International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 3375-3379

Received: 12-03-2018
Accepted: 16-04-2018

Neeti Lakhani
Division of Animal Nutrition, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India

Preeti Lakhani
Division of Animal Physiology, National Dairy Research Institute (NDRI), Karnal, Haryana, India

Correspondence
Neeti Lakhani
Division of Animal Nutrition, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India

Plant secondary metabolites as a potential source to inhibit methane production and improve animal performance

Neeti Lakhani and Preeti Lakhani

Abstract
Among all greenhouse gasses methane makes up 16% of total global GHG emissions which is probably the second most important gas after CO_2 contributing to global warming. Methane has 23 times more global warming potential than carbon dioxide. In ruminants, approximately 95.5% of CH4 generation is produced by fermentation of feed in the rumen. Reduction in methane emission is associated with enhanced efficiency of nutrient utilization as well as it reduces impact of methane on global warming. The methane released by ruminants and its contribution to greenhouse effects has compelled nutritionists and microbiologists to explore some antimethanogenic substances preferably through natural sources for eco-friendly animal production. Alternate hydrogen sink such as sulphate etc. might prove to be a suitable option as these compounds also supply NPN and macro mineral. However, plant secondary metabolites (PSM) are natural components of plants having the ability to modify rumen fermentation without microbial resistance and without any residual effect on animal products. Here the effect of terminal electron acceptor (sulphate) and plant secondary metabolites (PSM) will be discussed.

Keywords: greenhouse gasses, hydrogen sink, methane, secondary metabolites, ruminants

Introduction
Greenhouse gas emissions such as carbon dioxide, methane, nitrous oxide, moisture and ozone are of great concern worldwide due to their effects on global warming and climate change, and consequently on ecological and socio-economic vulnerability (IPCC,2007) [1]. Methane makes up 16% of total global GHG emissions (Scheehle and Kruger, 2006) [2] which is probably the second most important gas after CO_2 contributing to global warming (Van Nevel and Demeyer, 1996) [3]. Methane has 23 times more global warming potential than carbon dioxide (IPCC, 2007) [1]. Methane emissions from the agriculture sector represent about 40% of that produced by human-related activities (Steinfeld *et al.*, 2006) [4]. Methane emission from Indian livestock ranges from 7.26 to 10.4 MT/year of which the emission from buffalo is estimated to be 3.93 MT/animal/year. This contributes 42% of the total livestock methane emission in India.

In ruminants, approximately 95.5% of CH4 generation is produced by fermentation of feed in the rumen (AGO 2003). Agriculture is responsible for 47% of the total anthropogenic CH4 emissions, of which 32% is derived from enteric fermentation in livestock (IPCC 2007). Methanogenesis is an essential metabolic process in the rumen which acts as hydrogen sink. Hydrogen produced during feed fermentation is immediately used to produce methane which does not allow its accumulation in the gaseous phase of rumen, which otherwise might hamper feed fermentation in the rumen. It is an inescapable phenomenon of rumen fermentation and livestock production systems contribute greenhouse gas emissions to the atmosphere causing climate change and global warming. An adult cattle and buffalo emit approximately 200-300 liters of methane per day depending upon its physiological stage of life. According to Johnson and Johnson (1993) [5] methanogenesis is an energetically wasteful process because 2-12% of gross energy is diverted towards methanogenesis making animal poor in energy utilization leading to a big economic loss.

Reduction in methane emission is associated with enhanced efficiency of nutrient utilization as well as it reduces impact of methane on global warming. A number of chemical feed additives were tried to decrease methane production in the rumen.

