

Methanogenesis and recent techniques for mitigation of methanogenesis in ruminants

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Journal of Livestock Science (ISSN online 2277-6214) 5:35-48
Received on 18/06/2014; Accepted on 8 /07/2014

Abstract

Methane emission from ruminants reduces the efficiency of nutrient utilization. Therefore manipulation of rumen microbial ecosystem for reducing methane emission by ruminants to improve their performance is one of the most important goals for animal nutritionists. Reduction in methane emission from ruminants enhances the efficiency of nutrient utilization and augments productivity and also reduces methane impact on global warming. There are several methods to reduce methane emission from the rumen. These methods include processing of feeds, altering the type of ration, defaunation, supplementation of unsaturated fatty acids, organic acids, halogenated methane analogues, ionophores, microbial feed additives, non-ionic surfactants, sulphates and herbal products. Every method has its own merits and demerits. Herbs and herbal extracts such as neem (*Azadirachta indica*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), kadukka (*Terminalia chebula*) and amla (*Emblica officinalis*) contain secondary metabolites such as lignin, tannins, terpenoids, volatile essential oils, alkaloids, etc. have anti-microbial activity and inhibit rumen microbial growth in a very specific way and hence can be used for selective amelioration of rumen fermentation especially methanogenesis. Moreover, herbs or their extracts are unlikely to produce any residual effects in the animal products as they are organic and have been used for centuries by human race without adverse effects.

Key words: methane, rumen, methanogenesis, mitigation, herbs, extracts, nutrients.

Global warming

Global warming and ozone layer depletion due to increased emission of green house gases in the atmosphere have drawn world wide attention with an alarming stage of iceberg melting, increased ocean level, local and global eco-system upsets, changes in the rainfall patterns, changes in pathogenesis of plants, animals and human beings and alteration in life of the people (Kumar *et al.*, 2008). Several reports of the United Nations inter-governmental panels on climate changes (IPCC, 1994 & 1996) indicated the urgency of the problem. IPCC (2001) has warned that by the mid of this century the globe's temperature will rise just like anything up to 5.8⁰ C.

Methane (CH₄) is second major gas after carbon dioxide (CO₂) responsible for the warming of environment and ozone layer depletion. It is a potent green house gas as it has 23 times higher global warming potential than carbon dioxide (IPCC, 1996). Its present concentration of 1800 ppbv in atmosphere is more than double of about 750 ppbv estimated 100 years ago (Khalil *et al.*, 1993). Estimates of global methane production ranged between 350-820 T g/year (Khan *et al.*, 2001).

Greenhouse effect

The greenhouse effect is thought to be due to the absorption of solar infrared (IR) radiation by gases and the earth's surfaces, which, as a result, are heated and then re emit IR radiation at low frequency with a high absorptive power (Moss *et al.*, 2000). In fact greenhouse gases in the atmosphere are essential for maintaining life on earth, as without them the planet would be permanently frozen because all of the incoming heat from the sun would be radiated back into space by the earth's surface (Moss, 1993). Warming of the earth's surface is achieved by solar energy being radiated, mainly in the visible part of the spectrum (wavelength 0.4 to 0.7 μm) and passing through the atmosphere of the earth without being absorbed. Some of the solar energy is reflected back into space by clouds and about 7% is radiated in the ultra-violet region of the spectrum (below 0.4 μm), which is absorbed by the ozone layer in the atmosphere. The solar energy reaching the earth's surface warms the earth and is radiated back from the surface in the infra-red region of the spectrum (4–100 μm). Approximately 70% of this radiation is in the wavelength band between 7 and 13 μm, which can pass back through the atmosphere into space. The remaining radiation is absorbed, essentially by water vapour and carbon dioxide, thus there is warming of the lower layer of the atmosphere (troposphere), which in turn radiates heat, keeping the earth warmer than it would otherwise be (Gribbin,1988).

