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Review

An overview of the role of rumen methanogens in methane emission and its reduction strategies

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Methane is the most effective global warming greenhouse gas and methanogens are the key microbiota in methane emission. Emerging research focuses on ruminant methanogens due to their emission of methane globally; of which around 20% is from livestock. Enhanced techniques revealed the methangens diversity, adaptation in rumen, methanogenesis and their reduction strategies. Based on diet, geographical location, type of ruminant species, methanogen population shows vast diversity. Many strategies also interfere to reduce the methane emission worldwide such as dietary composition, vaccines, plant secondary metabolites, analogs and fungal secondary metabolites. This review gives a concise knowledge of methanogens' interference in methane emission and research and development techniques used for reducing methane emission.

Key words: Methane, plant secondary metabolites, ruminants, ionophores, lovastatin.

INTRODUCTION

Methane is a more potent greenhouse gas, having 21 folds greater global warming potential than carbon dioxide (Sirohi et al., 2013). Livestock are major source of methane emission contributing about 81 to 92 MT methane per annum globally (IPCC, 2007; Patra, 2012a). India has livestock wealth of 272.1 million cattle, 159.8 million buffaloes, 71.6 million sheep, 140.6 million goats and 13.1 million (GOI, 2012, Sridhar et al., 2014) other ruminants, which produce large amounts of CH_4 as a part of their normal digestive process. This constitutes about 20% of the world's ruminant population. The rumen of the dairy cow contains a rich and diverse population of microbes that produce significant quantities of methane during feed digestion; it contributes to greenhouse gas emissions (GHG). Methane emissions represent between 30 and 50% of the total GHG emitted from the livestock sector; with enteric methane from ruminant production systems representing by far the most numerically important source. It is responsible for approximately 80% of the methane emissions from the sector (Gill et al., 2010). Strategies for reducing methane provide opportunities to improve livestock productivity and reduce greenhouse gas emission. In order to develop the strategies, vast knowledge on methanogens' diversity and genomic capability is required. Enhanced research and technology on rumen metabolism revealed the rumen methanogen

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diversity, methane emission and mitigation. Rumen contains a microbial population of 10^{11} bacterial cells, 10^3 fungal cells and 10^6 protozoa cells. Methanogen cells are roughly present in 1 ml of rumen fluid (Sunil et al., 2012), but only 10% of the microbial population was identified (Pers-Kamczyc et al., 2011). Methanogen population varies based on the geological locations. Like in India, *Methanomicrobium* phylotype is the most dominant methanogens in buffaloes, whereas *Methanobrevibacter* phylotype is the predominant in Australia (Chaudhary and Sirohi, 2009).

RUMEN MICROBIOTA

Ruminants are mainly fed by lignocellulosic based bi-products which are rich in complex carbohydrates; hence the active microbial populations present are derivatives of this feed. The rumen epithelial or epimural bacterial community performs a vast diversity of functions necessary for host health including the hydrolysis of urea, scavenging of oxygen and the recycling of epithelial tissues (Cheng et al., 1979; Dinsdale et al., 1980; McCowan et al., 1978; Petri et al., 2013). *Fibrobacter succinogenes* (Hungate et al., 1950; Flint et al., 1990), *Ruminococcus flavefaciens* (Dehority et al., 1986), *Ruminococcus albus* (Dehority, 1967; Stewart, 1979; Bryant, 1986), *Clostridium cellobioparum* (Hungate, 1944), *Clostridium longisporum, Clostridium lochheadii* (Hungate, 1957), *Eubacterium cellulosolvens* (*Cillobacterium cellulosolvens*) (Bryant, 1958; Van Gylswyk, 1970) were the most active cellulose degrading microbes; *Butyrivibrio fibrisolvens* (Bryant, 1953; Bryant, 1956; Cotta, 1992), *Prevotella ruminicola* (Cotta, 1992), *Eubacterium xylanophilum,* and *Eubacterium uniformis* (Van Gylswyk, 1985) greatly participated in hemicelluloses degradation, while *Streptococcus bovis* (Latham et al., 1986), *Ruminobacter amylophilus* (*Bacteroides amylophilu*) (Hamlin and Hungate, 1956) and *Prevetella ruminicola* (*Bacteroides ruminicola*) (Cotta, 1992) were dominating group of starch degrading microbes.

