. However, the abatement of ruminant GHG emissions has not received adequate attention by the United Nations Framework Convention on Climate Change [11]. Even so, several research groups have been working to develop strategies to optimize ruminal functions in order to achieve the desired levels of production by enhancing feed conversion efficiency and simultaneously reducing methane emissions by manipulating the rumen microorganisms. It is essential to have a detailed knowledge of ruminal microbiome, their interactions among themselves and with the host to achieve these objectives, and to identify the new approaches for mitigation of GHGs emissions [12].

2. Livestock GHG emissions

Livestock emissions depend considerably on some of the environmental characteristics such as the mean annual temperature, geographic location and the economic level of the country. It has been observed that in developing and emerging countries, the dietary habits increase meat consumption contributing to these emissions [4, 13], nevertheless developed countries have a greater proportion of intensive animal production, which results in higher emissions of CH₄, which is estimated to be 150.7 g/cow/day by cattle [4]. Additionally, the size and productivity of animals affect their feed intake and enteric CH₄ emissions [14], which can vary by animal type, growth stage and composition of diet [15, 16]. Castelán-Ortega et al. [17] reported that the average CH₄ emissions by individual dairy cattle are higher in the tropics than in temperate regions, 319.1 and 283 g/day, respectively. This could be attributed to the elevated proportion of cellulose in tropical forages, which is reported to produce three times more CH₄ than hemicellulose.

The estimation of livestock emissions differs considerably between studies as different models are employed for their estimation. Some authors use their own models, but most of the authors follow the guidelines of IPCC [18]. However, the differences on estimations still continue. Tier I utilizes default global or regional emission factors. Tier II utilizes estimated regional or local emission factors and is used in some enteric fermentation studies, nevertheless Tier III is the most reliable model for enteric CH₄ emission and has several advantages compared to Tier II, because it represents mechanisms of enteric fermentation in more detail and can be expected to describe more of the variations caused due to nutritional and animal factors [8, 19].

Enteric fermentation in ruminants and manure management emissions contributes directly to around 9% of total anthropogenic emissions. In 1990, enteric methane global emissions were 84 Tg/year CO₂-eq (84×10^9 kg CO₂-eq), which increased to 92 Tg/year CO₂-eq in 2005. It is reported that the main sources of global enteric CH₄ emissions are Asia (33%), followed by Latin America (23.9%), Africa (14.5%), Western Europe (8.3%) and North America (7.1%) [14]. Beef trades also have a significant impact on GHG emissions. Emissions from beef trade represented 2% of total emissions traded internationally in 2010 and increased by 19% during the period between 1990 and 2010. The dominant global fluxes in 2010 were the exportation of emissions embodied in meat from Brazil and Argentina to Russia (2.8 and 1.4 Mt CO₂-eq (2.8 and 1.4×10^9 kg CO₂-eq), respectively), emissions embodied in US imports of meat from Canada were the same that emissions embodied in US exports to Mexico of 1.2 Mt CO₂-eq. Australian meat exported to South Korea also embodied substantial emissions of 1.0 Mt CO₂-eq. In European countries, meat exported from France to Italy and France to Greece embodied 1.4 and 1.2 Mt CO₂-eq emissions, respectively. Also Italian meat imported from Poland, Germany and Netherlands embodied 0.7, 0.6 and 0.7 Mt CO₂-eq emissions, while Chinese emissions embodied in beef exported were small in comparison with the other countries. Although emissions due to import of meat are considered insignificant, it is important to consider all livestock sectors that contribute to emissions [13].

With respect to the Mexico, total CH₄ emissions in 2006 were 8954.10 Gg, and agriculture sector was the highest contributor with significant input due to enteric fermentation and manure management [16]. Earlier, Rendón-Huerta et al. [18] has also reported that enteric CH₄ emissions are the major source of GHG emissions in Mexican livestock production systems. They calculated the GHG emissions from dairy cattle in Mexico for a period of time of 30 years using a Tier II of IPCC and reported that emissions of CH₄, N₂O and CO₂-eq during 1970 to 2010 increased from 144 to 270, 0.349 to 0.713 and 3704 to 6962 Mt/year, respectively. They observed that methane emissions per cow increased by 11%, while per liter of milk decreased by 30%. In the past 40 years, total N₂O emission increased by 104%, but N₂O/cow emissions increased only by 22% in the same period and decreased by 25% per liter of milk. The reduction in GHG emissions per liter of milk means an increase in the efficiency of production systems resulting in an augmentation of milk production per cow and consequentially diminishing the emissions [18]. Hernández-De Lira et al. [16] based on animal census data from 2012, reported that the methane emissions by enteric fermentation in Mexico were 1926.08 Gg CH₄, of which beef cattle produced 1651.8 Gg CH₄; while dairy cows generated only 172.70 Gg CH₄.

Emissions by manure management, mostly CH_4 and N_2O , are produced during the manure decomposition carried out by anaerobic microbial activities. These emissions depend on specific manure composition and quantity produced which, in turn is dependent on other factors as animal type, breed, weight, diet and climate conditions. Although CH_4 emissions from enteric fermentation are higher than those from manure [13, 16], manures also contribute to N_2O emissions due to volatile nitrogen losses, principally in form of ammonia (NH_3) and NO_x [13]. They have reported that CH_4 and N_2O emissions from manure would increase by 20 and 29%, respectively, from 2000 to 2020.

