International Journal of Livestock Research eISSN : 2277-1964 NAAS Score -5.36 | Vol 8 (11) Nov '18

Review Article

Enteric Methane Emissions and Abatement Approaches: A Review

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Rec. Date:	Feb 08, 2018 09:04
Accept Date:	Jun 15, 2018 18:36
DOI	10.5455/ijlr.20180208090406

Abstract

Production of enteric methane through microbial fermentation in ruminants has created attention, for its role as a greenhouse gas and loss of feed energy to the animal. The development and commitment to mitigate methane emissions are likely to influence the productivity of livestock systems, comprising animal nutrition. Curbing rumen methanogenesis is conceivable using advanced mitigation policies and their viability for practical applications are being researched around the world. It is possible to modify the microbial ecosystem of rumen to decrease the production of methane, but the manipulation of microbial components of the system has to be considered in an integrated way which differ in terms of viability, cost, and acceptance by end users. Therefore, their implementation should be based on the capacity to reduce methane emissions along with economic sustainability and improvement in animal performance. The present review highlights the enteric rumen methanogensis and strategies to mitigate the level of methane production.

Key words: Global Warming, Methane Mitigation, Rumen Methanogenesis

How to cite: Kashyap, V., Grover, C., De, S., Panwar, R., & Verma, S. (2018). Enteric Methane Emissions and Abatement Approaches- A Review. International Journal of Livestock Research, 8(11), 1-21. doi: 10.5455/ijlr.20180208090406

Introduction

Climate change, due to large scale industrialization has drawn worldwide attention. The rise in global human population, unlimited use of fossil fuels and urbanization are the key reasons for increased atmospheric concentration of greenhouse gases. These changes have led to global warming and depletion of ozone layer, have increased the penetration of ultraviolet rays, which is a great threat to the mother earth. Global warming has occurred over the past 120 years and has been especially rapid in the last 20 years. Among the various GHGs, carbon-dioxide (CO_2), nitrous-dioxide (NO_2), methane (CH4), tropospheric ozone, trichlorofluoromethane (CCl_3F) and dichlorodifluoromethane (CCl_2F_2) accumulating at a rate of 0.3-0.9% annually and methane is the second major GHGs among all of them (Patra, 2012). Moreover, it has been reported that globally 15% of total methane emits from rumen, where 2-12% of the ingested feed energy is lost as CH_4 (Knapp *et al.*, 2014).

India's livestock represents one of the largest zone in the world having a total of 529 million livestock, which makes India the largest contributor to global CH_4 emissions (FAO, 2007). Yet, approximately 90% of cattle and buffalo are native breeds having low productivity. Indian livestock region produces nearly 12Tg of CH_4 annually, of which more than 90% is emitted through enteric rumen fermentation (Chhabra *et al.*, 2013; Forabosco *et al.*, 2017). This is much higher than CH_4 emissions from the US livestock sector i.e. 7.85Tg yr^{-1} (Hristov *et al.*, 2014). The livestock sector of India accounts for 78% of total CH_4 emissions from the agriculture sector and about 50% of CH_4 emissions from all sectors in India (Swamy and Bhattacharya, 2006). Cattle have been documented to be a significant contributor of methane i.e. 53.5% trailed by buffalo (39%), goat (4.7%) and subsequently sheep (1.8%), where annual methane production by Indian livestock sector was estimated to raise by 15×10^9 kg in 2050 (Singhal *et al*., 2005; Patra, 2014). Representing Indian scenario, methane emissions related to livestock sector can cause increase in surface temperature up to 0.7-0.036 mili kelvin (mK) over the 20 and 100 year time periods correspondingly, which is crucial step for India to achieve its GHGs emissions reduction goals (Kumari *et al*., 2018). Understanding the current tendency, as well as impending projections of CH_4 emissions it is very important to identify policy gaps and to implement long-term policy mechanisms to reduce CH_4 emissions. Although a very limited data for representing studies concerning future prediction related to Indian livestock are available (ALGAS, 1998; Yamaji *et al*., 2003; Singh *et al*., 2012; Pathak *et al.*, 2013).

Microbiota of Rumen

Ruminants in India are served mainly on the diet containing high lignocellulosic agricultural by-products that mainly composed of cellulose, hemicellulose, lignin, starch, and protein. The rumen comprise various types of bacteria which are degrading these feed components. They interact among themselves and with other microbial groups to perform a synergistic effect on the production of volatile fatty acids and microbial proteins inside the rumen. Some of the common features of bacteria that reside in the rumen of animals fed on high roughage diet are as follows-

(a) Majority of the bacteria are Gram-negative, however, number of Gram-positive bacteria tends to increase on the increment of high-energy diets in the ration.
(b) Most of the bacteria are obligate anaerobes.
(c) Some of them are so sensitive to oxygen that these are killed upon exposure to oxygen.
(d) A few rumen bacteria require a very low redox potential indicating their high degree of anaerobiosis and grow at a redox potential of lower than –350 mV. The bacterial community residing the rumen is enormously diverse. The majority of these microbes have not yet been cultivated; however, development of molecular tools have estimated that bacteria alone belonged to at least 300-400 phylotypes containing, 5.0×10^6 to 6.0×10^7 CFU/mL rumen liquor of the total bacterial population (Nathani *et al.*, 2013).