METHANOGEN POPULATION IN RUMEN

Maximum rumen has anaerobic microbiota; hence it is very difficult to maintain them. Methanogens are very important for the functioning of rumen and to control hydrogen pressure maintenance. Archea can be found in the limb rumen 30 h after birth (Morvan et al., 1994). So far 113 species of methanogens are recognized in the ecosystem but only few species of methanogens are found in the rumen (Janssen and Kirs, 2008). *Methanobrevibacter spp*. were initially colonized methanogens in the limb rumen and less population of *Methanobacterium spp*. while seven weeks after birth, lambs contained only *Methanobrevibacter* spp. (Skillman et al., 2004); but, *Methanobrevibacter* disappeared 12^{th} to 19^{th} day after birth (Zhu et al., 2007). *Methanobacterium formicicum, Methanobrevibacter ruminantium*, *Methanosaricina barkeri*, *Methanosaricina mazei* and *Methanomicrobium mobile* are the predominant methanogens (Stewart et al., 1997; St-Pierre and Wright, 2012); hence *M. ruminantium* (Leahy et al., 2010), of the order Methanobacterials is predominant in the rumen (Jarvis et al., 2000).

METHANOGENESIS IN RUMEN

Feed components like complex carbohydrates, proteins and other organic substances are degraded to monomer components by the fibrolytic or primary anaerobes. These monomers are further converted into volatile fatty acids, carbon dioxide and hydrogen. Methanogens utilize H_2 and CO_2 as a substrate produced from the fermentation of feeds; these are the main electron acceptor and donor and produce methane. However, along with methanogens, other microbes also participate in methane emission either by involving in hydrogen metabolism or by affecting the methanogen population. The synthesis of methane contributes to the efficiency of the system in that it maintains the partial pressure of H_2 to levels that might inhibit the normal functioning of microbial enzymes involved in electron transfer reactions, particularly NADH dehydrogenase. This results in NADH accumulation, and ultimately reduces rumen fermentation (Morgavi., 2010) (Figure 1). The capturing of the H_2 produced by fermentative species to hydrogen utilizing species is referred to as interspecies H_2 transfer (Wolin et al., 1997). Attachment of methanogens to the external pellicle of protozoa has been reported by Krumholz et al. (Krumholz, 1983; Stumm et al., 1982). Some *in vitro* and *in vivo* studies demonstrated that the lack of the protozoal population in the rumen ecosystem has a significant effect on both the population of methanogens and the level of methane production (Cieslak et al., 2009a; Morgavi et al., 2012). The research also showed that sheep maintained without protozoa for more than 2 years have reduced methanogenesis in comparison with sheep kept without protozoa for only 2 months (Morgavi et al., 2012). Formate, which is formed in the production of acetate, can also be used as a substrate for methanogenesis, although it is often converted quickly to hydrogen and carbon dioxide instead (Hungate, 1970; Archer and Harris, 1986). By removing hydrogen from the ruminal environment as a terminal step of carbohydrate fermentation, methanogens allow the microorganisms involved in fermentation to function properly and support the complete oxidation of substrates (Sharp, 1998). The fermentation of carbohydrates results in the production of hydrogen and if this end product is not removed, it can inhibit metabolism of rumen microorganisms (Sharp, 1998).

