



Rumen microbial system, methanogenesis, and methane mitigation strategies in ruminants

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Abstract

Much of the biomass in this world is rich in fibre which is utilised by the ruminants with the help of rumen microbes to produce a good quality protein for human consumption. However, this conversion of fibre to high-quality protein is paralleled by the production of methane which represents the wastage of feed energy and is a powerful greenhouse gas harmful to the global climate. The microbial community in the rumen has co-evolved with their host animal in a symbiotic relationship over millions of years and methanogenesis has emerged as a result of the refinement of the fermentation process in the rumen. The one-to-one relationship between the methanogen population and the methanogenesis has not been established yet, which indicates the role of associated rumen microbiota, substrate availability, and other functional parameters of the rumen. This review has focused on the total rumen microbial structure, methanogen structure, rumen fermentation process, methanogenesis, factors affecting methane production, and methane mitigation strategies. The balance between the H₂ producers and H₂ consumers in the rumen determines the level of methane production in the rumen. Therefore, decreasing the availability of H₂ in the rumen by fostering alternative H₂ sinks, such as propionate production, is very instrumental in reducing the rumen methane emissions. Any strategy of methane abatement should concurrently consider the enhancement of propionate production to prevent the inhibition of rumen functions. Although a great deal of information regarding the rumen microbial community structure, rumen physiology, and methane mitigation strategies is currently available, more research is still needed. The majority of the *in vivo* experiments pertaining to methane abatement strategies discussed in this review are the short term experiments in which long term unwanted effects could not be precisely predicted. Therefore, there is a need for long-term experiments to draw valid and logical conclusions on the methane abatement strategies.

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1. Introduction

Methane is one of the major greenhouse gases other than carbon dioxide (CO₂) and nitrous oxide which have contributed significantly to the global climate change concerns. Methane constitutes about 40% of the total greenhouse gas emission from livestock settings and this 40% forms 6% of the total greenhouse gas emission of anthropogenic origin (Gerber et al. 2013; Ripple et al. 2014; Beauchemin et al. 2020). In terms of CO₂ equivalents, an annual estimate of 43 Gt were produced annually from the cattle population globally in 2019 with approximately 1500 kg of methane from each animal (FAO 2021; Davison et al. 2020). Majority of the hydrogen (H₂) production in the rumen is diverted towards the methane production in the rumen which acts as major H₂ sink in the

ruminants. This methane production keeps the partial pressure of H₂ from rising, which would otherwise impair the normal operation of the rumen microbial ecology. Ruminant methane emissions account for a significant feed energy loss of 2% to 12% of gross feed energy (Mayberry et al. 2019; Martin et al. 2010; Hook et al. 2010). Therefore, the enteric methane emission from ruminants needs to be decreased in order to decrease global warming, increase the feed energy availability for livestock production, and help sustain the ecosystems on the earth. Various preventive measures have been postulated and among those measures, the reduction of red meat consumption with the consequent reduction of the ruminant population has been proposed by certain sections of the society (Beyngelsson et al. 2016; Pais et al. 2020). However, this measure seems to be uncertain owing to the ever-increasing

demand for red meat because of expanding population and improving living standards in developing countries (FAO 2017).

Methane production is a matter of concern for its contribution to global greenhouse gas accumulation and wastage of feed energy. Its production is carried out by a special group of microorganisms called methanogens of phylum *Euryarchaeota* in the livestock gut, particularly in the rumen of ruminants (Morgavi et al. 2010; Hook et al. 2010). Therefore, methane mitigation strategies target the rumen microbes by various means such as immunization, defaunation, diet modifications, changes in management conditions, dietary supplementations, genetic selection of animals, etc. The permanent manipulation of rumen microbiota sustaining for longer periods has been reported to significantly reduce the emission of methane gas from ruminants (Abecia et al. 2013; Meale et al. 2021). However, these strategies targeting the methanogens have produced varied results which may be because of a complex microbial web in the gut in which microbes may be promoting the survival of methanogens either by producing conducive environmental conditions or by producing a substrate for methanogens (Morgavi et al. 2010).