DOI 10.5455/ijlr.20180208090406

In addition, protozoa, fungi, methanogenic archaea and bacteriophages also contribute to diversity and functioning of the rumen microbial ecosystem. Rumen ecosystem consists > 50 genera of bacteria with a cell population 10^{10}-10^{11} CFU/mL in ruminal fluid, 25 genera of ciliate protozoa (10^4-10^6 /mL), five genera of anaerobic fungi (10^3 - 10^5 zoospores /mL) and 10^8-10^9 PFU/mL of bacteriophages (Kamra, 2005). The extent may even be higher as many of the microorganisms residing in the rumen are non-culturable.

Bacteria

The rumen micro-biome is highly variable and change in feed components also result changes in diversity of microbial population. The environment of the rumen is strictly anaerobic which limit growth of invading microorganisms due to high buffering capacity and osmotic pressure. Some of the rumen microbes produce antimicrobial compounds which inhibit the growth of other microbes present in the eco-system (Odenyo *et al*., 1994). For optimum growth of microbes, rumen provide favorable temperature (39°C) and pH (6.0-6.9), respectively. Bacteria responsible for cellulose degradation in ruminal fluid comprised *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* (Sahu *et al*., 2004).

Protozoa

Cilliate protozoa of rumen were classified into two groups i.e. entodiniomorphid protozoa and holotrich protozoa (Hungate, 1986). Holotrich protozoa comprise different enzymes like invertase, polygalacturonase in huge quantities. Interestingly, some of the holotrich protozoa also carry enzymes for degradation of hemicelluloses and cellulose but the amount present are relatively low as compared to that in entodiniomorphid protozoa (Williams and Coleman, 1985). Major groups of ciliate population in cattle and buffalo include *Entodinium*, *Elytroplastron*, *Ostracodinium*, *Isotricha*, and *Dasytricha* (Leng *et al*., 2011). The key role of ciliate protozoa in the rumen is stabilization of pH as rumen is always having low pH (Santra *et al*., 1995). Elimination of cilliate protozoa from rumen resulted in decrease in ammonia nitrogen and methanogenesis (Newbold *et al*., 2015).

Fungi

The presence of obligate and anaerobic fungi play a significant role in the degradation of fibrous diet with help of their various enzymes (Paul *et al*., 2003). It was observed that the diet possess high fiber content stimulate the growth of ruminal fungi as compared to the diet enriched in carbohydrates (Kamra *et al*., 2003). Fungi get access very easily into the lignocellulosic diet due to the presence of different enzymes like cellulases, hemicellulases, proteases, and esterases as compared to the cellulose-degrading bacteria (Fonty and Joblin, 1990).

DOI 10.5455/ijlr.20180208090406

Bacteriophages

Bacteriophages are responsible to infect specific bacteria which are not beneficial at different feeding regimes. Studies on bacteriophage role to infect bacteria indicate that *Siphophages* can infect methanogens *viz, Methanobacter*, *Methanobrevibacter*, and *Methanococcus* species but these have not been isolated from the rumen (McAllister and Newbold, 2008). Hence bacteriophage can be used as an alternative approach to control the increasing population of methanogens in the rumen. However, relative abundance of prophage i.e. 0.1 % in Phylum *Euryarchaeota* have been observed in metagenomic study of phage-bacterial interactions (Miller *et al*., 2012). Another limiting factor is that bacteriophage are host specific in nature. Rumen comprise of a large population of methanogens as compared to bacteriophage, therefore unable to deal with such situation, which is a major drawback in using this strategy to reduce methane (Janssen and Kirs, 2008).

Microbial Fermentation in Rumen

The rumen provides an anaerobic and methanogenic environment, where CO_2 and H_2 are produced from the fermentation of feeds, where they act as electron acceptor and donor (Fig. 1). In this environment, organic matter get degraded by the activity of different groups of anaerobes consisting of primary and secondary fermenters. The primary anaerobic fermenters degrades the monomers which were initially ingested by the animal in form of organic polymers. These monomers are further converted into volatile fatty acids, CO_2 and H_2 by both the primary fermenters and secondary fermenters.

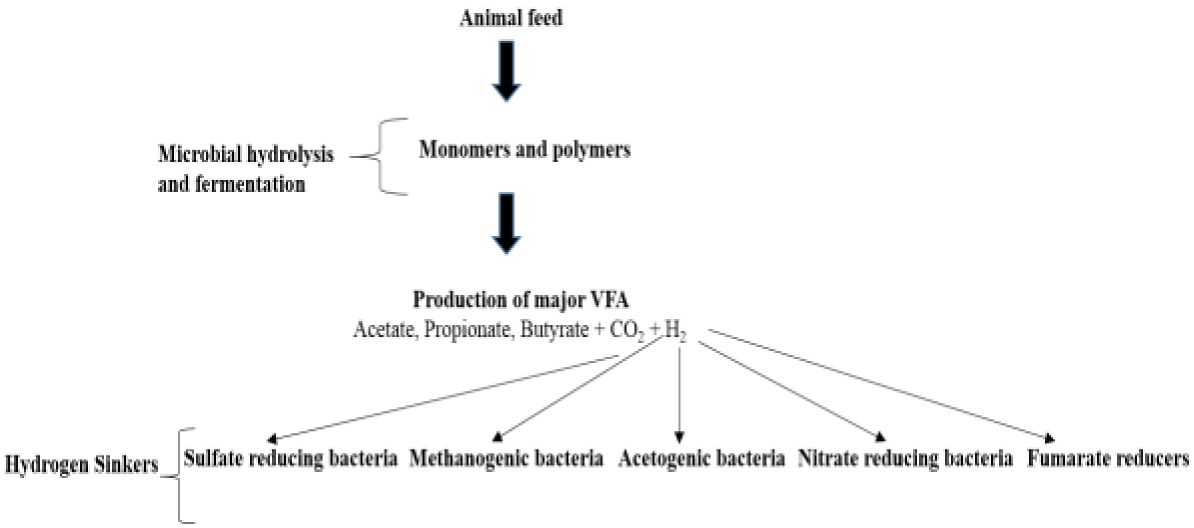

Fig. 1: Schematic microbial fermentation of feed polysaccharides and H_2 reduction pathways in the rumen (Morgavi *et al*., 2010).