Asia, particularly China, Western Europe and North America are the regions with the highest GHG emissions from manure management [14]. According to EPA [20], global GHG emissions from manure management were 446 million tonnes of CO_2 -eq, of which the share of CH_4 and N_2O was 53 and 47%, respectively, while FAO [3] estimated global GHG emissions from manure management were 368 million tonnes of CO_2 -eq. In case of Mexico, CH_4 emission from manure was 62.24 Gg CH_4 , where beef cattle and dairy cow emitted with 29.49 and 2.42 Gg CH_4 , respectively [16]. Similarly, FAO [3] reported that Asia, Central and South America, Sub-Saharan Africa, Western Europe, North America, Eastern Europe and the Commonwealth of Independent States were the regions with the highest emissions of N_2O due to manure [14].

3. Rumen environment

Ruminants are herbivorous mammals considered as latecomers in evolution. Their fore-stomach is a very complex environment, which allows them to convert plant tissues into nutritious and useful products. The digestive tract of ruminants is formed by various compartments such as reticulum, rumen, omasum, abomasum, small intestine, cecum, colon and rectum [21]. The ruminant stomach is composed by three pregastric fermentation chambers (rumen, reticulum and omasum) [22] (**Figure 1**). Environmental conditions such as temperature (38–42°C), redox potential (250 to 450 mV), pH (5.5–7) controlled by buffer in saliva and osmolarity (260–340 mOsm) [23] provide the ideal conditions for the digestion of plant material by microorganisms. Fibrous components are hydrolyzed and fermented by the interactions among different microbial communities inhabiting the rumen, producing mainly acetate, propionate and butyrate, CO_2 , H_2 and CH_4 . VFAs are the most important source of energy for the animal (75% of the total amount of the digested energy) [24]. Moreover, microbial cell biomass is the major source of protein and amino acids [25]. Microbial population also synthesizes vitamins B and K and employs detoxification mechanisms for phytotoxins and mycotoxins [26].

Microbial ruminant ecosystem is composed by a high microbial population density, predominantly obligate anaerobic microorganisms. Bacteria are the most abundant microorganisms and more than 50% of the cell mass in the rumen are comprised of at least 50 bacterial genera (10^{10} – 10^{11} ml^{-1}), followed by 25 genera of ciliate protozoa (10^4 – 10^6 ml^{-1}), six genera of fungi (10^3 – 10^6 ml^{-1}), methanogenic archaea (10^7 – 10^{10} ml^{-1}) and bacteriophages (10^8 – 10^9 ml^{-1}) [27–29], nevertheless only 10% of these microbiome have been identified and described [30].