2. Rumen microbial structure and methanogen composition

The rumen microbial system, consisting of bacteria, protozoa, fungi, archaea, and bacteriophages, is extremely diverse and a majority of them are yet to be defined. For millions of years, these microbes have co-evolved with host animals and have assumed highly specific metabolic functions which are inevitable for the normal growth and development of the animal (Morgavi et al. 2010). The organic matter in the rumen of animals is degraded by primary and secondary fermenters in succession which results in the production of volatile fatty acids, CO₂, and H₂. In this succession, the methanogen archaea come in the last which utilise these fermentation end products as substrate (Morgavi et al. 2010). Rumen microbial structure consists of a diverse group of microorganisms that act on cellulose, starch, proteins, and sugars as the substrates for the fermentation process. They form a complex microbial web in which some are net producers and some net utilisers of H₂ in the rumen. The most dominant bacterial phyla in the rumen are Firmicutes, Bacteroidetes, and Proteobacteria (Henderson et al. 2015). The cellulolytic bacteria such as *Ruminococcus* and *Eubacterium* spp. are the most commonly identified H₂ producers, whereas, members of phylum Bacteroidetes are net H₂ utilisers and the members of *Fibrobacter* genus do not produce H₂. Therefore, the abundance of net H₂ producing microbes determines the methane production in the rumen. A study on sheep characterised the rumen methanogens into low and high methane-producing ruminotypes (Kittelmann et al. 2014). The propionate producing *Quinella ovalis* and lactate/succinate producing *Fibrobacter* spp., *Kandleria vitulina*, *Olsenella* spp., *Prevotella bryantii*, and *Sharpea azabuensis*

were associated with low methane production. The high methane-producing ruminotypes were characterised by Ruminococcaceae, Lachnospiraceae, Catabacteriaceae, Coprococcus, other Clostridiales, Prevotella, other Bacteroidales, and Alphaproteobacteria. Similarly, in beef cattle (Wallace et al. 2014) and dairy cows (Danielsson 2016) high methane production was characterised by the four-fold lesser abundance of Proteobacteria spp. corroborating these observations. The treatment of rumen microflora in goats by halogenated methane analogues inhibited methanogenesis by fostering the abundance of H₂ consuming *Prevotella* and *Selenomonas* spp. (Denman et al. 2015). An interesting observation from the above studies was that some *Prevotella* species were associated with high methane production and others with low production.

The Proteobacteria, mainly represented by the family of Succinivibrionaceae, are highly abundant in low methane emitters (Danielsson et al. 2017). This is because the members of Succinivibrionaceae produce succinate, an intermediate product in the propionate production process which does not produce H₂ and thus, results in lower methane production in the rumen of animals. The members of phylum Actinobacteria, mainly represented by the Bifidobacteriaceae and Coriobacteriaceae families, were abundantly found in high methane emitters (Danielsson et al. 2017). Lactic acid and acetic acid, produced by *Bifidobacterium*, are more oxidised products compared to propionic acid which results in more H₂ production with consequently higher methane production. In addition to rumen bacteria, fungi and protozoa significantly contribute H₂ to the rumen environment along with CO₂, formate, and acetate (Gruninger et al. 2014). Several fungal genera have been detected in the rumen environment and new taxa are being added as a result of the identification by advanced molecular techniques. Although in a meta-analysis study a decrease in fungal abundance was observed as a result of defaunation with a consequent decrease in methane production (Newbold et al. 2015), no differences were observed in the composition of the rumen fungi in a study on sheep related to methane emission (Kittelmann et al. 2014). Methanogens have been found to have a strong association with ruminal fungi, however, their impact on the methane production process has not been established yet. On the other hand, methanogens are intimately associated with rumen protozoa either intracellular or on their surfaces (McAllister et al. 2015; Newbold et al. 2015; Belanche et al. 2014). A meta-analysis study revealed a positive relationship between the protozoal population and the methane emission expressed per unit of dry matter intake by animals (Guyader et al. 2014).

Methanogens, falling under phylum *Euryarchaeota*, lack cell wall peptidoglycan. In *Methanobrevibacter* and *Methanobacterium* it is replaced by pseudomurein, in *Methanosarcina* by heteropolysaccharide, and in *Methanomicrobium* by protein (reviewed by Hook et al. 2010).