The synthesis of methane contributes to the efficiency of the system where it avoids an increase in the partial pressure of H_2 to a stage that might interfere the path for the usual functioning of microbial

DOI 10.5455/ijlr.20180208090406

enzymes that are involved in electron transfer reactions, especially NADH dehydrogenase which results in the accumulation of NADH and eventually reduce rumen fermentation. The capturing of the H_2 formed by one microbial species and used by another is normally referred as interspecies H_2 transfer and this phenomenon includes a syntrophic association between two microorganisms (Wolin *et al*., 1997). Type of feed and occurrence of electron acceptors excluding CO_2 in the rumen have a major effect on the presence and activity of H_2 producers and utilizers. This well-known pathway other than methanogenesis can also consume H_2 and thus potentially compete with methanogens and decrease methanogenesis in the rumen.

Ruminal Methanogenesis

Livestock has become a livelihood of millions of dairy farmers both in developed and developing nations. Ruminants belong to tropical countries such as India mainly rely on highly fibrous plant resources as feed, where these are utilized in rumen followed by their breakdown via multifarious microbial population (Sirohi *et al*., 2012; Balamurugan *et al*., 2018). The rumen customs into a greater portion of the reticulo-rumen, which is an effective chamber to retain ingested feed stuff (Russell and Rychlik, 2001). Large particles of digest are propelled up into the esophagus and mouth during contractions of the reticulum. Ruminants thus provide suitable habitat and a continuous supply of fresh nutrients to fibrolytic rumen microorganisms that convert plant cell wall polysaccharides into fermentation products such as protein, vitamins, volatile fatty acids i.e. acetic, propionic, butyric and short chain organic acids and these are absorbed across the rumen epithelium (Mitsumori and Sun, 2008). The rumen microbes such as, bacteria, fungi, and protozoa along with their fiber degrading enzymes help the fibrous plant materials to get digested, where microbial proteins are responsible to create maximum amino acids reaching to the small intestine (Janssen, 2010). A variety of rumen bacteria produce end products including formate and hydrogen which are subjected to secondary fermentation by other microbial species (Mitsumori and Sun, 2008). The rumen provide strict anaerobic habitat in which substrates are partially oxidized. Thus, removal of hydrogen gas derived from reducing equivalents (NADH), is a critical feature of rumen fermentation, since the build-up of hydrogen with the rumen is thermodynamically unfavorable for plant fiber fermentation (Morgavi *et al*., 2010). Rumen methanogens are capable of using hydrogen, formate, and methanol to produce methane via a pathway coupled to ATP synthesis. Efficient hydrogen removal by methanogens has a profound effect on the functioning of the rumen fermentation system as the build-up of hydrogen inhibits the re-oxidation of co-enzymes involved in redox reactions with in bacterial cells creating a less favorable environment for volatile fatty acid formation (Janssen, 2010). Methanogens produce methane mainly by using two major substrates; CO_2 and methyl group containing compound. The hydrogenotrophic methanogens that use CO_2 as their

DOI 10.5455/ijlr.20180208090406

carbon source and hydrogen as the main electron donor play a prominent role during methanogenesis in the rumen (Fig. 2).