Consequences of the greenhouse effect on our environment

The consequences of the increase in concentration of the gases that generate the greenhouse effect are that average global temperatures will rise, along with many consequences on human life. The degree to which these changes are projected to occur is dependent upon a reliable greenhouse gas policy model and a range of scenarios for the levels of greenhouse gas emissions. By the year 2030, the world is likely to be 1–2°C warmer than today, although given the full range of uncertainties, the range could be from 0.5°C to 2.5°C. The concomitant rise in global mean sea level is 17 to 26 cm, with a full range of 5 to 44 cm, due to mainly thermal expansion of the oceans and increased melting of ices in the Arctic and Antarctic areas (Moss *et al.*, 2000).

The projected climatic changes in the next century due to the greenhouse effect are likely to have an effect on water supplies and the increase in temperature will induce a new distribution of deserts and wet areas in the world and will alter the range or numbers of pests that affect plants or diseases that threaten animals or human health. Also of interest are the effects on unmanaged ecosystems, mainly forests (Moss *et al.*, 2000). The rising concentration of methane is correlated with increasing populations and currently about 70% of methane production arises from anthropogenic sources and the remainder from natural sources. Agriculture is considered to be responsible for about two-thirds of the anthropogenic sources (Duxbury and Mosier, 1993). Biological generation in anaerobic environments is the major source of methane, although losses associated with coal and natural gas industries are also significant. The major agricultural sources of methane are flooded rice paddies, enteric fermentation and animal wastes. Decreasing methane emissions from these sources by 10 to 15% would stabilize atmospheric methane at its present level and is a realistic objective (Duxbury and Mosier, 1993).

Various sources of methane emission

Very recent radiocarbon [^{14}C] isotope measurements on atmospheric methane indicate that between 20 and 30% is of fossil origin. The remaining 70 to 80% of atmospheric carbon is derived from sources that yield contemporary carbon: enteric fermentation (animals and insects), natural wetlands, biomass burning, oceans and lakes, rice production, and waste treatment (Cicerone and Oremland, 1988).

Methane emission from livestock

Livestock are one of the largest single sources of methane emission with 80–115 million tonnes per year, equivalent to 15–20% of total anthropogenic methane (IPCC, 2001). Ruminant microorganisms are responsible for the emission of methane from livestock (cattle, buffalo, sheep, goats, camel, *etc.*). The global cattle population is responsible for 73% of methane emissions of all livestock and in tropical regions, where at least half of the global ruminant livestock population is located and is fed mainly low-quality diets, 10-12 per cent of the gross energy ingested is lost through methane (McCrabb and Hunter, 1999). Grasslands in the tropics constitute a large natural feed resource, which is mainly suited for grazing by ruminants. Tropical grasses are of low to moderate digestibility (on average 13% lower dry matter (DM) digestibility than temperate grasses) and are often deficient in critical nutrients such as protein and phosphorus (Minson, 1990). Under such conditions, methane produced during ruminal fermentation represents a loss of 10–11% of gross energy intake (McCrabb and Hunter, 1999). The enteric methane contributes approximately 30-40 per cent of total methane produced from agricultural sources (Moss *et al.*, 2000).

Methane production during ruminal fermentation as a result of methanogenic bacteria and protozoa is an unavoidable and inefficient product of rumen fermentation (Johnson and Johnson, 1995). In ruminants, feed is converted to short chain fatty acids in the rumen, which are used as a source of energy and the hydrogen generated as an intermediate is converted rapidly to methane by the methanogens (Stewart *et al.*, 1997). The methane synthesis in rumen is usually associated with decreased propionate production and increased acetate to propionate ratio (Russell, 1998). Rumen microbial utilization of carbohydrates in the gut of animals results in the production of volatile fatty acids, microbial protein, CO_2 and CH_4 with little hydrogen (H_2). Methane generation should be viewed as an energy sink where H_2 from all rumen microorganisms drains, allowing a greater total yield of ATPs. Reaction: $4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ is the most common mode of methane production in the rumen.