The composition of methanogens is dependent on the host species and the ration fed. The identification of rumen methanogens in sheep revealed that the majority belong to *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales* orders with *Methanobrevibacter* the most dominant genus (Wright et al. 2004; Nicholson et al. 2007; Wright et al. 2008). In bovine species, *Methanobrevibacter ruminantium* is the most dominant methanogen species followed by *Methanosphaera stadtmanae* in dairy cattle on a mixed ration regimen. Whereas, in grazing cattle, *Methanomicrobium mobile* and *Methanobacterium formicicum* were the most dominant methanogen species and *Methanobrevibacter* spp. could not be identified (reviewed by Hook et al. 2010). In another study on feedlot cattle, the corn diet resulted in the predominance of *Methanobrevibacter ruminantium*, *Methanobrevibacter thaueri*, *Methanobrevibacter smithii*, and *Methanosphaera stadtmanae* methanogen species and potato by-product diet resulted in the predominance of *Methanobrevibacter smithii*, *Methanobrevibacter ruminantium*, and *Methanobrevibacter thaueri* species (Wright et al. 2007). The feed efficiencies in ruminants show a significant correlation with the type of methanogens in the rumen. There has been the predominance of *Methanosphaera stadtmanae* and *Methanobrevibacter* spp. in less efficient beef cattle with no difference in the total population of methanogens (Zhou et al. 2009). In another study, *Methanobrevibacter smithii* was found only in animals of higher feed efficiency (Zhou et al. 2010). Also, the animals fed a high-energy diet revealed the dominance of *Methanobrevibacter smithii* and *Methanobrevibacter* spp. and low-energy-diet fed animals showed the predominance of *Methanobrevibacter ruminantium*.

3. Rumen fermentation processes and methanogenesis

Fermentation is a complex process starting from the breakdown of complex polymers like carbohydrates, proteins, and lipids which results in the generation of oxidized cofactors – NAD, NADP, and FAD along with the release of CO₂ and H₂. Broadly, there are three groups of microorganisms involved in rumen fermentation (Millen et al. 2016). The first group, consisting of hydrolytic bacteria/protozoa/fungi, is involved in the breakdown of complex organic polymers into monomers which are further absorbed or catabolized into volatile fatty acids (VFA), CO₂, H₂, and alcohols. The methanogens constitute the second group that utilize H₂ to reduce CO₂ into methane or acetate and this is the dominant pathway of rumen methanogenesis. The third less significant group of homoacetogens produce acetate from H₂ and CO₂. The common monomer of carbohydrate polymers is glucose and the fermentation process of one mole of glucose yields one mole of butyrate and two moles of acetate and propionate. However, the H₂ output associated with these end products is different. In the production of butyrate and acetate H₂ is

produced, whereas, the propionate production pathway acts as a sink for H₂ produced in the rumen. Though methanogenesis is the major H₂ sink, other minor sinks such as unsaturated fatty acids, sulphur, and nitrates also consume the excess H₂ in the rumen (Millen et al. 2016). The most efficient pathway of VFA production from glucose is propionate with no methane production and the least efficient is the production of one mole of methane from one mole of glucose with high acetate production. The rumen fermentation process is regulated by a complex web of interactions between the rumen microflora to balance the end products produced in the rumen. For example – the protein degrading microbes produce branched-chain fatty acids and ammonia which is essential for fiber degrading microbes, lactic acid produced in the rumen is balanced by lactate producing and lactate utilising microbes, and the accumulation of succinate, produced by fibrolytic/amylolytic bacteria, is prevented by succinate utilizing bacteria such as *Selenomonas ruminantium* by converting it into propionate (Millen et al. 2016). The interaction between H₂ producers and H₂ consumers is another microbial interaction vital for the sustained fermentation process and continuous energy (ATP) supply.

In ruminants, the rumen is a fermentation chamber where the microbial population, living in a symbiotic relationship, exchange their metabolites to promote the survival of each other – a process called cross-feeding (reviewed in Kobayashi 2010). Methane production operates on this principle of cross-feeding where H₂ producing microbes and H₂ consuming methanogens interact with each other. Though the primary utilizers of H₂ and CO₂ are methanogens, there is a genus *Methanosarcina* that strongly prefers methanol and methylamines as the substrate for methane production over H₂ and CO₂. Therefore, methane production is carried out *via* two major pathways – one is the hydrogenotrophic pathway consuming H₂ and CO₂ and the other pathway consumes methanol and methylamines (Martin et al. 2010; Poulsen et al. 2013; McAllister et al. 2015). The major archaea producing methane by hydrogenotrophic pathway is the genus *Methanobrevibacter* along with other genera – *Methanosphaera*, *Methanimicrococcus*, and *Methanobacterium*. On the other hand, methylamines and methanol are used as the substrate for methane production by *Methanosarcinales*, *Methanosphaera*, and *Methanomassiliicoccaceae* (reviewed in Tapio et al. 2017). The VFA are potential substrates for methanogenesis because they can be converted to H₂ and CO₂ as well, but their conversion is too lengthy for a comparatively shorter rumen turnover (reviewed in Hook et al. 2010). In the process of carbohydrate fermentation by rumen microbes, the removal of H₂ is the last vital step carried out by methanogens for optimum rumen function, which will otherwise lead to inhibition microbial function. However, the archaeal population had either no correlation (Morgavi et al. 2012; Zhou et al. 2011; Danielsson