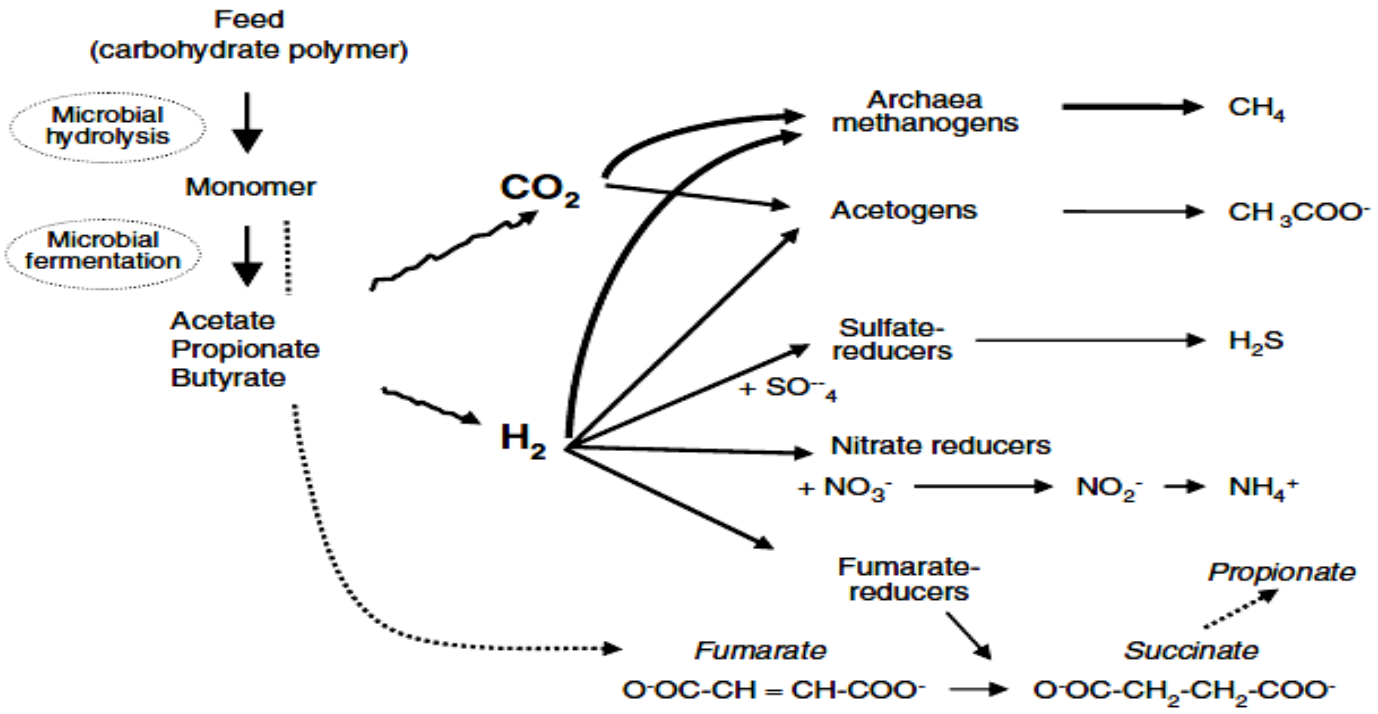

Figure 1. Methane production/ synthesis in the ruminants (Adapted from Morgavi et al., 2010).

STRATEGIES INVOLVED IN METHANE REDUCTION

Methane mitigation depends on the relationship methanogens have with other organisms in the rumen. Mitigation is caused either by attacking the methanogens directly or indirectly by the substrate available for methanogenesis (Hook et al., 2010). Some of the strategies to reduce methane production are given in Table 1.