CH₄ from enteric fermentation by ruminants is not only an important greenhouse gas associated with environmental problems, but it also represents a loss of feed energy (20–150 kJ/MJ) intakes (Singh *et al.*, 2005). Therefore, developing feeding strategies to minimize CH₄ emission is desirable in long-term mitigation of emission of greenhouse gases into the atmosphere and for short-term economic benefits.

Micro-organisms involved in methane production in rumen

Methane is produced by strict anaerobes belonging to the sub-group of the Archaea domain. There is a large phylogenetic diversity of methanogens in natural media. Also, the different genera and species of methanogens have various shapes and physiological characteristics: cocci, rods, spirilla, and thermophilic and mesophilic species, motile and nonmotile cells (Woese *et al.*, 1990). Methanogens like *Methanobacterium formicicum*, *M. ruminantium*, *M. bryanti*, *Methanobrevibacter ruminantium*, *Methanosarcina barkeri*, *Methanomicrobium mobile* and *Methanoculleus olentangyi* are present in the rumen in a large number in rumen liquor depending upon the type of diet given to animals, especially the fibre content in the ration. On a fibre rich diet, production of acetic acid is more coupled with more production of methane (Baker, 1999). Rumen methanogens grow only in environments with a redox potential below –300 mV. More than sixty species were isolated from various anaerobic habitats like sanitary landfills, peat bogs, waterlogged soils, salt lakes, thermal environments, and intestinal tracts of animals. Only five of these species belonging to *Methanobrevibacter* and *Methanosarcina* genera, were isolated from rumen digesta. Only two of these species have been found at a population level greater than 10⁶ ml⁻¹ of rumen liquor (Steudler *et al.*, 1989).

Methanogenesis by methanogens

The reactions involved in methane production in the rumen, which have been described by Rouviere and Wolfe (1988), are their sole energy-generating mechanism. They show that specific co-factors are needed for the methane to be produced and inhibition of some of them could be a way to reduce the activity of methanogens. Although, H₂ is one of the major end products of fermentation by protozoa, fungi and pure monocultures of some bacteria, it does not accumulate in the rumen, because it is immediately used by other bacteria which are present in the mixed microbial ecosystem. The collaboration between fermenting species and H₂-utilising bacteria (e.g. methanogens) is called “interspecies hydrogen transfer”. Some physical associations between fermentative species and H₂-users may facilitate interspecies transfer in the rumen. Attachment of methanogens to the external pellicle of protozoa has been reported by Krumholz *et al.* (1983) and Stumm *et al.* (1982). The molar percentage of volatile fatty acids (VFAs) influences the production of methane in the rumen. Acetate and butyrate promote methane production, while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen.

Reduction of methanogenesis in ruminants

The mitigation of methane emission by the rumen microbes using various techniques have been investigated extensively with varying degree of success.

Feed processing technologies

The processing can improve the feeding value by increasing its digestible energy content and / or by increasing feed intake. Therefore, an attempt to increase feed intake may

reduce methane emission. These techniques are chopping and grinding of straws, alkali/ammonia treatment of straws and feed residues, urea-molasses blocks. These processing techniques are reported to depress the methane emission from rumen by 10%. Reduction in methane is associated with increased propionate production (Johnson & Johnson, 1995).

Type of ration

The quantity and quality of ration consumed by ruminants have a major influence on the proportion of energy lost as methane since acetate: propionate ratio is influenced by feed quality and quantity as well as with roughage: concentrate ratio. Methane emission would be less when high grains are fed as a result of higher production of propionic acid. Methane emission fall down drastically to as low as 2-3 per cent (Johnson and Johnson, 1995).

Johnson *et al.* (2000) concluded that the most effective way of reducing methane output per unit of product was through production systems, which increased both animal growth and reproductive rates. Factors such as dry matter intake and diet composition are critical to the amount of methane produced in the rumen. Johnson and Johnson (1995) reported that there is a relationship between the level of dry matter intake and diet composition, so that providing carbohydrates of high digestibility associated with high ingestion levels might result in a decrease in gas production. The comparison between dry matter intake (DMI) and methane emission (g/day) evidenced that gas emission was related to the increase in DMI in animals, and the treatments with concentrate addition resulted in higher methane emission.