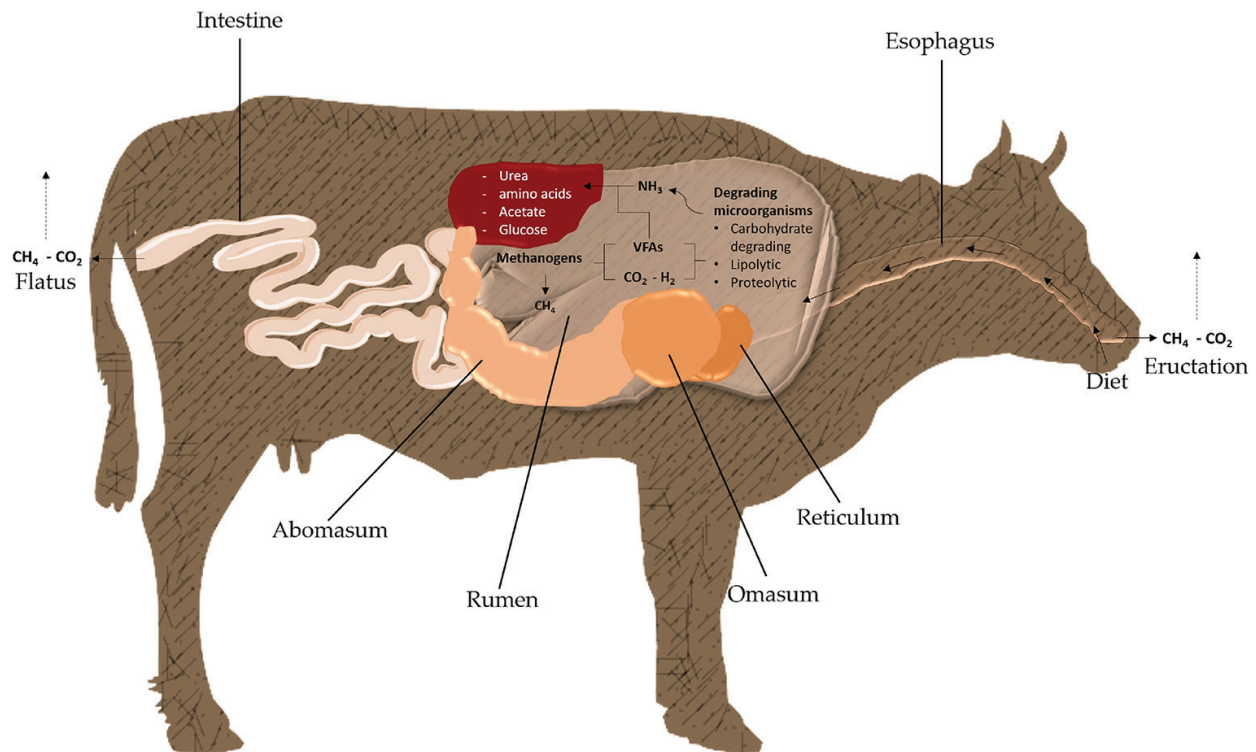


Figure 1. Ruminant digestion process. Note: Gastrointestinal tract of ruminants and main biochemical processes occurring in it.

The interactions of these microorganisms are widely different, namely mutualism, commensalism, syntrophy, competition and depredation [31, 32].

Hydrolysis of plant polysaccharide material is the first step in the enteric fermentation process, and 80% of plant cell material degradation is carried out by bacteria and fungi, and the rest 20% is by protozoa [33]. In the second stage, monomers are fermented to VFAs, branched chain VFAs, organic acids (lactate), alcohols, CO₂ and H₂. VFAs are absorbed by the rumen and omasal walls of the host animal for its nutrition [10]. Though several parameters such as rumen fluid, volume, pH and VFAs, concentration can disturb this absorption [34]. Free acids can be oxidized by obligate hydrogen producing bacteria to acetate, albeit this reaction is thermodynamically non-favorable, and hence are carried out only in syntrophic association with hydrogen consuming bacteria or archaea, which diminish the partial pressure of H₂. When the conditions are not favorable, VFAs are accumulated, decreasing the pH and inhibiting rumen microbiome [35, 36]. NH₃ is produced due to proteolysis and can be used by microorganisms to build their own proteins. The excess of NH₃ is absorbed by the rumen wall and transported by the animal blood [37]. The digested proteins, lipids and the carbohydrate constituents of microbial cells are exploited in the small intestine for the maintenance of the animal and the production of meat and milk. During enteric fermentation, a large quantity of CO₂ is produced due to diverse biochemical processes. A part of this CO₂ produced is released through eructation or normal respiration, and other part is reduced with H₂ to CH₄ by hydrogenotrophic methanogens. Methane produced is primarily released through eructation and approximately 10–15% is emitted by normal respiration and via flatus [10].

CH₄ production can be accomplished by the reduction of acetate and methyl-containing C1 compounds, nonetheless these pathways are not common in the rumen [38]. About 2–12% of gross energy intake (GEI) produced in the rumen by fermentation is converted to methane, which apart from leading to the loss of the feed energy, results in the emission and consequently, global warming [39].

4. Microbial diversity and abundance in rumen

As explained above, microorganisms present in gastrointestinal tracts (GIT) of ruminants and their relationship yield several benefits to the host. The composition of microbiome in GIT varies according to several conditions. Microbial populations can be affected by factors such as type and race of animal, age of the host, diets, feeds, farming practicing and geographical regions [40].

The microbial diversity presents in ruminant's changes across different points of the GIT. Mao et al. [41] studied the microbial population of 10 distinct sites of the GIT in dairy cattle and observed that the microbial diversity differed for the analyzed points. They reported 21 different phyla belonged to Firmicutes (64.81%), Bacteroidetes (15.06%) and Proteobacteria (13.29%). At genus level, the most abundant genera in cattle GIT included *Prevotella*, *Treponema*, *Succinivibrillum*, *Ruminococcus*, *Acetivibrio*, *Mogibacterium*, *Butyrivibrio* and *Acinetobacter* as well as many different unclassified genera, among which *Prevotella*, unclassified Ruminococcaceae, unclassified Rikenellaceae, unclassified Christensenellaceae and unclassified Bacteroidales were predominant.

A study carried out by Henderson et al. [42] determined the rumen microbiology of 32 species or subspecies of animals from 35 different countries of seven world regions and evaluated the differences among them. Seven bacterial groups comprised around 67.1% of the total bacterial sequenced, they corresponded to *Prevotella*, *Butyrivibrio* and *Ruminococcus*, as well as unclassified *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidales* and *Clostridiales*, but were not present in the same proportions in all animal species tested. The abundance of archaea worldwide was similar in all the sampled analyzed, and all belonged to methanogens and corresponded to *Methanobrevibacter gottschalkii* and *M. ruminantium*. *Methanospira* sp. and two *Methanomassiliicoccaceae*-affiliated groups, contributing to 89.2% of total archaeal community in rumen. Even in the same region, the age of the animal is other important factor that contributed to considerable differences in microbial diversity. It has been demonstrated that the ruminal microbiota of young dairy cattle is more heterogeneous than microbial community of those cows reaching maturity (2 years). In general, microbial communities in the rumen of dairy cows have been dominated by bacteria (>90%), followed by eukarya (2–8%) and a small abundance of archaea (1.0%). Similarly, a metagenomic study of the rumen microbiome in Holstein dairy cows reported 26 bacterial phyla belonging to Bacteroidetes (61–80%), followed by Firmicutes (12–23%), Proteobacteria (3–10%), Spirochaeta, Fibrobacteres and Actinobacteria (up to 2%). Again, they reported that *Prevotella* from Bacteroidetes was the most abundant genus (>50%), followed by *Bacteroides* (10.91%) and *Parabacteroides* (1.73%). In the case of Firmicutes, the predominant genera were *Abiotrophia*, *Acetivibrio* and *Acetohalobium*.