Dietary composition impact on methane emission

The type of diet composition and the carbohydrate rate in diet are very important in methane synthesis. Diet can alter the pH of the rumen by rumen microbial composition (Johnson and. Johnson, 1995). Corn silage based diet increased the propionate concentration but decreased ruminal pH, CH_4, L/kg of dry matter intake, and concentrations of acetate and butyrate (Benchaar, 2013). The compositional basis of a cow's diet has been known to have effects on methane expulsion, with corn and soybean meal concentrate diets generally resulting in less gas production than forage diets. Concentrate and forage diets also affect ruminal pH differently, which may contribute to the activity of the enteric methanogens. The levels of methane expulsion from forage-fed and concentrate-fed cows in relation to ruminal pH showed that cows fed with all-forage diet maintain pH of more or less constant around 6.7 to 6.9; meanwhile concentrate-fed cows' ruminal pH decreased dramatically to as low as 5.45 immediately after feeding. Mixed ruminal bacteria from the forage-fed cow converted carbon dioxide and hydrogen to methane, while no methane was produced by the concentrate-fed cow (Kessel and Russell., 1996). Yan et al. (2010) studied the relationship between methane emission, animal production and energy utilization in lactating dairy cows fed with diet containing grass silage. They concluded that dairy cows capable of high milk yielding and energy utilization efficiency are effective for reducing methane emission from lactating cows.

Ionophores as methane mitigators

Ionophores are highly lipophilic ion carriers. They pass through the permeable peptidoglycan layer of gram-positive bacteria and penetrate into the lipid membrane. Therein, they destroy ion gradients at the expense of ATP, ultimately resulting in the depletion of energy reserves, impaired cell division, and the likely death of the microorganism (Tedeschi et al., 2003). Microbiota which produces hydrogen and formate is gram negative and sensitive to ionophore, thereby preventing the formation of necessary substrates for methanogens. This leads to an effective dramatic reduction in methanogen population in the rumen. Many ionophores will not inhibit the propionate-producing bacteria, resulting in an increased proportion of this volatile fatty acid (Callaway et al., 2003). Propionate is efficiently utilized by ruminants,

Table 1. Different types of Nutritional substrates used for reduction strategies of methane

Substrate	Quantity	Method applied	Incubation period	Digestibility	Methane	Reference
Nitrate supplement	3% in diet	Open-circuit respiration chambers	6 weeks	NA	35.4%	Hegarty et al., 2012
50 : 50 forage : concentrate ratio diet *ad libitum*	375 g/day	Sulphur hexafluoride tracer gas technique	93 days	No significant change	39%	Jordan et al., 2006
50 : 50 forage : concentrate ratio	250 g/day coconut oil	Sulphur hexafluoride tracer gas technique	105 days	No significant change	18%	Jordan et al., 2006b
10 : 90 forage : concentrate ratio diet	10% soya oil / 12% whole soya bean	SF_6 tracer technique	103 days	Reduced	40%/ and 25%	Jordan et al., 2006
60 : 40 forage : concentrate ratio diet	3% Soya oil on DM bases	Open-circuit respiratory chambers	60 days	Reduced	14%	Mao et al., 2010
45 : 55 forage : concentrate ratio diet	sunflower seeds (SFS), linseed oil (LO) or rapeseed (RS) oilseeds (3.3% of DM)	Respiration chambers	112 days	Increased	18%	Beauchemin et al., 2008
maize silage, grass hay and concentrate,	linseed oil (6.6% of DM	Respiration chambers	63 days	Increased	10% reduction	MacHmüller et al., 2000
maize silage, grass hay and concentrate	sunflower seed (6.0% of DM)	Respiration chambers	63 days	Reduced	27%	MacHmüller et al., 2000
Alfalfahay (4.2 kg/DM/cow) and rye grass silage (6.6 kg/DM/cow)	48% cottonseed (CS)	SF_6 tracer technique	84days	NA	23%	Grainger et al., 2010
grass silage, grass silage plus concentrate (GS+C), maize silage (MS) with monensin	120 mg feed DM/syringe.	Hohenheim Gas Test	24 h	NA	30%, 17%, and 18%	Gerald Wischer et al., 2012

Table 1. Contd.