Protein supplementation in the diets increased the nutrient digestibility and decreased significantly methane production in rumen (Mehra *et al.*, 2006). The higher efficiency of energy utilization is cited by O'hara *et al.* (2003) as the most efficient strategy to reduce methane emission per kilogram of milk or meat in ruminants. Moss and Givens (2002) reported that higher animal performance might reduce methane emission because the number of animals in the production system might be decreased.

Defaunation

The methanogenic bacteria have an eco-symbiotic relationship with ciliate protozoa and remain attached to the outer surface of the protozoa. Protozoa in the rumen are associated with a high proportion of H₂ production, and are closely associated with methanogens by providing a habitat for up to 20% of rumen methanogens (Newbold *et al.*, 1995). In defaunated ruminants, the methanogenic bacteria do not get the symbiotic partner and methane synthesis is partially inhibited. On defaunation the methane production is reduced by 20-50% (Van Nevel and Demeyer, 1996) depending on the various factors in the diet of the animal.

Supplementation of unsaturated fatty acids

As methane is produced in the rumen to act as a hydrogen sink during the fermentation of carbohydrate, So poly unsaturated fatty acids(PUFA) having double or triple bonds have potential to be used as hydrogen sinks, because these bonds will get saturated by hydrogen and less hydrogen will be available for methane production. Various trials have shown the potential of feeding PUFA rich diet such as vegetable oils for methane reduction. Methane emissions can be decreased by supplementing the diet with certain additives and ingredients. Adding fats to the diet can reduce methane emission by lowering ruminal fermentability, and to a lesser degree, through hydrogenation of the unsaturated fats (Johnson and Johnson, 1995). Adding 4.6% canola oil, a source of unsaturated fat, to a high-forage diet was an effective suppressant of methane, with daily methane emissions decreased by 32%

and methane emissions as a percentage of GE intake decreased by 21 per cent (Beauchemin *et al.*, 2006).

Dohme *et al.* (2000) observed in a *in vitro* study that canola oil added to the diet at 5.3% of dry matter (DM) decreased total methane production by 20%. McGinn *et al.* (2004) reported that adding a similar amount (5% of dietary DM) of sunflower oil to a backgrounding barley-based diet also decreased methane, as a percentage of GE intake, by 21%. Sunflower oil is rich in oleic acid (45%) and linoleic acid (40%), whereas canola oil contains 54% oleic acid, 22% linoleic acid, and 11% linolenic acid (NRC, 2001). Fat feeding has also been shown to reduce methane through a reduction in protozoal numbers, although protozoal numbers were not measured in this study. Methanogenic bacteria are metabolically associated with ciliate protozoa (Newbold *et al.*, 1995), and feeding oil can cause substantial decreases in protozoan populations (Ivan *et al.*, 2004).

Organic acids

Alternative natural feed additives that shift ruminal fermentation may show promise in mitigating methane production (Wallace, 2004). Addition of fumaric acid decreased methane emissions *in vitro* (Asanuma *et al.*, 1999) and *in vivo* (Bayaru *et al.*, 2001) but not in all studies (McGinn *et al.*, 2004), possibly because of the level of supplementation used. Dietary supplementation of dicarboxylic organic acids such as malate, fumarate, aspartate etc. reduces methane production (Martin, 1998). Malate, a potent methane inhibitor is present in animal feeds like alfalfa (2.9-7.5% of DM) and bermuda grass (1.9-4.5%) but its level varies with variety and stage of maturity. These organic acids are converted to succinate or propionate by reduction process and less hydrogen will be available for methane production. *In vitro*, continuous rumen culture experiments have shown that adding graded levels of fumarate to fermentations of ryegrass pasture substrate linearly depressed methane production whilst linearly increasing propionate production (Kolver *et al.*, 2004).