In the archaeal community, the genera *Methanobrevibacter*, being the predominant genera, and accounted 0.5% of the total microbial abundance [43].

Earlier, Kim et al. [44] analyzed the diversity of bacteria and archaea based on 16S ribosomal RNA (rRNA) and reported 13,478 bacterial and 3516 archaeal sequences, which correspond to 7000 and 1500 species of bacteria and archaea, respectively. Among nineteen phyla of bacterial domain, the most abundant were *Firmicutes* (57.9%), *Bacteroidetes* (26.7%) and *Proteobacteria* (6.9%). Within *Firmicutes*, the most abundant class was *Clostridia* (>90%), and the rest belonged to *Bacilli*, *Erysipelotrichi* and unclassified *Firmicutes*. In the *Clostridia* class, the predominant genera were *Butyrvibrio*, *Acetivibrio*, *Ruminococcus*, *Succiniclasicum*, *Pseudobutyrvibrio* and *Mogibacterium*. In the *Bacteroidetes* phylum, the predominant class was *Bacteroidia*, and *Prevotella* represented the most abundant genera. All the five classes of *Proteobacteria* were represented in the rumen bacterial sequences. More than 99% of the archaeal sequences correspond to the phylum *Euryarchaeota*, followed by 11 sequences of the phylum *Crenarchaeota*. About 94% of all archaeal sequences were assigned to the classes *Methanobacteria*, *Methanomicrobia*, *Thermoplasmata* and *Methanopyri*, all of them within phylum *Euryarchaeota*. However, this microbial abundance in rumen can be considerably different between the extremely high and low methane emitters. While archaea are 2.49 times more, bacteria are less (0.98×) in high emitters. In addition, *Euryarchaeota* and *Crenarchaeota* recorded an increase in high emitters (2.48× and 3.00×, respectively), and at genus level, *Methanobrevibacter* and *Methanosphaera* have been found more abundant (2.44× and 2.54×, respectively). In case of bacterial domain, there were no significant differences between *Firmicutes* and *Bacteroides* between high and low emitters, but *Proteobacteria* was 0.24 times less in high emitters. At genus level, *Desulfovibrio* was two times more in high emitters than low emitters. However, a higher abundance of *Succinovibrionaceae* was recorded in low emitters along with a change in acetate and hydrogen concentration profile, resulting in a low methanogenesis [45]. These microbial dynamics in animals of different types and from different regions clearly demonstrate that it is possible to develop strategies to mitigate livestock methane emission through microbial manipulation strategies. Various studies [46, 47] have suggested that it is possible to adapt the rumen microorganisms by manipulating the feeding management in the young animal, which have been found to persist in their later life. These results suggested that the methane emissions can be decreased considerably by manipulation of rumen microbiome through feed alterations.

As mentioned earlier, the composition of population in rumen is affected by the age and diet of the animal. Li et al. [47] evaluated the rumen microbiota of pre-ruminant calves of 14- and 42-day-old calves fed milk replacers based on 454-pyrosequencing of 16S rDNA and reported a total of 170 bacterial genera in the developing rumen of 14-day-old calves. They, further demonstrated that microbiota changed according to their dietary modifications and physiological changes in the host. Moreover, the transition from 14 to 42 days had a significant impact on the ruminal microbial composition. The most abundant phylum, *Bacteroidetes*, increased significantly his abundance from 45.7 (14 days) to 74.8% (42 days), the phylum *Synergistetes* also increased, while the abundance of *Firmicutes*, *Proteobacteria* and *Fusobacteria* decreased during this time. The results of these two age groups are different from those based on the rumen of 12-month-old animal, where the most abundant

phyla were *Bacteroidetes* (52%), *Firmicutes* (42.7%), *Spirochaetes* (2.3%) and *Fibrobacteres* (1.9%). This study clearly demonstrated that the changes in feed affect and change the dynamics of ruminal microbiome. Petri et al. [48] studied the impact of diet and its impact of an acidotic challenge on the composition of six different bacterial targets from heifers fed forage, mixed forage, high grain, post-acidic challenge (4 and 12 h) and recovery. They observed that all of the bacterial target groups were affected by dietary treatment, with exception of *S. bovis*, *Ruminococcus* spp. and *Fibrobacter succinogenes* represented a large percentage of the bacterial population present in the mixed forage diet. *Prevotella* corresponds to the most abundant genera in the acidotic challenge, but the lowest in the animal fed forage. *Megasphaera elsdenii* was present in abundance in the sample of 12 h after acidotic challenge, but its abundance decreased during recovery, while at the same time *S. ruminantium* increased in proportion. Both *S. ruminantium* and *M. elsdenii* accounted the smallest proportion of the bacterial population in heifers fed forages.