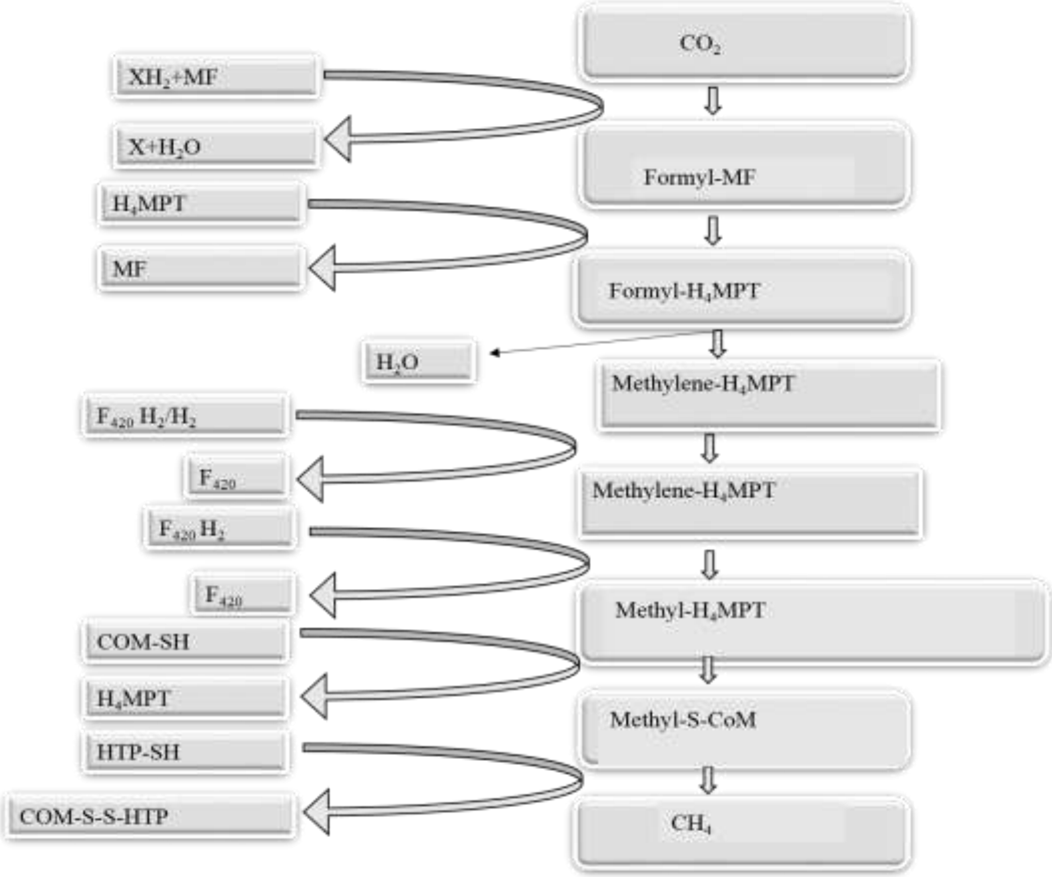

Fig. 2: Enzymatic pathway for the formation of methane in methanogens. Formyl- MF, formylmethanofuran; Formyl-H$_4$MPT, *N*^{5}-formyl-tetra-hydromethanopterin; Methenyl-H$_4$MPT, *N*^{5}, *N*^{10}-methenyl-tetra-hydromethanopterin; Methylene-H$_4$MPT, *N*^{5}, *N*^{10}-methylene-tetra-hydromethanopterin; Methyl-H$_4$MPT, *N*^{5} methyl-tetra-hydromethanopterin; Methyl-S-CoM, methyl coenzyme M; F$_{420}$, coenzyme F$_{420}$; F$_{420}$H$_2$, reduced coenzyme F$_{420}$; H$_4$MPT, methanopterin; H-S-HTP, *N*-7-mercaptohetanoyl-*O*-phospho-L-threonine; X named as unidentified electron donor (Attwood and McSweeney, 2008).

During the oxidation of sugars via the EMP pathway in bacteria, fungi and protozoa, electron carrying cofactors such as NADH must be reoxidized to NAD$^+$ to allow fermentation to carry on. Under the anaerobic conditions of the rumen, where it is impossible to use oxygen as an acceptor of electron transfers to regenerate NAD$^+$, the reduction of CO$_2$ permits recycling of reduced cofactors (Morgavi *et al*., 2010). In hydrogenotrophic methanogenesis, CO$_2$ is initially carried by methanofuran and reduced to formate. The electrons involved in this first step are donated by ferrodoxin reduced with H$_2$. The formyl group from formyl tranferase is transferred to tetrahydromethanopterin (H$_4$MPT), forming formyl-H$_4$MPT in a reaction catalyzed by formyl methanofuran: H$_4$MPT-formyl transferase. The formyl group then positively reduced to methenyl- H$_4$MPT and then to methylene-H$_4$MPT in a reaction catalyzed by 5, 10-methenyl H$_4$MPT

DOI 10.5455/ijlr.20180208090406

cyclohydrolase and methylene-H_4MPT: coenzyme F420 oxidoreductase, respectively. In the next step, a reaction catalyzed by methyl-H_4MPT: HSCoM methyltransferase transfers the methyl group to H_4MPT, forming methyl- H_4MPT. Methyl-CoM is reduced to methane by methyl coenzyme M-reductase in the last phase of methanogenesis. Substrate, methyl-coenzyme M (methyl-SCoM, 2- (methylthioethanesulfonate) and coenzyme B (CoBSH, N-7-mercaptoheptanoylthreonine phosphate) are transformed to methane and a heterodisulfide (CoBS-SCoM) and afterwards reduced to generate the CoB-SH and CoM-SH thiols. The methyl transfer from H_4MPT and CoM as well as reduction of heterodisulfide are both exergonic reactions that are highly encouraging for ATP synthesis (Liu and Whitman, 2008).

Mitigation Strategies for Rumen Methanogenesis

Methane is a potent greenhouse gas that has a high urgency for mitigation because of its harmful global warming effect and because of its combination with tropospheric ozone and carbon, it becomes lethal that could reduce the life expectancy of 3.1 billion people worldwide (USEPA, 2014). Under usual feeding conditions, CH_4 production is an unavoidable consequence of the fermentation of organic matter (OM) in the digestive tract of ruminants. Theoretically, the generation of CH_4 in the rumen can be reduced by following ways-

(1) Promoting a shift in fermentation in such a way that it could generate more reduced volatile fatty acids (VFAs), e.g. propionate, acetate, fumarate, and butyrate;
(2) Enrichment of animal diet with significant amount of rumen degraded protein, so that this fraction of protein could be used directly by rumen microorganisms;
(3) Addition of feed additives that inhibit methanogenesis e.g. bromochloro methane (BCM) or compounds which can favor bioreduction e.g. long-chain unsaturated fatty acids;
(4) Immunization against methanogens;
(5) Defaunation (removal of protozoa population of the rumen), as protozoans are represented as the hydrogen fuel supplier to methanogens;
(6) Stimulation of the growth of bacteriophages that infect and lyse methanogens;
(7) Supplementation of diet with compounds that exactly promote the growth of bacteria and/or archaea that utilize compounds such as nitrates and sulfates and have a higher affinity for H_2 than methanogens;
(8) Direct-fed microbial (DFM), a new approach have been focused recently, which include the use of live, naturally occurring microorganisms.