Halogenated methane analogues

Various halogenated methane analogues so far tried as methane inhibitors are such as carbon tetrachloride, chloral hydrate, trichloroacetamide, DDT, trichloroacetaldehyde, bromochloromethane, chloroform, methylene chloride, methylene bromide, nitrapyrin, hemiacetyl of chloral and starch etc. (Haque, 2001) generally inhibit methanogens. Favourable effects of these had been reported only in those animals fed on high roughage diets, as prevalent in Indian livestock. Chloral hydrate is converted in the rumen to chloroform prior to inhibiting methanogens. Bromo chloromethane is believed to inhibit methane production by reacting with reduced form of Vitamin B₁₂ which inhibits methanogenesis.

Ionophores

Ionophores are generally used as feed additives in order to improve the efficiency of digestion in ruminants, such as tetronasin, monensin, lasalocid, salinomycin, narasin, lysocellin etc. These ionophores antibiotics are carboxylic polyether compounds produced by various strains of *Streptomyces* eg. Monensin by *S. cinnamonensis* and lasalocid by *S. lasoliensis*. Monensin is moderately active against gram positive bacteria, certain mycobacteria and coccidian, while lasalocid is specifically active against hydrogen producing bacteria and results in higher propionate production which in turn is related with low methane production (Kobayashi *et al.*, 1992).

Microbial feed additives, probiotics and prebiotics

The use of acetogenic bacteria as microbial feed additive along with some anti-methanogenic compound may be effective in methane inhibition, as acetogenic bacteria may not be able to compete with methanogenic bacteria due to poor affinity with hydrogen. Probiotics such as yeast cultures are used to stimulate bacterial activity in the rumen. The probiotics have been shown to stabilize rumen pH, increase propionate levels and decrease the amount of acetate, methane and ammonia production. Addition of *Sacchromyces cerevisiae* reduced methane production *in vitro*. (Mutsvangwa *et al.*, 1992).

Non-ionic surfactant (NIS)

Tween 80, a non ionic surfactant, given at a concentration of 0.05 percent (v/v) in growth medium *in vitro*, increase growth rate of rumen bacteria and fungi and rate of cereal digestion, succinate and lactate dehydrogenase activities and polysaccharides degrading enzymes activities (Lee and Ha, 2003).

Sulphate supplementation

In the rumen fermentation, three H₂ utilizing microbes are the sulphate reducing bacteria, methanogens and carbon dioxide reducing acetogens, which have a threshold value of H₂ (m mole/ liter) as 0.0013, 0.067 and 1.26 respectively at which these bacteria act as the dominant electron acceptors. Thus it appears that sulphate-reducing bacteria have the highest affinity to utilize hydrogen in the rumen, even better than methanogens, but the availability of sulphate in the rumen appears to be a limitation. Kamra *et al.* (2004) observed that sulphate supplementation helps in increasing the production of fibre degrading enzymes and fibre degradation in the rumen. As sulphate / sulphite have high affinity for utilization of hydrogen for its reduction to sulphide, therefore, a fibre diet, as prevalent in Indian livestock, sulphate / sulphite supplementation can be a good mode of rumen amelioration for improving fibre degradability and inhibiting methanogenesis, but a proper dose will have to be optimized, keeping in view the toxic levels of sulphide generated on sulphate reduction.

Herbs/herbal extracts in mitigation of methanogenesis

Plant extracts have been used for centuries for various purposes (traditional medicine, industrial applications, food preservatives) because of their antimicrobial properties (Davidson and Naidu, 2000) and because most of them are categorized under GRAS (Generally Recognized as Safe) for human consumption (FDA, 2004).

Herbal extracts and their active principles having potential in reducing methanogenesis

The phytochemical screening of *Psidium guajava* (guava) leaves revealed the presence of alkaloids, saponins, steroidal rings and deoxy sugars characteristic of cardenolides (Elekwa *et al.*, 2009).