5. Methane mitigation strategies

The necessity to implement abatement strategies for enteric GHG emissions has been expanded in conjunction with the increase in the population and food demand. There are two concerns over methane emissions by livestock ruminants. First, the release of methane is considered a loss of energy for the animal, resulting in a decrease in animal productivity between 2 and 12%. Second, the calorific potential of methane released has a negative impact on climate change. There are several publications on strategies to reduce methane production [49–52]. The main target of these strategies is on methanogenic archaea by decreasing their substrate availability either directly or indirectly. Overall, abatement strategies include mechanisms such as modifications in dietary composition, and/or by supplementation of diet with chemical inhibitors, lipids or plant compounds, some of these strategies are shown in Figure 2.

5.1. Dietary composition

The quantity of enteric methane production is directly related to the quantity and quality of the feed consumed by the animal. The loss of GEI was augmented with an increase in high feed quantity. Animals with a low feed efficiency increase environmental impact due to the loss of GEI in form of methane.

The most common feeding mechanisms for the ruminants are based on pasture (grazing) and harvested forages. Hay and silage are the most common cattle forages. Hay has been recognized as superior feed than silage, but in cold and wet weather, silage is most used due to its major productivity. Silages for ruminants in temperate areas are usually based on cereals and legumes such as grass, maize, lucerne and red clover, which provide carbohydrate, protein and lipid sources for the animal [53]. It has been extensively reviewed that the replacement of ruminant forage diets with high grain diets can reduce methane production [9, 27, 54, 55]. Fermentation of cereal grains with high starch content increased the voluntary intake and reduced the residence time in the rumen, promoting post-ruminal digestion. Starch also