Any method or technique which are quite stable and effective to lower on-farm CH_4 emissions should be practical and must not have negative effect on the viability of ruminant livestock production. Alteration in diet arrangement to encourage useful modifications in rumen fermentation characteristics remains the most feasible approach to reduce the level of methane production. Dropping CH_4 production per unit product over the lifespan of a ruminant should be seen as the major objective to reduce the GHG emission from livestock sector. This highlights the need for integrated solutions that result in improved digestive

DOI 10.5455/ijlr.20180208090406

efficiency, reproductive performance and animal health to extend the productive lifetime of growing or lactating ruminants. Some of the effective methane abatement strategies are enlisted in Table 1.

Alternate Hydrogen Sinker as Direct Fed Microbial (DFM)

Management of H_2 inside the rumen is the most important aspect to be taken into consideration while developing strategies to control ruminant methane emissions (Joblin, 1999). Unfortunately, till date the focus tends to be primarily on the amounts of methane produced rather than considering the disposal of H_2 that impairs digestion and fermentation in the rumen if it stores.

Table 1: Methane abatement strategies, their mechanism and selection criteria

Formulations	Mechanism	Concerns	References
Addition of starch rich diet	Reduced ruminal pH, Induce greater proportion of propionate versus acetate	Risk of sub-acute ruminal acidosis (SARA)	Hino and Hamano, (1993)
Addition of Lipids	Biohydrogenation occur which inhibit methanogens and protozoa, Induce greater proportion of propionate versus acetate	Long period studies required	Czerkawski *et al.*, 1966; Jordan *et al.*, 2006b; Alexander *et al.*, 2008
• Fatty acids			
• Oils			
• Seeds			
Defaunation	Provide less hydrogen for methanogenesis	Adaptation of microbiota may occur	Abecia *et al.*, 2012; Zhou *et al.*, 2013;
• Chemical- Bromo-chloro-methane (BCM)			
• Feed additives- Lauric acid			
Methanogen Vaccine	Enhance host immune response to methanogens	Vaccine formulations and their targets	Subharat *et al.*, 2016
rGT2 Protein formulated with saponin			
AdditionofIonophores	Inhibits protozoa and gram-positive bacteria	Banned in the European Union	Sauer *et al.*, 1998
Addition of plant secondary metabolites	Reduced hydrogen availability to methanogens	Optimum dosage and long-term studies required	Bodas *et al.*, 2012
Addition of organic Acids	Greater proportion of propionate, versus acetate	Varies with diet	Wood *et al.*, 2009

However, a decline in methane production by 20-50% is known to be achievable without reducing feed intake and this should increase an energetic efficiency of digestion by 2-5%, and possibly more on high roughage diets (Tomkins and Hunter, 2003). Therefore, an understanding of the microorganisms and main metabolic pathways in the rumen is helpful in devising strategies to manage reducing equivalents flow in the rumen for decreased methanogenesis (Jeyanathan *et al.*, 2014). In the rumen, methanogens assume the role of terminal reducers of carbon by using the hydrogen by-products generated from fungal, bacterial and protozoan energy. This process is called "inter species hydrogen transfer" and is important in maintaining the microbial fermentations and plant fiber degradation that occurs in the rumen by oxidizing and reusing reduced cofactors such as NADH. In rumen other than methanogens, the hydrogen evolved during microbial fermentation are utilized by a number of bacteria that act as alternate hydrogen sinker e.g. sulfate reducing bacteria, methylotrophs, propionate forming bacteria, nitrate/nitrite reducing bacteria, homoacetogens, and capnophiles. Among the different strategies studied, one promising option to produce less CH_4 is using alternate hydrogen sinkers as direct fed microbial (DFM) for manipulation of biochemical pathways prevailing in the rumen. Direct fed microbial have been effectively used in rumen to prevent digestive disorders like acidosis and to reduce pathogenic load in young animals in such a way that they increase the overall productivity of ruminant system (Krehbiel *et al*., 2003; Adams *et al*., 2008; McAllister *et al*., 2011; Lettat *et al*., 2012b; Kalebich and Cardoso, 2018). They are recognized as the substitute to the use of antibiotics and chemical elements that may bring a risk of antibiotic resistance and residues in animal products. Though, to date there is little evidence to suggest the efficacy of DFM to control the production of CH_4 in ruminants. Inside the rumen dominating community of methanogens consumes H_2 to reduce CO_2 to CH_4. Some rumen methanogens can consume formate or methyl group containing compounds such as methanol and methylamine (Janssen and Kirs, 2008). Carbon dioxide constitutes up to 65% of total gas in the rumen and it is not a limiting substrate of rumen methanogenesis (Ellis *et al.*, 1991). Therefore, H_2 is a key compound for controlling CH_4 production. The major biochemical pathways explored to decrease CH_4 emissions from ruminants by using DFM are the redirection of H_2 away from methanogenesis and decreased the production of H_2 during feed fermentation are discussed below.