et al. 2012; Danielsson 2016; Kittelmann et al. 2014; Shi et al. 2014) or very weak correlation (Wallace et al. 2014) with the level of methane emissions. It was supposed to be the composition of the archaea, not the absolute number, which determines the methane emission level (Shi et al. 2014; Tapio et al. 2017).

4. Determinants of methane production

The acetate and butyrate production in the rumen fermentation process occurs largely from structural carbohydrates along with the production of H₂ gas. On the other hand, propionate is the predominant product of non-structural soluble carbohydrates which consume H₂ produced in the rumen. It has been observed that diets rich in starch content result in less methane emission because of lower H₂ production by starch consuming bacteria (Jenssen 2010). Therefore, the type of carbohydrate present in the diet influences the methane production in the rumen. The archaeal population was reported to have either no correlation (Morgavi et al. 2012; Zhou et al. 2011; Danielsson et al. 2012; Danielsson 2016; Kittelmann et al. 2014; Shi et al. 2014) or very weak correlation (Wallace et al. 2014) with the level of methane emissions. However, a positive correlation between the relative abundance of genus *Methanobrevibacter* and methane emissions was reported (Zhou et al. 2011; Danielsson et al. 2012; Shi et al. 2014; Danielsson 2016). Therefore, it can be the composition of the archaea, not the absolute number, which determines the methane emission level. It has also been observed that the diet as well as the geographical location of the host animal determines the methanogen composition and in turn the methane production level in the rumen of cattle (Wright et al. 2007). Methanogens live in a symbiotic relationship with rumen microorganisms, particularly rumen protozoa, for H₂ cross-feeding while methane production. Large size protozoa are heavily colonized by methanogens (Belanche et al. 2014) but H₂ production per unit biomass, and hence methane production, is less compared to smaller protozoa (reviewed in Tapio et al. 2017). For example, an *in vitro* study revealed that the smaller *Entodinium* spp. were more associated with methane production than the larger species like *Polyplastron multivesiculatum* (Ranilla et al. 2007). However, in contrast to these observations, a study reported no correlation between methane emissions and the relative abundance of different protozoa types in the rumen (Kittelmann et al. 2016).

5. Methane mitigation strategies

The methane production process is a major H₂ sink in the rumen, therefore consideration of alternative H₂ sinks is mandatory while attempting the methane reduction. The propionate production is the second major H₂ sink in the rumen and the other minor H₂ sinks are nitrate/nitrite reduction, reductive acetogenesis, and unsaturated fatty acid biohydrogenation (Mitsumori and Sung 2008; Kobayashi et al. 2010). Therefore, for optimum rumen function methane

reduction strategy must be paralleled by the enhancement of propionate production. Any methane reduction strategy adopted must – reduce H₂ production without compromising feed digestion, stimulate H₂ utilizing pathways for better economy of feed energy, and inhibit population and activity of methanogens (Martin et al. 2010). Following are the possible methane reduction strategies:

5.1 Immunization/vaccination

Vaccination represents the most economical option for methane reduction in ruminants with a theoretical requirement of one or two vaccinations in the entire life span of an animal. A number of attempts have been made to reduce the methane emissions by vaccination against various methanogens in the rumen with extremely variable results ranging from a 20% increase to a 69% reduction in methane production (Baca-Gonzalez et al. 2020). However, Wedlock et al. 2013 postulated that a maximum of 20% reduction in methane output is possible if all the common motifs of the entire archaea are targeted. An *in vitro* study revealed agglutination of methanogens and reduction in growth and methane production due to the serum from sheep vaccinated against *Methanobrevibacter ruminantium* M1 (Wedlock et al. 2010). A broad range vaccine against methanogens in sheep developed significant specific IgG titres in plasma, saliva, and rumen fluid, but no change in methane production and methanogen population was observed (Williams et al. 2009). A vaccine targeting three designated methanogens produced 8% reduction in methane production in sheep, however, testing with a different set of methanogen species or at a different geographical location did not elicit a same positive response (Wright et al. 2004). The differential response to vaccination against methanogens as a result of different diet and geographical location makes a single-target approach less viable (Wright et al. 2007). The possible reasons for vaccination failures in reducing methane output are diversity of methanogens, variable animal rearing conditions, and replacement of rumen environment void by targeted species by another set of methanogens (Wright et al. 2007; Williams et al. 2009). Therefore, for successful vaccination against methanogens, a much more broad-spectrum approach is required with a greater understanding rumen methanogen population.