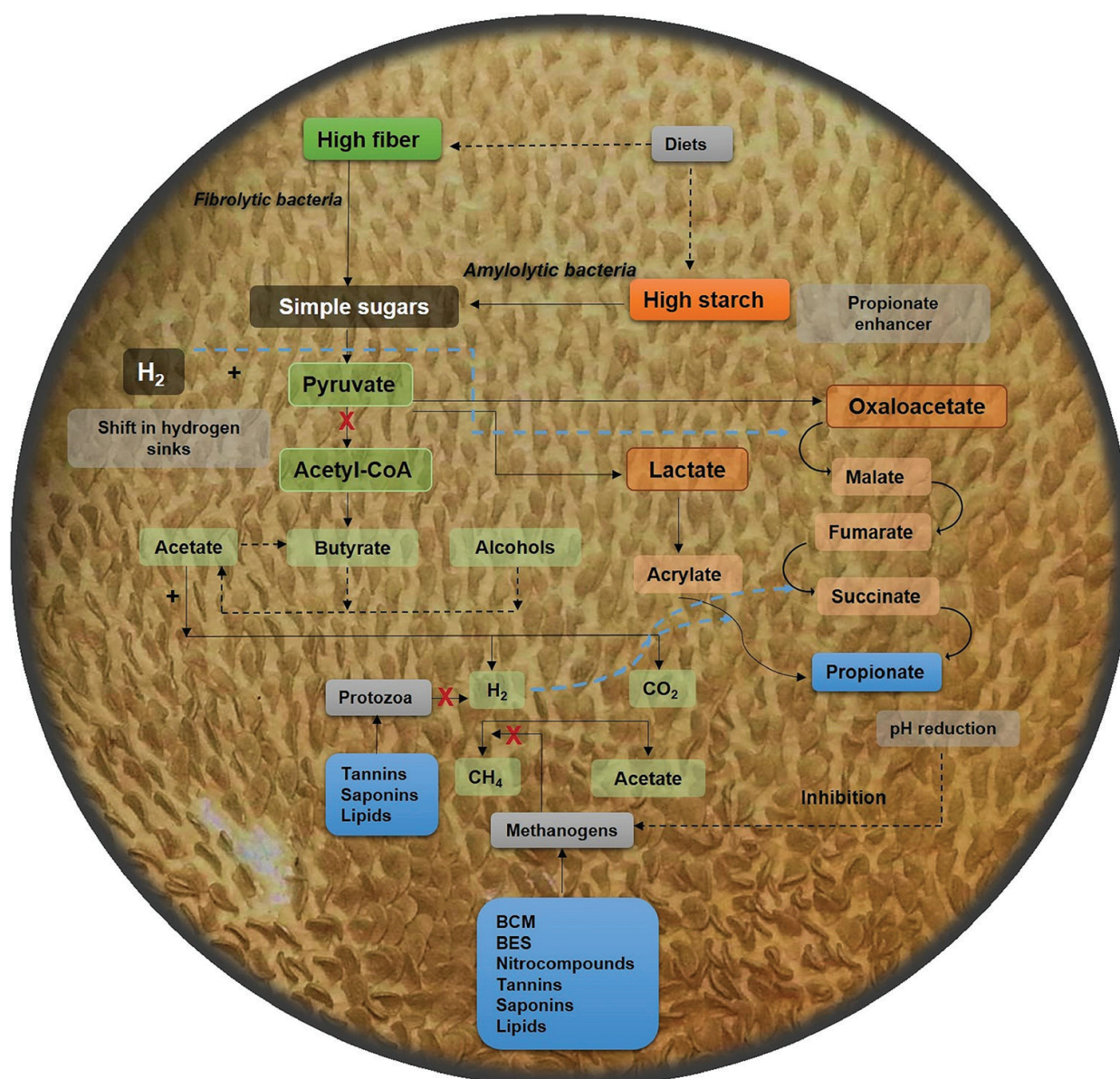


Figure 2. Mitigation strategies on methane emission by rumen microbiome manipulation through change in diets. Note: The main pathways and products formed when high fiber diet is used are represented in green color. The effect of high starch diets, which enhances propionate production due to shifting of hydrogen sinks, is presented in orange color. Dietary supplements and their main targets in order to reduce methane production are indicated in blue color.

enhanced propionate production, which depleted H^+ , and thereby decreased its availability for hydrogenotrophic methanogens. Moreover, propionate production decreased the pH, causing an inhibitory effect on methanogens and protozoa [56]. The loss of GEI with grain-based diets is commonly 4%, while it is 6.5% or more in forage-based diets.

Lettat et al. [57] reported that starchy diets, apart from increasing the propionate concentration, decreased the concentrations of acetate and butyrate and consequently methane production (-14%). Diversity and richness of bacterial community were reduced with increase in the starch content of the diet, however, the total bacterial population, *Prevotella* spp. and *M. elsdenii* were favored. The bacterial group *Prevotella* has been identified as amylolytic and propionate

producer and the dominant within the rumen [58, 59], while *M. elsdenii* is a well-known lactate-utilizing and propionate-producing bacteria. CH_4 reduction has been linked to the decrease in protozoan populations since protozoa are known as hydrogen producers and are in symbiotic relationship with methanogens. Hence, with a decrease in protozoan population, there is a decrease in the hydrogen transfer between them and methanogens, and this decreased the methane production. However, metabolic activity of archaea and methanogenic population increased when methane production decayed, demonstrating the cDNA-qPCR method to estimate archaeal growth and activity is unreliable to reflect changes in ruminal methanogenesis. However, there should be sufficient care before adopting this as a wholesale strategy. It has reported that changes in dietary composition not only can affect microbial diversity but also can generate animal disorders, producing a negative effect on the host. Saleem et al. [60] reported that high grain diets increased the concentrations of several toxic compounds such as putrescine, methylamines, ethanolamine and VFAs in the rumen fluid. VFAs accumulation can decrease the pH lower than 5.5 and produce subacute ruminal acidosis, which is a common and disturbing problem for farmers [61]. High grain diets have been commonly observed in favor of amylolytic microorganisms and against fibrolytic microorganisms. Petri et al. [48] reported that rumen of Angus heifers fed with high grains diet recorded a higher abundance of *Prevotella* spp., *S. ruminantium* known also as amylolytic bacteria, and *M. elsdenii*. Whereas, a higher abundance of the fibrolytic bacteria *Ruminococcus* spp. and *F. succionogenes*, and the lactate-producing *S. bovis* was observed with forage diet. Kittelmann et al. [62] observed a positive correlation between the occurrence of methanogens and fibrolytic bacteria. *Methanobrevibacter ruminantium* is found to be correlated with the family Fibrobacteraceae and *M. gottschalkii* with the family Ruminococcaceae. *Ruminococcus* spp. is known to produce large amounts of H_2 , while *Fibrobacter* spp. produces formate, which is substrates for methanogens. Therefore, the abundance of fibrolytic bacteria could be related with methanogenic communities and consequently with methane production.

5.2. Dietary supplementation

5.2.1. Chemical inhibitors

Compounds nontoxic to animal, but inhibitors to methanogens have been used to reduce methane production. Although these compounds inhibit-specific enzymes involved in methanogenesis pathway, it has been reported that they could also have an impact on other microbial groups present and could affect the uptake of feed by the animal [5, 27]. The most used and effective compounds are the analogous of coenzyme M, inhibitors of methanopterin biosynthesis, nitrocompounds and halogenated compounds [63–65].

Bromochloromethane (BCM), a methane analogue, has been extensively used to decrease methane production [65–67] but has a limited use due to its great ozone depleting capacity [66]. This compound reduces vitamin B12 and inhibits the cobamide-dependent methyl transferase step of the biosynthesis pathway of methyl coenzyme M, involved in methanogenesis pathway. After 12 h of supplementation, BCM-cyclodextrin (0.5 g/100 kg live weight) decreased the methane production of steer by 29%, and without adversely affecting the animal productivity [65]. Mitsumori et al. [67] studied the effect of different concentrations of BCM-cyclodextrin (BCM-CD) on the rumen microbial population of goats. Doses of BCM-CD

were of low (0.5 g/100 kg live weight LW), medium (2 g/100 kg LW) and high (5 g/100 kg LW), which decreased the methane emissions by 4.64, 71.46 and 91.23%, respectively. Denman et al. [68] analyzed the microbial diversity of the samples from the above study and reported that the relative abundance of Bacteroidetes increased with the BCM-CD doses, while Firmicutes, Synergistetes and Lentisphaerae phyla decreased. In the case of control animal, Bacteroidetes (60%) was dominant, followed by Firmicutes (24%), Synergistetes and Lentisphaera (both contributed ~4%). Administration of BCM also reduced considerably methanogenic diversity, however, *Methanobrevibacter* species were the most abundant in all treatments. Based on phylogenetic binding and functional assignment, the major genera were *Prevotella* and *Selenomonas* which were associated with the propionate production by the randomizing succinate pathway. This pathway was the primary route of H₂ consumption and decreased H₂ availability for methanogens.