Propionate-Forming Bacteria

Type of diet given to animal affects the key volatile fatty acids (acetic, propionic, and butyric acid) produced in the rumen. The concentrate-based diet offered to ruminants results high propionate level as compared to high forage diet, which yields more acetate. Propionate formation consumes reducing equivalents ($FADH_2$, NADH), where pyruvate is reduced to propionate, therefore it is considered as H_2 -utilization pathway, while in H_2 formation, protons (H^+) are reduced to H_2 (Baldwin *et al.*, 1963). As H_2 is the main precursor for CH_4 production, increase in propionate formation was stoichiometrically associated with a decrease in CH_4 production, as propionate biochemical pathway is majorly followed by bacterial DFM in ruminant production (Seo *et al*., 2010). The *Propionibacterium* species had been used as DFM in rumen to increase the animal productivity (Ghorbani *et al*., 2002; Adams *et al*., 2008). Further, *Megasphaera elsdenii* and *Propionibacterium* species were used to prevent rumen acidosis in concentrate-fed animals as well as *Lactobacillus* species have been used to reduce pathogenic counts in young animals. However, CH_4

DOI 10.5455/ijlr.20180208090406

production status was not indicated in any of these studies (Aikman *et al*., 2011; Lettat *et al*., 2012b; McAllister *et al*., 2011). The decrease in CH_4 emission was recently observed in lactating cows receiving a mixture of *Propionibacterium jensenii* - *Lactobacillus* species. The DFM approach showed potential to mitigate rumen CH_4 emission (Lettat *et al*., 2012a).

Nitrate/Nitrite-Reducing Bacteria

The metabolic pathway representing nitrate metabolism in the rumen is expected to be dissimilatory nitrate reduction in which nitrate is reduced to ammonia in two-step processes: nitrate to nitrite and nitrite to ammonia. The effective use of nitrate to reduce the rumen methanogenesis has been restricted due to toxicity of the nitrite. Rumen microbes quickly reduce the nitrate into nitrite, but the rate of reduction of nitrite into ammonia is slower, which can cause nitrite accumulation in the rumen (Iwamoto *et al*., 1999). Having the capability to reduce nitrate or nitrite, *Wolinella succinogenes* and *Selenomonas ruminantium* are required to be present at a concentration of 10^6 cells/ml of rumen fluid (Asanuma *et al*., 2002; Yoshii *et al*., 2003). The methanogens are prevailing in the rumen at the concentration of about 10^9 cells/ml, therefore to compete with them it may be useful to rise the activity of nitrate/ nitrite reducing bacteria in the rumen (Jeyanathan *et al*., 2011). Bacteria that have the ability to reduce nitrate or/and nitrite are more active when nitrate is included in the diet. *In-vitro* trials suggested that incorporation of nitrate increased the number of nitrate reducing bacteria such as *Wolinella succinogenes* and *Veillonella parvula*, but could not satisfactorily reduced methane production (Iwamoto *et al*., 2002). Therefore, providing nitrate/nitrite-reducing bacteria as DFM along with nitrate may improve the nitrate reduction process thereby avoid nitrite toxicity. Nitrate as feed additive can decrease rumen methanogenesis in different ruminant species and production conditions (Sophea *et al*., 2010; Van Zijderveld *et al*., 2011; Hulshof *et al*., 2012; Olijhoek *et al*., 2016). In rumen, during microbial protein synthesis, nitrate could replace urea as a nitrogen source in low nitrogen diets. In an *in-vivo* study replacement of 1.5% of urea by 3% calcium nitrate reduced CH_4 emission (Li *et al*., 2012). However, the possible negative impact of long-term supplementation of nitrate on animal health has to be explored extensively.

Homoacetogens

Homoacetogens have been described in diverse environments including rumen, with the ability to produce acetate via heterotrophic and autotrophic growth. They propagate heterotrophically by utilizing sugars and autotrophically by utilizing formate, CO, and H_2/CO_2. Promotion of autotrophic growth of homoacetogens is thought to be a competitive pathway to methanogenesis as the same substrates are used. Acetate is a valuable nutrient for the host and for other microbes present inside the rumen. The total count of homoacetogens observed from rumen is inconstant (undetectable to 10^7 CFU/mL of rumen liquor) reliant on feed, age of the animal, and time of sampling (Fonty *et al*., 2007). Bearing in mind for preparation of

DOI 10.5455/ijlr.20180208090406

acetogens as DFM, has some limitations such as tendency of methanogens to sequester H_2 from rumen fermentation is more as compared to acetogens and it was found that energy yield from methanogenesis was greater than that of acetogenesis (Thauer *et al*., 1977). Studies have been reported where homoacetogens sustained efficient metabolisim inside the rumen and inhibited methanogenesis in absence of methanogens, to stimulate acetogenesis (Nollet *et al*., 1997; Fonty *et al*., 2007). However, the thermodynamic stability for acetogens in capturing the H_2 from fermentation (28-46%) were comparatively less than methanogens (>90 %) which may affect the whole fermentation system in the rumen (Gagen *et al*., 2012). Latterly, Acetogenic bacterium was isolated from rumen, having ability to grow on low threshold concentrations of hydrogen (Boccazzi and Patterson, 2011). Such acetogens could participate with methanogens inside the rumen. Different studies have been accompanied in different environments (gut microbial ecosystem of humans, rodents, macropods, and wood-digesting termites) where homoacetogenesis demonstrated their active role in reducing methane emissions (Breznak and Switzer, 1986). The homoacetogen population observed in the forestomach of *Tammar Wallaby* (*Macropus eugenii*) was different when compared to the population found in ruminants, which could justified the reason for lowering of methane production (Gagen *et al*., 2010).