Substrate	Quantity	Method applied	Incubation period	Digestibility	Methane	Reference
defaunation with fumaric acid defaunation with fumaric acid, Barely, grain39%, Berseem hay 40%, Wheat straw 20.14%, Vitamin and mineral premix 0.30%	200 mg	*In vitro*	24 h	NA	43.07%	Abdl-Rahman, 2010
Hay: concentrate (1:1)	10.2 and 20.4 g/kg of *Knautia arvensis* extract	*In vitro*	24 h	No significant effect on TVFA, A/P and methanogens	5.8 and 7.1%	Makkar and Becker, 2008b
Barley silage: concentrate (51:49)	15, 30, 45 g/kg DM of *Quillaja saponaria*	Serum bottle	24 h	IVDMD and A/P decreased; TVFA unaffected	5.33% 4.43%	Holtshausen et al., 2009
Hay: concentrate (1:1)	14.8 and 30.4 g/kg DM of *Trigonella foenum-graecum*	*In vitro*	24 h	No significant effect on TVFA, A/P and methanogens	2.21 and 2.21%	Makkar and Becker, 2008b
Lucerne hay: concentrate (1:1)	0.5 g/L of *Yucca schidigera*	RUSITEC	22 days	No significant effect	12.8%	Wang et al., 1998
Grass silage and hay: barley (77:23)	0.001 and 0.02, and 0.1 g/kg DM of effective sarsaponin of *Medicago sativa*	RUSITEC	10 days	IVDMD, TVFA, A/P, total bacteria unaffected	-5.16, 3.87 and 1.29%	Sliwinski and Machmuller, 2002
Hay: concentrate (32:68)	1.65 g/l or 174 g/kg Substrate of *Sesbania sesban*	*In vitro*	24 h	50.5% reduction in protozoa	11.9%	Makkar and Becker, 2008b
Wheat straw: Concentrate (1:1)	0.2 g/kg DM *of Acacia concinna*	*In vitro*	24 h	TVFA & IVDMD unaffected, A/P and protozoa numbers decreased	3.8 and 18.6%	Patr, and Agarwal, 2006
Wheat flour: wheat straw (4:1)	0.2 g/kg DM of *Sapindus mukorossi*	*In vitro*	24 h	IVDMD, A/P and protozoa decreased (70-90%), TVFA unaffected	22- 96%	Agarwal and Patra, 2006

Table 1. Contd.

Substrate	Quantity	Method applied	Incubation period	Digestibility	Methane	Reference
Corn starch	1.2–3.2 g/l or 180–480 g/kg substrate of *Medicago sativa*	Serum bottle	24 h	TVFA increased, A/P decreased, protozoal numbers decreased	36.0–64.1%	Lila et al., 2003
Corn grain/Chinese wild rye (50:50)	0.30, 60, 80 g/l cultural media of *Tribulus terrestris*	*In vitro*	24 h	TVFA, acetate and Ammonia decreased, propionate and A/P increased, protozoa decreased	23.43%	Feng et al., 2012
Lucerne hay: concentrate (60:40)	5 g/kg DM of *Camellia sinensis*	*In vivo*	21 days	No significant effect	8.71%	Yuan et al., 2007
Wild rye: concentrate (60:40)	4.1 g/kg DM	*In vivo*	60 days	TVFA increased; A/P unaffected; protozoal and methanogen decreased	27.2%	Mao and Liu, 2010
Hay: concentrate	0, 400, 600, 800 mg/kg DM of *Ilex kudingcha*	*In vivo*	10 days	No significant effect	ND	Zhou et al., 2012
Hay : concentrate (1:1)	10.2, 20.4 g/kg DM of *Medicago sativa*	*In vivo*	14 days	TVFA,A/P,methanogens unaffected	5.8- 7.1%	Makkar and Becker, 2008a
Corn: corn silage	0.25-1.5% DM of *Quillaja saponaria,*	*In vivo*	22 days	ND	No effect	Li and Powers, 2012
Ryegrass hay: concentrate (3:2)	13.5 g/kg of diet or 16.1 g/day of *Q. saponaria*	*In vivo*	18 days	TVFA decreased, digestibility, A/P, protozoa not affected	21.7%	Pen et al., 2007
Barley silage: concentrate (51:49)	10 g/kg of DM	*In vivo*	28 days	No significant effect	7%	Holtshausen et al., 2009
Forage: concentrate (49.2–56:21)	5 g/kg body wt of *Sapindus saponaria*	*In vivo*	21 day	Digestibility, A/P and protozoa decreased; TVFAandmethanogens increased	7.8%	Hess et al., 2004
3 kg concentrate mixture and chopped maize fodder (*Zea mays*)	fumaric acid @ 2% of DMI	*In vivo*	21 days	No change in digestibility	20.7%	Mohini et al., 2008
Wheat straw based diet.	2 ml of neem leaf extract in 30ml of medium	*In vitro*	24 h		40%	Malaiyappan et al., 2012