5.2 Defaunation

The removal of rumen protozoa, called defaunation, has been reported to reduce methane emissions by as high as 50% (reviewed in Hegarty 1999). The protozoa are large producers of H₂ in the rumen and their intimate association with methanogens favours the cross-feeding of H₂ in the process of methane production by methanogens. The reduced methane output in defaunated animals persisted for more than two years, indicating a persistent shift brought on by defaunating agents (Morgavi et al. 2008). Between 9% and 37% of methane is produced as a result of the intracellular and extracellular

methanogens associated with rumen protozoa (reviewed in Martin et al. 2010; Hook et al. 2010). However, this decrease in methane generation is not a regular occurrence (Hegarty et al. 2008). The defaunation process decreases the population of protozoa as well as associated methanogens. The 26% decrease in methane production per kg dry matter intake of protozoa-free lambs was associated with a decrease in the methanogen population (reviewed in McAllister and Newbold 2008). Whereas, in another study, a 20% decline in methane production in sheep was associated with an absence of protozoa with no change in abundance of methanogens (Morgavi et al. 2008; Mosoni et al. 2008) which indicates the decreased H₂ production as an underlying cause of decreased methanogenesis. However, the judicious use of the defaunation strategy for methane reduction is required because it may negatively affect the normal rumen functions and in turn the performance of animals.

5.3 Use of ionophores and organic acids

The ionophores renowned for selecting gram-negative bacteria, like monensin and lasalocid, have been shown to increase animal production efficiency and decrease methane emission from animals (reviewed in Martin et al. 2010; Hook et al. 2010). Additionally, it has been claimed that they have an impact on rumen protozoa, whose alterations perfectly match the modifications in methane production (Guan et al. 2006). The ionophores do not affect the methanogens, but inhibit gram-positive bacteria and protozoa and thus reduce the supply of H₂ for methane synthesis. The selection for gram-negative bacteria shifts the rumen fermentation process towards propionate production (Martin et al. 2010; Hook et al. 2010) which results in less acetate, butyrate, formate, and H₂ production. The monensin treatment reduced the methane production by 7-9% compared to control for a long period of six months with no adaptation of target microbes in dairy cattle (Odongo et al. 2007) and no changes were observed in the abundance of rumen methanogens (Hook et al. 2009). Thus, it is the curtailment of H₂ supply by ionophores that decreases the methane production in the rumen. However, the effect of ionophores on the emission range and the persistency of reduction remained highly variable ranging from no effect to 25% reduction with a persistency of a few days to six months (reviewed in Martin et al. 2010). The effects of organic acid supplementation on the methane production in ruminants remains inconclusive and variable. From no changes in methane production (Beauchemin and McGinn 2006; Molano et al. 2008) to a 16% reduction in beef cattle (Foley et al. 2009), and to a reduction as high as 75% (Wallace et al. 2006) or 76% (Wood et al. 2009) in lambs was reported. However, the methane reduction by organic acid supplementation is influenced by the diet with a greater reduction in animals fed high concentrate diet (Foley et al. 2009). The reason for greater methane reduction is more propionate production from a high concentrate diet which acts as a H₂ sink. Though the organic

acids are good at methane reduction but studies on persistency and optimal conditions of use are needed.