2-bromoethanesulfonate (BES) is another common and successful compound to decrease methane emissions, which is an analog of coenzyme M. In an *in vitro* mesocosm study with cow manure and anaerobic digester sludge, a 89 and 100% decrease in methane production was observed at 0.5 and 10 mmol/L, respectively. Relative abundance of *Methanosaeta* and *Methanosarcina* decreased considerably at 10 mmol/L. Moreover, a decrease in *mcrA* expression, which encodes the α subunit of the methyl coenzyme M reductase and due to it is used for the relative measure of methane metabolites and methanogenic abundance in different environments [69], was observed with the increment of BES. A decrease in syntrophic-bacteria *Syntrophomonas* was observed too at both concentrations of BES. It is known for oxidation of butyrate and other fatty acids in syntrophic association with H₂-consuming bacteria and/or hydrogenotrophic methanogens and could explain the decrease in methanogenic activity [70].

The inhibitory effect of chloroform is attributed to its capacity to target the corrinoid-containing MtrA subunit of the large multimeric membrane enzyme methyl tetrahydromethanopterin:coenzyme M methyltransferase [71]. Martínez-Fernández et al. [72] studied the inhibitory effect of chloroform-cyclodextrin (CCD) by way of supplementation; as low (1 g/100 kg live weight LW), medium (1.6 g/100 kg LW) and high (2.6 g/100 kg LW) dose along with two diets (roughage:concentrate (60:40) or roughage hay) in eight steers. All three doses decreased the methane production by 14, 37 and 55%, respectively. Changes in microbial community were observed too, archaeal abundance was negatively correlated with CDD levels, Methanobacteriaceae family and Methanoplasmatales order were found to be decreased. Protozoan population increased with CCD doses with roughage:concentrate diet, while chloroform did not have any effect on fungi community. Bacterial population was also affected, relative abundance of *Bacteroidetes* increased, while *Firmicutes*, *Synergistetes* and *Verrucomicrobia* phyla were decreased. While methanogenesis was inhibited, an increment in the production of amino acids, organic and nucleic acids was observed. All of these metabolic changes modified the ruminal microbiome, increased the *Bacteroidetes:Firmicutes* ratio and decreased archaea and *Synergistetes*. Although abundance of fibrolytic bacteria, protozoa and fungi was not affected, methanogenesis was inhibited by 30%. They concluded that the use of chloroform as methanogenic inhibitor did not adversely affect rumen metabolism and could redirect H₂ to another pathways producing non-methane end products.

Apart from the compounds mentioned, nitrocompounds are also being used *in vivo* to mitigate methane emissions. These compounds target specific sites of MCR due to its molecular shape and oxidative potential and inhibit the last step of methanogenesis pathway. It has been reported that 3-nitrooxypropanol (NOP) at 40–80 mg/kg, decreased methane emissions around 30% and also increased body weight gain considerably without affecting feed intake or milk characteristics [73]. Duin et al. [74] reported that only 0.1 μ M NOP is needed to inactivate completely MCR, and 1 μ M to inhibit the methanogenic population. It was also reported that bacterial population was not affected by the addition of NOP, while methanogenic population decreased and protozoal abundance increased [75]. The decrease in methane production (–59.2%) by NOP (2 g/day) could be related directly to the reduction in the population of methanogens. The reduction in methanogen populations due to the addition of nitrocompounds need not always result in an increase in protozoan populations, since the compounds could also affect the symbiotic methanogens-protozoan association and thereby could result in decreased protozoan populations.

5.2.2. Plant bioactive compounds

Plant secondary metabolites have also been extensively used in the reduction of methane emissions. The most common used are tannins, saponins and essential oils, and they can affect methanogens either directly or indirectly. Further, they reduce protozoal population and thereby reducing symbiotically associated methanogens, apart from decreasing fiber digestibility and H_2 production [76].