Table 1. Contd.

Substrate	Quantity	Method applied	Incubation period	Digestibility	Methane	Reference
Wheat straw containing diets	40R:60C	*In vitro*	24 h	Propionic acid levels increased, no significant changes in digestibility	22.60%	Sirohi et al., 2011
Myristica fragrans fruit powder	roughage 50% and concentrate 50%	*In vitro*	24 h	decreased	48%	Sirohi et al., 2012

and thus may enable increased derivation of energy from feed. The efficacy of ionophores in ruminant diets is examined (Guan et al., 2006).

Methane analogs as inhibitors

Methanogens can be inhibited by the addition of methane analogues such as commonly 2-bromoethanesulphonate (BES), a structural analog to coenzyme M, 3-bromopropanesulfonate (BPS). It mimics methyl-coenzyme M lumazine, and ethyl 2-butynoate. Some inhibitors, however, are more effective against certain species of methanogens than others, and some only offer short-term protection (Ungerfeld et al., 2004). *M. ruminantium* was the most sensitive to the effects of BES, *M. ruminantium* was most sensitive to ethyl 2-butynoate, *Mm. mobile* was somewhat sensitive, and *M. mazei* was unaffected. Lumazine is a structural analogue of some important co-factors in methanogenesis, but slight methanogen recovery was observed six days post-feeding, jeopardizing the chance of significant long-term benefits. Cell envelope differences may be related to the differences observed in toxicity of the methanogens to ethyl 2-butynoate. The presence of an S-layer in *M. mazei* and *M. mobile* (absent in *M. ruminantium*) may have conferred some resistance, which is a problem for the practical use of this inhibitor *in vivo* (Ungerfeld et al., 2003). Like BES, selective resistance to ethyl 2-butynoate among different species may favor these species over long-term, rendering obsolete any initial decreases in enteric methane production. Dihydrogen (H_2) is the key element that maintains methane production in the rumen. Among H_2 producers, protozoa also play prominent role. This is strengthened by their close physical association with methanogens, which favors H_2 transfer from one to the other. H_2, formate and ethanol can accumulate during the process of ruminal methanogen inhibition. By the addition of precursors the formation of these products would be avoided and the electrons would be relocated. A case in point is the butyrate precursor that can relocate the electrons into butyrate. But, the butyrate precursors were ineffective as electron acceptors because they were not completely converted to butyrate and were also metabolized through other pathways (Ungerfeld et al., 2006).