5.4 Plant extracts

The use of plant extracts, consisting of tannins, saponins, essential oils, etc., has generated considerable interest in methane mitigation because of the rising antimicrobial resistance against the antimicrobials used for methane reduction (Begum et al. 2021). The condensed tannins bring about the reduction of methanogenesis in two ways – directly inhibiting the methanogens and indirectly limiting H₂ availability because of decreased feed degradation (Tavendale et al. 2005). The feeding of tannin-rich plant material such as *Lespedeza cuneata* in goats (Puchala et al. 2005), *Acacia mearnsii*, *Callinada calothyrsus*, and *Fleminga macrophylla* in sheep (Carulla et al. 2005; Tiemann et al. 2008) significantly reduced the methane production in the range of 13% to 57%. However, the tannin extract of *Schinopsis quebrachocolorado* (Beauchemin et al. 2007) and tannin-rich sorghum silage (de Oliveira et al. 2007) revealed no effect on the methanogenesis in cattle. Saponins are the plant constituents that directly inhibit the rumen microbes, particularly protozoa. Saponins are believed to reduce protein breakdown and at the same time promote microbial protein and biomass synthesis (reviewed in Martin et al. 2010) which limit the H₂ supply for methanogenesis (Dijkstra et al. 2007; Guo et al. 2008). Under *in vitro* conditions, the supplementation of saponin rich *Yucca schidigera* and *Quillaja saponaria* powder at 15 g/kg of dry matter reduced the methane production (Holtshausen et al. 2009). However, owing to the negative effects on the digestibility of feed at such higher levels (Guo et al. 2008) these saponin rich powders were fed to cows at 10 g/kg of dry matter and no changes were found in the methane output of animals. Therefore, it was concluded that saponins result in methane reduction because of the reduced feed digestion and fermentation (Holtshausen et al. 2009). Similar to the ionophore antimicrobials, the essential oils exert their antimicrobial effect by targeting the gram-positive bacteria (Calsamiglia et al. 2007) with which the H₂ supply for methanogens gets curtailed. The garlic oil, owing to the presence of toxic diallyl sulphide and allicin, was shown to reduce methane production under *in vitro* conditions (Busquet et al. 2005; Macheboeuf et al. 2006). However, in an *in vivo* study on heifers, the supplementation of essential oil had no effect on the methane production but negatively affected the digestibility of feed (Beauchemin and McGinn 2006). Therefore, further research is warranted under *in vivo* conditions to optimise the dose and delivery of tannins, saponins, and essential oils for the reduction of methanogenesis without compromising the feed digestibility.

5.5 Forage species, stage of maturity, and processing

The effect of forage species and stage of maturity on methane production in ruminant animals can be attributed to the

variability in forage chemical composition. A decrease of 21% in methane production was noted when the timothy hay was substituted by lucerne (Benchaar et al. 2001) and a decrease of 10% was observed in grazing beef cattle when a mixture of lucerne and grasses (70:30) was fed to animals (McCaughy et al. 1999). The higher dry matter intake with increased digestibility was observed by these authors resulting in the faster gut passage of feed and higher malate production which were responsible for lower methane emissions. However, these observations could not be replicated with all other legume fodders (Van Dorland et al. 2007). There are certain legumes and shrubs such as sainfoin, lotus, sulla, and *Leucaena*, rich in tannins, which substantially reduce the population of methanogens and H₂ producing rumen microbes resulting in the reduction in methane emission (Ku-Vera et al. 2020; reviewed in Waghorn 2007). The cattle grazing on *Leucaena* along with pastures has shown improved growth performance with lower methane emission compared to cattle grazing on pastures alone (Harrison et al. 2015; Taylor et al. 2016; Molina et al. 2016; Pineiro-Vazquez et al. 2018; Ramirez-Aviles et al. 2019). In controlled feeding trials, a linear decline up to 60% in methane emission was observed when low-quality grasses were replaced by *Leucaena* up to 80% level (Pineiro-Vazquez et al. 2018) and a 7% reduction in methane emission was observed when 20% fresh *Leucaena* was included in the diet of feedlot cattle (Kennedy and Charmley 2012). In an *in-vitro* trial with a tannin-rich legume – *Desmanthus*, a 26% reduction in methane production was observed compared to Rhodes grass (Vandermeulen et al. 2018) and at a 31% dietary inclusion level of *Desmanthus* there was a 10% decline in methane emission per kg dry matter intake in cattle (Suybeng et al. 2020). With increasing maturity, the fibre content increases and soluble sugars decrease in forages. An increase in methane production was observed when dairy cows grazed on forages of high maturity (spring vs summer) (Robertson and Waghorn 2002) but the same was not observed in the cows grazing on four different maturity stages of timothy pastures (Pinares-Patino et al. 2003). Lesser methane output by feeding young fresh forages has been ascribed to higher soluble sugars and essential fatty acids (reviewed in Martin et al. 2010). The finely ground or ensiled forages tend to produce lower methane compared to simple dried or coarsely chopped forages (Boadi et al. 2004; Beauchemin et al. 2008). However, the documentation of forage processing and preservation concerning methane emission in ruminants is sparsely available and thus, needs further research.