The seeds of *Embilica officinale* (amla) yield a fixed oil (16%) which is brownish yellow in colour. It has linolenic acid (8.8%), linoleic acid (44%), oleic acid (28.4%), stearic acid (2.15%), palmitic acid (3%) and myristic acid (1%) (Thakur *et al.*, 1989).

Pods of *Acacia concinna* (shikakai) have been found to contain triterpenoids and steroid saponins, acacidol, acacic acid and sonumin (Chevallier,1996). The inhibitory effect of these extracts on protozoa could be due to their saponin content.

Chao *et al.* (2000) reported that roots of *Zingiber officinale* (ginger) are rich in camphene (14.1%), β -bisabolene (22.1%) and ar-curcumene (14.5%).

The seed kernel of *Azadirachta indica* (neem) contains bitter compounds such as nimbin, nimbidin and azadirachtin and is being used in human medicine (Brahmachari, 2004).

The ferula genus from the family of Umbelliferae has been found to be a rich source of gum-resin (Fernch, 1971). This genus presents interesting phytochemical features such as the occurrence of sesquiterpenes and sesquiterpene coumarins (Abd El-Razek *et al.*, 2001).

Yield of some common herbal extracts having potential in reducing methanogenesis

The yield of guava leaves methanol extract was 4.2% and that of water extract was 8.02% (Elekwa *et al.*, 2009). The yield of assafoetida resin methanol extract was reported to be 10.8% (Abbas Ali *et al.*, 2009). Kadukka seeds had a methanol extract yield of 31% (Klika *et al.*, 2004) and water extract yield of 32.6% (Anwesa *et al.*, 2009). Garlic bulbs methanol extract recorded a yield of 4.6% (Adeniyi *et al.*, 2006) and its water extract yielded 56% (Ekwenye and Elegalam, 2005). The yield of ginger rhizome water extract was 70% (Ekwenye and Elegalam, 2005). Neem seed kernels methanol extract recorded a yield of 34% (Sanguanpong, 2003) and its water extract recorded a yield of 10% (Garcia, 2006).

Herbal extracts in reducing methane production

The use of plant extracts appears as one of the most natural alternatives to the antibiotic use in animal nutrition. Plant extracts offer a unique opportunity in this regard (Wallace, 2004), as many plants produce secondary metabolites, such as saponins and tannins, which have antimicrobial properties. These compounds have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Hristov *et al.*, 1999).

Methanol extract of seed pulp of *Terminalia chebula* (kadukka) and methanol, ethanol and water extracts of the bulb of *Allium sativum* (garlic) reduced methane production significantly in the rumen liquor of buffaloes (Patra *et al.*, 2006).

The presence of tannins in *Terminalia chebula* might be responsible for reduction in methane emission. 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 (Asiegbu *et al.*, 1995).

Garlic oil is a complex mixture of many secondary plant products including allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan (Busquet *et al.*, 2005). The decrease in methane production observed in garlic oil and its compounds confirms their ability to inhibit methanogenesis (Busquet *et al.*, 2005).

Neem cake has been recommended as a feed supplement for ruminants after water processing (Agarwal *et al.*, 1987) as the water extract from neem seed kernel cake was reported to stimulate *in vitro* enzyme activity in rumen bacteria related to fibre degradation (Agarwal *et al.*, 1991). *In vitro* studies by Patra *et al.* (2006) showed that adding water extracts of neem seeds decreased total rumen volatile fatty acid (VFA) digestibility and also reported antiprotozoal activity of the water extract of neem kernels.

There are numerous essential oils and plant extracts each with varying effects on ruminal fermentation and feed digestion (Kamra *et al.*, 2005). Some essential oils exhibit antimicrobial properties (Helander *et al.*, 1998), and this has motivated recent interest in developing essential oil products as natural feed additives for cattle (Wallace, 2004). Recent studies have shown that some essential oils beneficially affect ruminal fermentation, causing an increase in VFA (Castillejos *et al.*, 2005) and decrease in the rate of amino acid deamination (McIntosh *et al.*, 2003). The antimicrobial activity of essential oils and secondary plant metabolites is highly specific, which raises the possibility that these compounds can be used to target methanogens.