Effect of lipids on methane emission

Lipids such as fatty acids and oils also show some effect on the rumen methanogens. Fatty acids inhibit methanogens by binding to their cell membrane and disturbing their membrane transport (Dohme, 2001). In the meta-analysis of methane, lipid supplemented in the diet of lactating dairy cows showed a 2.2% decrease in methane per 1% of supplemented lipid in the diet (Eugene, 2008). 5.6% methane reduction per percentage unit of lipid added to the diet was observed in cattle and sheep (Beauchemin et al., 2008). Methane was reduced by 22% in sheep fed with myristic acid in a 58% concentrate based diet (Machmuller et al., 2003). Plant extracted oils naturally contain a medium to long chain fatty acids (Soliva et al., 2004). Refined soy oil based diet fed to beef bulls reduced methane by 39% (Jordan, 2006). Sunflower oil also had good impact on methane production; it resulted in 11.5 to 22.0% reduction in methanogenesis (McGinn, 2004). Linseed oil supplemented at a level of 5% of DM to lactating dairy cows resulted in a 55.8% reduction in grams of methane per day (Martin, 2008). Garlic (*Allium sativum*), Eucalyptus (*Eucalyptus globules*) and Neem (*Azadirachta indica*) oils were tested *in vitro* for methane emission, but garlic oil with low fiber diet reduced methane by 55.8% (Sirohi et al., 2012). Fatty acids, with medium chain length such as coconut oil, canola oil, kernel oil, sunflower oil reduce the methane emission in ruminants (Machmuller and Kreuzer, 1999; Dohme et al., 2000). Supplementation of coconut oil (7%) with 100 g/day of garlic powder increased the end products

and improved rumen microbial population; and 9% methane gas was reduced (Kongmun et al., 2011). According to Kumar et al. (2009), in vitro inclusion of eucalyptus (E. globules) oil (EO) at 1.66 µl/ml showed positive effect by reducing 56% methane mitigation, but has negative effect on fatty acid; 0.33 µl/ml of EO reduced 10% methane but had no effect on fatty acid synthesis. Szumacher-Strabel et al. (2011)'s experiment proved methane mitigation was reported only in wild dog rose seeds oil treatment, but had no negative impact on the rumen. Also, there was no change in rose seed residue.

Plant extracts as effective methane mitigators

Plants secondary metabolites such as, saponins, tannins and oils have anti-microbial activity, which can be used as alternative additives to reduce methanogen population in the rumen (Kamra, 2008). Herbal plant extracted products have a prominent effect on rumen microbiota either directly changing the methanogens or indirectly affecting protozoa. It has the ability to change the methane emission (Navneet et al., 2012). Saponins mitigate methane by reducing the protozoa population; tannins and essential oils have toxic effect on methanogens (Cieslak et al., 2013). Methanol extract of Terminalia chebula reduced 95% methane and double level of the extract was inhibited completely. Phenolic acids such as p-coumaric acids, ferulic acids, cinnamic acids and phloretic acids and some monomeric phenolics have been found to decrease methane, acetate and propionate production (Ushida et al., 1989; Asiegbu et al., 1995). The ethanol extract of Emblica officinalis fruit and methanol extracts of the fruits inhibited methanogenesis significantly ($P < 0.05$). The anti-methanogenic and anti-protozoal activity of the saponins has to be further investigated by long term in vivo trials on different feeds; as earlier reports indicated that the rumen microbes get adapted to saponins by prolonged feeding of such feeds (Wallace et al., 2002). Supplementation of coconut oil with garlic powder improves the ruminal fluid fermentation of volatile fatty acids and reduces the methane emission along with protozoal population (Kongmun et al., 2010). Zmora et al. (2012)'s 24 h study on *in vitro* dry matter digestibility (IVDMD) showed that Xanthohumol inhibited the rumen methanogens directly. Cieslak et al. (2012) showed that *Vaccinium vitis idaea* tannin had antimicrobial activity potential to indirectly mitigate methane and thereby ammonia.