Capnophiles

The capnophiles require high levels of CO_2 for their growth. The rumen is an anoxic chamber, where CO_2 is one of its major gas. The occurrence of capnophiles in the rumen is therefore predictable but their use as scavengers of CO_2 to mitigate methanogenesis remained doubtful as this gas is not a regulating factor for methanogenesis. Hypothesis has been given regarding two types of CO_2 requirement among rumen bacteria: (a) Biosynthesis type, in which CO_2 is required for cell growth e.g. *Streptococcus bovis*; (b) Bacteria that are forming succinate in addition to biosynthesis e.g. *Succinivibrio dextrinosolvens*, *Mannheimia succiniciproducens*, and *Actinobacillus succinogenes* (Dehority, 1971). During succinate production, CO_2 is attached to the three-carbon phosphoenol pyruvate, an end product of glycolysis, to generate the four-carbon compound, oxaloacetate. The Oxaloacetate accepts two pair of electrons, when reduced into succinate. As such, both CO_2 and H_2 are used during this succinate formation and may have an impact on rumen methanogenesis. During the propionate production, succinate is also an important intermediate product. The *Tammar Wallaby* yields only one-fifth of the amount of CH_4 produced by ruminants per unit of digestible energy intake (Kempton *et al*., 1976). A physiological difference such as shorter retention time of feed in the foregut partially describes this. The occurrence of a novel group of acetogenic bacteria may be the reason for the reduction in CH_4 emission and the existence of capnophiles might be a potential contributor to this opinion (Gagen *et al*., 2010). The 16S rRNA clone library of capnophiles was analyzed from foregut samples of *Tammar Wallaby* and allocated to a group within the

DOI 10.5455/ijlr.20180208090406

family *Succinivibrionaceae*. A member from this group was isolated and its genome sequence was analyzed, which proved that it was a capnophile, dependent on CO_2 to support its metabolism by means of succinate biosynthesis (Pope *et al.,* 2011). However, clear recognition of their metabolic pathway and analogous environmental conditions are needed to assess their potential as rumen CH_4 mitigants.

Sulfate-Reducing Bacteria (SRB)

Sulfate reducing bacteria are obligate anaerobes which are present in diverse anaerobic enviornments like estuarine, saltmarsh and acidic sediments, landfills, freshwater lakes, sludge, and sewage drains (Andrea *et al.*, 2015; Brand *et al.*, 2015; Colin *et al.*, 2017; Cui *et al.*, 2017; Kashyap and Grover, 2017; Kharrat *et al.*, 2017; Pimenov *et al.*, 2014; Xia *et al.*, 2014; Yang *et al.*, 2017). Competitive relationships between methanogens and sulfate-reducing bacteria have been also described in the rumen (Paul *et al.*, 2011). In anaerobic environments, sulfate reducing bacteria competes with methanogens for their conjoint substrates (formate, H_2 and acetate). As the energy requirement of sulfate reduction was slightly more favorable ($\Delta G = -152$ kJ/mol) than methanogenesis ($\Delta G = -131$ kJ/mol), encouraging competition between these two groups for common substrate i.e. hydrogen and thus, can decreases methanogenesis theoretically according to this process (Gibson *et al*., 1993). The synergestic association between methanogens and SRB is another illustration of interspecies H_2 transfer. In sulfate-depleted environments syntrophic association of SRB and methanogens to generate and utilize H_2 have been observed (Muyzer and Stams, 2008). The population of SRB in the rumen is small (10^5 to 10^6 CFU/mL of rumen liquor) and represents mainly the genus *Desulfovibrio* and *Desulfotomaculum* (Campbell and Postgate, 1965; Huisingh *et al*., 1974). Recently, a sulfate-reducing bacteria belonging to genus *Fusobacterium* have been isolated from buffalo suggesting that there might be other not-yet-cultured SRB in the rumen (Paul *et al*., 2011). Introduction of sulfate into the rumen can enhance the capability of SRB. As such, sulfate reduction might be facilitated by the introduction of SRB when sulfate was used as an additive to reduce methanogenesis (Cummings *et al*., 1995). Only limited studies have been done on the effect of sulfate supplementation in rumen methanogenesis (Morvan *et al*., 1996; Van Zijderveld *et al*., 2010). The toxic end product i.e. H_2S resulting from the sulfate reduction might be the major reason for the lack of studies on this option. Using sulfate alone as an additive in animal diet cannot be an alternative for reduction of ruminal methanogenesis as can cause sulfide toxicity. Yet, SRB are flexible microorganisms and published information indicates that they might possess some characteristics favoring rumen CH_4 mitigation. For instance, a decrease in CH_4 emission was observed in an *in-vitro* study using the newly identified SRB, *Fusobacterium* species as a DFM with a high sulfate diet. After 3 days of incubation period methane production reduced from 2.66 to 1.64 mmol/g digested dry matter (DM) without accumulation of H_2S (Paul *et al*., 2011). No accumulation of sulfide was reported in the previous study which might be due to its rapid consumption by other microbes

DOI 10.5455/ijlr.20180208090406

such as cellulolytic bacteria for production of sulfur-containing amino acids or *Fusobacterium* species itself might be able to oxidize sulfide into sulfate as defined in the termite gut (Bryant, 1973; Droge *et al*., 2005).