5.6 Concentrate level and type

As it has been discussed in the previous section that the level of methane production is the function of the level of structural carbohydrates present in the ration of ruminants. Increasing the concentrate level in the ration of ruminants decreases methane emissions (reviewed in Martin et al. 2010) because of the increase in the fraction of soluble sugars compared to the

structural carbohydrates in the ration. Increasing the ratio of soluble sugars to structural carbohydrates in the diet of animals is associated with higher feed intake, rate of passage, and rumen feed turnover which shift the rumen fermentation process towards propionate production (Martin et al. 2010). As a result of this shift, the H₂ sources decrease and sinks increase which results in a reduction in methane production. Substantiating these facts, a meta-analysis of previous studies has revealed a curvilinear relationship between concentrate level in the diet and methane production by ruminants (Sauvant and Giger-Reverdin 2007). A constant methane loss to the tune of 6-7% of total gross energy intake was reported up to a concentrate level of 30-40% and thereafter a rapid decline to as low as 2-3% was observed at 80-90% concentrate level in cattle (Lovett et al. 2003; Beauchemin and McGinn 2005; Martin et al. 2007a). Furthermore, the source of concentrate has a significant effect on the level of methane production in the rumen. An interesting observation was made by Beauchemin and McGinn (2005) when they used two different sources of grains for feedlot cattle – maize and barley. They observed that the slow degradable starch source, maize, resulted in lower methane emission compared to a rapidly degradable starch source, barley, during the finisher phase. This reduction in methane output due to the maize diet has been attributed to its stronger rumen pH lowering ability which fosters a microbial community more involved in propionate production.

5.7 Composition of diet

As it has been stated in an earlier section that the type of carbohydrate in the diet of animals is strongly correlated with methane emissions because it determines the rumen pH and the consequent microbial community structure (reviewed in Hook et al. 2010). The digestibility of structural carbohydrates such as cellulose and hemicellulose is strongly correlated with methane emission in animals. Precise predictions of methane emissions in cattle have been made based on the dry matter intake, neutral detergent fibre, and lignin content of the animal feed (Ellis et al. 2007). Increasing the dietary soluble sugar content by adding the concentrate level to 80-90% of the total diet decreases the methane output to as low as 2-3% of total gross energy intake (Sauvant and Giger-Reverdin 2007). The higher content of structural carbohydrates in the diet increases methane production by enhancing acetate production compared to propionate and vice-versa. As described in the previous section, the fine grinding of forages and/or increasing the rapidly fermentable carbohydrates in the diet increases the rate of passage in the gut and decreases the rumen pH which contributes to the decreased rumen methanogenesis (reviewed in Hook et al. 2010). In a feeding trial on sheep, the supplementation of red alga (*Asparagopsis taxiformis*) in sheep diet up to the level of 3% linearly decreased the methane emission up to 80% and no recurrence of the original level of methane emission was observed which suggested that

methanogens could not adapt to the alga in the diet (Li et al. 2016). In recent experiments with cattle, the supplementation of dried *Asparagopsis* at 0.2-1% level of organic matter reduced methane emission by 55-98% with no negative effects on the other growth parameters and product quality (Rouque et al. 2019; Kinley et al. 2020; Stefenoni et al. 2021).

5.8 Dietary lipids

The inclusion of dietary lipids in the ration of ruminants is a promising nutritional strategy to mitigate the methane production without any negative effects on the rumen function. The addition of lipids in animal diets reduces the methane production in the rumen because they enhance propionic acid production and biohydrogenation of unsaturated fatty acids, and they are not fermented by rumen microbes, and thus reduce the total organic matter available for fermentation. Furthermore, the medium-chain fatty acids potentially reduce rumen methanogens and polyunsaturated fatty acids inhibit the cellulolytic bacteria and protozoa which significantly contribute to the process of methanogenesis in the rumen (reviewed in Martin et al. 2010). However, fluorescence *in situ* hybridization technique did not reveal any significant differences in the methanogen population of dairy cows supplemented with flaxseed, and thus it was stated that the fatty acids might be exerting their effects on the methanogen activity, not the methanogen number (Kong et al. 2010). The meta-analysis of earlier studies revealed a reduction of 3.8% (reviewed in Hook et al. 2010), 2.2% (Eugene et al. 2008), and 5.6% (Beauchemin et al. 2008) in methane output for every 1% addition in supplemented fat in the diet of ruminants. The polyunsaturated fatty acids supplied in the diet of ruminants act as efficient alternative H₂ sinks to methane production. *In vivo* trials have revealed a dose-dependent decline of methanogenesis by supplementing extruded linseed in dairy cows (Martin et al. 2007b and 2009) and coconut oil in heifers (Jordan et al. 2006a). Along similar lines, a decline of 52% and 37% in methane emissions were observed at dietary supplementation of 5.8% linseed oil (Martin et al. 2008) and 6% soybean lipids (Jordan et al. 2006b), respectively. However, the effect of dietary lipids on the methanogenesis is determined by the type of diet, ruminant species, and lipid form, as well as the results are not always uniform. The supplementation of linseed in dairy cows along with hay produced a greater decline in methane production compared to linseed with maize silage (Martin et al. 2009). Along similar lines, the supplementation of coconut oil in beef cattle (Lovett et al. 2003) and myristic acid in sheep (Machmuller et al. 2003) along with a concentrate diet resulted in a greater decrease in methanogenesis compared to the forage diet. The practical repercussions of lipid inclusion in the diet of ruminants as a methane mitigation strategy are its effects on the palatability of diet, feed intake, animal performance, and final product quality (reviewed in Hook et al. 2010).