Vaccines and antibiotics

Vaccines are used to prevent or control disease for a particular period, but the utilization of vaccines reduces methanogens population and increase productivity is a current topic. The anti- methanogen vaccine triggers the immune system of ruminants and produces antibodies against methanogens in the ruminants. A vaccine against three selected methanogens has been developed in Australia. Immunization in sheep lowered CH_4 production by 8%, while further testing failed to confirm its efficacy in other geographical regions (Wright et al., 2004). *Streptomyces cinnamonensis* secondary metabolite known as monensin inhibits the gram positive bacteria, which is responsible for supplying substrate to methanogens. Monensin acts on the cell wall of the gram positive bacteria; it interferes with ion flux and decreases the acetate-to-propionate ratio in the rumen, effectively decreasing CH_4 production. The effect of monensin on lowering CH_4 emission is dose-dependent: at lower doses (10 to 15 ppm), it results in the production of profitable milk, but has no effect on CH_4 (Grainger et al., 2008; Waghorn et al., 2008); but at higher doses (24 to 35 ppm) (McGinn et al., 2004; Sauer et al., 1998; Van Vugt et al., 2005), it reduces CH_4 production by up to 10% (g/kg DMI). However, there have been unanswered questions over the perseverance of CH_4 suppression (Johnson and Johnson, 1995).

Role of a fungal secondary metabolite, lovastatin in methane mitigation

Lovastatin ($C_{24}H_{36}O_5$) is a secondary metabolite of idiophase of the fungi with a molecular weight of 404.55 (Lai et al., 2003). It inhibits the key enzyme of cholesterol biosynthesis such as enzyme 3-hydroxy-3-ethyl glutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) (Alberts, 1988). Isoprenoid is a central component in Archeal cell wall and it is an intermediate step in cholesterol synthesis (Konrad and Eichler, 2002). As an inhibitor HMG-CoA reductase, lovastatin can suppress isoprenoid synthesis, thereby cell wall synthesis in archeal cell membrane and methanogen population (Smit and Mushegian, 2002). The Fermented Rice Straw Extract of lovastatin significantly reduced total CH_4 production by rumen methanogenic Archaea after 48 h of incubation by 19.47% (Juan et al., 2012). Biological control strategies such as bacteriophages or bacteriocins could prove effective for directly inhibiting methanogens and redirecting H_2 to other reductive rumen bacteria such as propionate-producers or acetogens (McAllister and Newbold, 2008). However, most of these options are in the early stages of investigation and still require significant research over an extended period to deliver commercially viable vaccines and biological control options that will be effective over a range of production systems and regions.

Potential of *genetics* to reduce *methane* emissions in ruminants

The key microbiota Archea is a very small population and it emits large portion of methane in rumen. Molecular

analysis provided that methyl coenzyme-M reductase gene (Martino et al., 2013) is a genetic marker common for the Methanogenic population. De Haas et al., (2011) analyzed the association between cumulate enteric methane emission and Genome wide Single Nucleotide Polymorphism. Though SNP effect could be identified, no large regions were significantly associated. The cows with lower residual feed intake have lower predicted methane emission grams/day. Hence, it is possible to reduce methane emission. Genetic variation suggests that 11 to 26% methane mitigation in 10 years could be more in a genetic selection program.

CONCLUSION

For more than 20 years, research has been done on rumen methanogens. Along with key enzymes methane emission, which causes global warming, made an important task to reduce methanogen population. Various strategies have been implemented to mitigate methane such as by changing diet, especially by providing diet rich in oil seed or proteins rather than carbohydrates. Ionophores, antibiotics and vaccine also have positive effect on methane mitigation, but chance of developing resistance to vaccines is also there. Fungal secondary metabolites such as lovastatin and plant extracts had significant effect on methane emission and a vast deal of information have revealed mitigation strategies. Genomic analysis showed that methyl coenzyme-M reductase is a marker gene for methane production and correlation between food intake. SNP in the genome and breed selection has significant results against methane emission. Now, more work has to be done on the direct effect on rumen methanogens to mitigate methane.

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Conflict of interest

The authors did not declare any conflict of interest.

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