Tannins are polyphenolic compounds which form complexes with metal ions, amino acids and polysaccharides, and thereby reduce ruminal fermentation. They can be divided into hydrolysable and condensed tannins. Hydrolysable tannins at high concentrations may be toxic to ruminants, while condensed tannins can make several nutrients unavailable to the animal due to irreversible binding [77]. Moreover, they can bind to the gastrointestinal tract, causing negative effects [78]. However, they have been found to be effective in reducing methane emissions. Condensed tannins have been reported to reduce methane by around 16% based on dry matter intake (DMI) [79]. Total methanogen population decreased by 22.3–36.7% when purified hydrolysable (HT) and condensed tannins (CT) (1 mg/ml) were tested *in vitro* conditions. Hydrolysable tannins were found to be more effective than with condensed tannins in reducing methane formation [80]. On the contrary, Bhatta et al. [76] reported that CT had a greater effect on methane reduction (–5.5%) than HT (–0.6%) and its inhibitory effect on methanogens (–28.6%) was more than HT (–1.6%). Protozoan populations also decreased by 12.3% with HT diets. However, a combination of HT+CT diets had a more significant effect and a 36.2% decrease was reported. Although tannins reduced total VFA concentrations was found to increase propionate concentrations and decrease iso-acids, which could have a negative effect on methanogenesis. In previous studies, a reduction in total and cellulolytic bacteria in response to tannins was observed along with the reduction in VFA production and also H_2 production, contributing to methane inhibition [81, 82].

Saponins are complex and diverse molecules which are divided in triterpene and steroid glycosides [83]. They are considered effective compounds to suppress methane production

due to their anti-protozoan properties [54]. Their anti-protozoan properties are attributed mainly to the formation of complexes with sterols in the membrane surface of protozoans [84]. However, this is pH dependent and composition of diet with addition of saponins [85, 86]. Moreover, saponins are potential defaunation agents and could result in the reduction in enteric CH₄ production by eliminating protozoa [9]. Nevertheless, they have an effect on the whole ruminant microbiome and animal digestion process, and not specifically targeting protozoan populations.

5.2.3. Lipid supplementation

Supplementation of lipids in ruminant diets is found to improve microbial metabolism of rumen, decreasing enteric methane emissions. Reduction in methane production could be due to the direct effect of fatty acids on methanogens, or indirectly due to the inhibition of the protozoan communities and associated methanogens due to enhanced propionate production. Beauchemi et al. [54] calculated that CH₄ (g/kg DMI) is reduced by 5.6% for each percentage unit of lipid, while Eugène et al. [87] estimated the methane reduction to about 2.3%.

Lipids commonly supplemented to reduce enteric fermentation are calcium salts of fatty acids, hydrogenated fats, and fats of animal origin, extracted plant oils, oilseeds and wastes from processing plants with high fat content [88]. Based on a meta-analysis of 27 publications on the effect of fatty acids in ruminant diets, fatty acids C12:0 and C18:3 demonstrated a significant inhibitory effect on methanogenesis without affect the productivity in dairy cattle [89]. Patra and Yu [90] analyzed *in vitro* the effect of five essential oils (EO) such as clove oil (CLO; from *Eugenia* spp.), eucalyptus oil (EUO; from *Eucalyptus globulus*), garlic oil (GAO; from *Allium sativum* L.), origanum oil (ORO; from *Thymus capitatus* L. Hoffmanns & Link) and peppermint oil (PEO; from *Mentha piperita* L.) on methane production, fermentation and ruminal microbiome. CLO, EUO, GAO, ORO and PEO significantly reduced the methane formation by 34.4, 17.6, 42.3, 87 and 25.7%. Further, decrease in relative abundance of ruminant microbial population such as archaea, protozoa and major cellulolytic bacteria *F. succinogenes*, *R. flavefaciens* and *R. albus* was recorded. Microarray analysis by RumenBactArray showed that the effect of each oil tested was unique. *Firmicutes* phylum was decreased by addition ORO and GAO, but increased by PEO. While, *Bacteroidetes* phylum, mainly *Prevotella* OTUS were found to be increased by addition of ORO and PEO. EO decreased the abundance of several microorganisms, *Syntrophococcus sucromutans*, *Succiniclasticum ruminis* and *Lachnobacterium* and members of *Lachnospiraceae*, *Ruminococcaceae*, *Prevotellaceae*, *Bacteroidales* and *Clostridiales*. This was correlated with feed degradability, ammonia concentration and molar percentage of VFAs, which directly affect microbial communities, their metabolic interactions and hence the methane production.

Beauchemi et al. [91] studied the effect of addition of saturated and unsaturated long-chain fatty acids to cattle basal diet, consisting mainly of whole-crop silage. Lipids of animal origin (tallow) and sunflower oil at 34 g/kg, and oilseed (whole sunflower seeds) at 89.3 g/kg were added to bring the total dietary fat content to about 59 g/kg of dry matter. On basis of dry matter intake, diets containing tallow or sunflower oil decreased methane emissions by 11%, while sunflower seeds by 23%. Based on digestible energy intake, all lipid sources decreased methane emissions by 17%. Previously, coconut oil has also been reported as an effective

inhibitor of methane production. Jordan et al. [92] reported a 39% decrease in methane emission at a concentration of 375 g/day.

Although supplements are being used primarily in reducing methane emission from livestock, their use in increasing efficiency in feed conversion and animal productivity, based on GEI, animal weight gain, meat and milk production has also been reported [73]. However, few other studies also have reported the negative effect of supplements on the quantity and quality of animal products such as milk and meat [60, 61]. This contradiction could be due to the reason that rumen microbial diversity is dependent on type and amount of feed, which in turn influences the nutrient absorption by animal. This implies that further studies on the relation between rumen microbiome and metabolomics of rumen are essential in order to understand the variations in relation to animal products due to supplements.

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