Kamra *et al.* (2005) reported that the extract of a number of plants reduced methane production *in vitro*. McIntosh *et al.* (2003) reported that growth of the methanogen *Methanobrevibacter smithii* was inhibited but only when the concentration of essential oil

product exceeded 1,000 ppm. Some plant extracts have high content of flavonoids which decreased methane production and stimulated microbial metabolism as it increased both degradability of crude protein and cell wall constituents resulting in increased biomass production (Broudiscou *et al.*, 2002).

The tropical plants containing high amount of saponins have been found to have antiprotozoal and antimethanogenic activity (Sliwinski *et al.*, 2002). There are some feeds or forages plants, which contain saponins such as *Alfalfa* (3-5%), *Sapindus rarak*, *Sapindus mokorossi*, *Yucca schidigera* (4%), *Quillaja saponaria* (10%), *Acacia concinna*, *Embllica officinalis* etc. caused a decrease in methane production from 2

0-60% on different substrate accompanied with a decrease in ammonia nitrogen and the numbers of protozoa. The molar proportion of acetate was found to be decreased and that of propionate was increased. Saponins reduces the protozoal population which reduces the inter species hydrogen transfer to the methanogenic bacteria attached to the protozoa, thereby decreases the hydrogen availability to the methanogens. Given the detrimental effect of saponins on protozoa and that around 25% of ruminal methanogens live in association with protozoa, the potential effect of saponins on methanogenic archaea and methane production in the rumen has drawn interest lately. (Newbold *et al.*, 1997).

Tannins have been found to be toxic for many of the rumen microbes, especially ciliate protozoa, fibre degrading microbes and methanogenic bacteria. As a result of this property the methanogenesis in the rumen is also reduced (Kamra, 2006). There are reports indicating a decrease in CH₄ emission with dietary addition of condensed tannins or inclusion of condensed tannin- containing forage (Carulla *et al.*, 2005). Negative effects on ruminal fiber digestion, which may relate to decreased number of cellulolytic bacteria, formation of condensed tannins complexes that are resistant to enzymatic digestion and impaired substrate adhesion by fibrolytic microbes would reduce H₂ availability to lessen methanogenesis (Carulla *et al.*, 2005).

In vitro studies in mitigation of methanogenesis using herbs or herbal extracts

The effect of inclusion of peppermint (*Mentha piperita*) oil (at 0, 0.33, 1.0 and 2.0 µl/ml of incubation medium) on gas and methane production, fermentation of feed and microbial profile was studied in a *in vitro* gas production test, using 200 mg of wheat straw and concentrate mixture in equal proportion as substrate in a 100 ml graduated syringe. The buffalo rumen liquor was used as inoculum and the observations were recorded at 24 hours of incubation. Methane emission was reduced (P<0.001) by 19.9%, 46.0% and 75.6% at 0.33, 1.0 and 2.0 µl levels, respectively (Agarwal *et al.*, 2009).

Different plants, herbs and spices (158 samples) were tested in a screening trial to assess their potential to modify ruminal fermentation *in vitro*, in particular their effectiveness for decreasing methane production. Most of the plants tested did not cause any noticeable effect on the fermentation parameters studied. However, rhizomes and roots of *Rheum officinale* (rhubarb), bark of *Frangula alnus* (frangula or alder buckthorn) and bulbs of *Allium sativum* (garlic) decreased methane production and acetate to propionate ratio (García-González *et al.*, 2008).

The effect of a commercially available aqueous allicin product was studied in the rumen simulation technique (RUSITEC) at 2 and 20 mg/l allicin. There was no effect on daily total VFA or ammonia production but a decrease (94% at 20mg/l allicin addition) in methane production was observed (Hart *et al.*, 2006).

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