5.9 Genetic selection

Genetic selection for low methane emissions is a recent and interesting aspect of methane mitigation strategies in ruminants. The individual variations in methane emissions do exist in animal populations for similar feed intake or product output (Pickering et al. 2015; Black et al. 2021). And, the reasons for such lower emissions in certain individuals of the population are assumed to be the smaller rumen volume, the faster rate of digesta flow, and lesser fermentation of ingested feed because of different community structure (Goopy 2019; Danielsson et al. 2017). Though the heritability of methane emission in ruminants is moderate (Pickering et al. 2015; Goopy 2019; Donoghue et al. 2013; Herd et al. 2014), the smaller genetic variation among the individual animals compared to other production traits makes it a less viable methane mitigation strategy compared to others. Another thwarting aspect of genetic selection for lower methane emission is its possible negative correlation with other production traits. Therefore, the success of selection for lower methane emission depends upon the selection pressure imposed on it with respect to other production traits (Black et al. 2021). The genetic progress for lower methane emission through direct selection in beef cattle as well as sheep is very low (0.2-0.4% annually) (Fennessy et al. 2019). Whereas, in contrast to beef cattle and sheep because of the widespread use of artificial insemination in dairy cattle an annual reduction of 2-2.6% of methane emission is possible theoretically at the cost of 0.6-1.8% reduction in production traits (Gonzalez-Recio 2020). However, keeping in view the economics of animal production the selection for a reduction in methane emissions will always be in conflict with the production traits of the animal, thus making genetic selection a less viable strategy for methane reduction in ruminants.

5.10 Miscellaneous strategies

Proper management of the animal populations has been advocated as a possible strategy for reducing methane emission per unit of animal product. The reduction in maintenance requirement of the animals, and improvement in reproductive and productive performance of animals potentially reduce the methane emissions per unit of animal product (Eckard et al. 2010; Black et al. 2021). The improved grazing management of beef cattle in Australia has witnessed a reduction of about 20% in methane emissions (DeRamus et al. 2003). An advisory in Australia has proposed management practices to reduce methane emissions from cattle populations – increasing the ratio of live weight to animal age, reducing the average age of the herd, culling unproductive animals, and changing the relative numbers of each livestock class (reviewed in Black et al. 2021).

6. Conclusion

Over the millions of years, the microbial population co-evolved with its host animal in a symbiotic relationship, and methanogenesis cropped up as a result of the rumen

fermentation process being improved. The lack of a direct correlation between the methanogen population and methanogenesis suggests that other rumen functional factors, such as substrate availability, associated rumen bacteria, etc may also play a role. The amount of methane produced in the rumen depends on the balance between H₂ producers and H₂ consumers. As a result, one of the most effective ways to lower rumen methane emissions is by encouraging the synthesis of alternative H₂ sinks like propionate production. To avoid inhibition of rumen activities, any methane reduction strategy should also take into account the enhancement of propionate production.

Presently, there is plenty of knowledge available regarding the rumen microbial community structure, physiology, and methane mitigation techniques, but more research is still required. The majority of the *in vivo* studies regarding the methane abatement techniques addressed in this review are short-term studies in which it is impossible to predict the long-term adverse consequences. Therefore, long-term studies are required to reach reliable and logical conclusions about methane abatement strategies.

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