Other Dietary Factors Affecting Methane Production

India possess the largest livestock production in the world i.e. of 520.6 million and ranked one in biggest number of cattle production that shares 16.1 % of world cattle population, buffaloes (57.9%), whereas second largest in number of goats (16.7%) and third largest in number of sheep (5.7%) (FAO, 2007). However, it undergoes a great challenge to provide feed both in a green and dry form required for increasing their number. There are several factors, which influence the methane production by the ruminants such as pH, volatile fatty acids, diet, levels and frequency of feeding, animal species, and environmental factors. The various factors responsible to affect the frequency of rumen to produce methane are mentioned below.

Dietary Components

Diet has a significant role on methanogens and methane production as both the quality and quantity of feed changes the efficiency of fermentation process. When a high concentration of carbohydrates diet are fed, a high quantity of methane emits, but highly digestible diet released low fraction of methane concentration (Johnson and Johnson, 1995). Diet comprising 30-40% concentrate exhibited 6-7% of gross energy (GE) intake whereas, diet comprising 80-90% concentrate exhibit only 2-3% of gross energy intake (Martin *et al*., 2010; Nampoothiri *et al.,* 2018). It clearly shows that, increasing in concentration of concentrate in animal feed reduces the methane emissions. Forages carries complex carbohydrate components which comprises structural carbohydrates (digested by microorganisms) i.e. cellulose and hemicellulose and non-structural carbohydrates i.e. starch and sugars (digested by the enzymes). To minimize the methane emissions from rumen, energy-rich structural carbohydrates must be replaced with non-structural carbohydrates. This will results in upturn in feed intake, greater rates of ruminal fermentation and accelerated feed turnover. With help of starch-fermenting microbes, shift of VFA production will occur from acetate towards propionate metabolic pathway and due to decline in relative quantity of ruminal hydrogen sources it reduce the CH_4 production (Kumar *et al*., 2013).

Plant Secondary Metabolites

Condensed tannins and saponins are significant ruminant feed additives due to its natural origin and can be used specifically for methane mitigation approach, as methane reduction capability has been recognized in both sources (Wanapat *et al*., 2013; Morales *et al.,* 2018). There are two type of approaches that tannins can perform against methanogenesis i.e. direct and indirect influence on rumen methanogen and hydrogen production. It was also observed that after utilizing condensed tannin, not only it reduced methane level but also assisted amino acids to get absorbed in small intestine (Chanthakhoun *et al*., 2011). Saponins are

DOI 10.5455/ijlr.20180208090406

basically natural occurring glycosides which can be found in many plants. Efficiency of feed utilization depends upon the quantity of protozoan population present in the rumen as their decreased quantity may improve the flow of microbial protein. In the interest of effective results, attention has been increasing in use of saponin-containing plants as a potential approach for suppressing or eliminating ruminal protozoa (Wanapat *et al.*, 2013).

Organic Acids

To enhance nutritional requirement and their effect on methane reduction, use of organic acids have been investigated in animal feed (Morgavi *et al.*, 2010; Kara *et al.*, 2018). However carrying similar conditions varied and insufficient data has been recognized from *in-vivo* and *in-vitro* trial as no variations in methane reduction were observed in beef heifers when compared to beef cattle which showed 16% decreases in methane emission with supplementation of organic acids (Beauchemin *et al*., 2008; Foley *et al*., 2009). Unexpectedly, giant shift in CH_4 reduction has been observed when encapsulated fumarate was used in the diet of lambs without any negative effect on animal growth. However, further research are needed to use such a product as additive (Wallace *et al*., 2006). It has been advised that the high malate content in early growth stage of fresh forages, especially lucerne, could lead to significant alterations in rumen microbial profile (Martin, 1998; Martin *et al*., 2010)

Lipids

Dietary fat could be a favorable nutritional substitute to lower ruminal methanogenesis without disturbing the ruminal parameters (Wanapat *et al*., 2013; Duthie *et al.*, 2018). Lipid supplementation can lower the methane production either by reducing fiber digestion, lowering the dry matter intake, inhibiting the population of rumen protozoa via biohydrogenation, use of oils can provide a practical methodology to reducing methane in circumstances where animals can be given day-to-day feed supplements. However, surplus oil can be harmful to fiber digestion and animal production. Oils carrying medium chain fatty acids can act directly on methanogens and ciliate protozoans. Thus, using the mixture of coconut oil and garlic powder showed tendency to reduce methane and improving VFA profile (Kongmun *et al.*, 2010). Many aspects need to be measured such as the form and type of oils well as its cost which has increased intensely in recent years due to their increased demand.

Conclusion

Looking at the evidences in a broad manner, profiling of rumen methanogens appears to be a significant tool for confirming the sustainability of ruminant-based agriculture production systems. Effective methane mitigation policies should be developed and adopted in such a way that systematic understanding of the microbial interaction of rumen methanogens can be understood. Approaches based on DNA analysis

DOI 10.5455/ijlr.20180208090406

provide assistance in categorizing and exploring that how rumen microbes can influence rumen fermentation profile without affecting the animal's production potential. Connecting the microbial profiles of animals to recognize the microbial shifts in rumen can be significant initiative among methane mitigation policies. Some of the dietary approaches used in different studies have made alterations in rumen microbial communities as exposed by profiling assays. The assessment of the rumen micro flora can be directly or indirectly interconnected to the methane mitigation policies for ruminants. In addition, advancement in genetic approaches and management practices for increasing ruminant productivity and abating methane emissions, in combination with other strategies, would provide new insights in reducing methane emission from ruminant system globally.

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