Many feed additives such as antibiotics, ionophores and defaunating agents have been used to modify rumen fermentation with an aim to enhance the efficiency of ruminant production and reduce methanogenesis. However, an increasing awareness of hazards associated with chemical feed additives, i.e. many of these chemical additives are either toxic to host animals or have a transient effect on methanogenesis (Moss *et al*., 2000) [6], presence of chemical residues in animal derived foods and development of bacterial resistance to antibiotics has diverted the research on feed additive technology towards exploiting natural products as feed additives. The methane released by ruminants and its contribution to greenhouse effects has compelled nutritionists and microbiologists to explore some antimethanogenic substances preferably through natural sources for eco-friendly animal production. Alternate hydrogen sink such as sulphate etc. might prove to be a suitable option as these compounds also supply NPN and macro mineral. However, plant secondary metabolites (PSM) are natural components of plants having the ability to modify rumen fermentation without microbial resistance and without any residual effect on animal products (Wallace *et al.*, 2002) [7]. Here the effect of terminal electron acceptor (sulphate) and plant secondary metabolites (PSM) will be discussed.

Sulphate and PSM as Rumen Modifiers

The animal body contains about 0.15% sulfur. It is a component of amino acids cysteine, taurine, methionine, homocystein, of the B-vitamins thiamine and biotin and the antioxidant glutathione, and some inorganic sulfur (Underwood and Suttle 1999) [8]. In ruminants, many inorganic forms of sulphur (e.g. potassium sulphate and sodium sulphate) can be used. Sulphate is reduced to hydrogen sulfite by SRB (Kung, 2008) [9]. The SRBs are natural inhabitant of rumen but their number is low. They reduce sulphate to hydrogen sulfite (Kung, 2008) [9]. Van Zijderveld *et al.* (2010) [10] showed that an enhanced level of sulphate in the diet increases the number of SRBs. Paul *et al*. (2012) [11] demonstrated reduction in *in vitro* methane production and a decrease in production of hydrogen sulphide and increased fibre digestibility by including live culture of the SRB isolates (from buffalo rumen) in the incubation medium. The isolates were identified as *Fusarium* sp. The inhibition of methanogenesis due to the presence of sulphate has also been reported for anaerobic digesters (Kroiss and Wabnegg, 1983) [12].Sulfate, either alone or in combination with nitrate and/or saponin, did not reduce digestibility (Van Zijderveld *et al*., 2010) [10] and did not alter VFA concentration.

Plant secondary metabolites (PSM), are non-nutritive plant metabolites which are essential for plant survival and proper growth and reproduction (Patra and Saxena, 2009b) [13]. These phytogenic feed additives show health promoting functions and also affected rumen ecosystem of ruminants which resulted in reduced methanogenesis and increased feed utilization efficiency (Kamra *et al*., 2008) [14]. These contain bioactive products such as essential oils, saponins and tannins which have antimicrobial properties. Numerous studies have been made to exploit these PSM like tannins, saponins, essential oils, terpenoids, flavenoids etc as natural feed additives to improve the efficiency of rumen fermentation such as enhancing protein metabolism, decreasing methane production and improving rumen ecology (Kamra, 2008; Patra *et al.* 2006b) [14, 15]. The tropical plants rich in saponins have been found to suppress and/or eliminate protozoa from the rumen and a reduction in methane and ammonia production. Tannins, especially condensed tannins (CT), decrease methane production in the rumen (Patra, 2011) [16]. Similarly, some plant extracts having high content of flavonoids decrease methane production and induce extensive stimulation of microbial metabolism which increases both degradability of crude protein and cell wall constituents. Essential oils are found to be beneficial for ruminal microbial metabolism reducing (Castillejos, 2006) [17] the risk of rumen acidosis, decreasing intra ruminal nitrogen turnover and nitrogen excretion and also inhibit methanogenesis (Sirohi, 2012) [18].

Effect on *in vitro* fermentation

Methanogenesis

Napasirth *et al.* (2013) [19] demonstrated the effect of 0.2 or 0.4% of ammonium sulfate, copper sulfate and sodium lauryl sulfate on methane production using *in vitro* gas production technique. They concluded that sulfate-containing compounds decreased the total gas and CH_4 production with maximum inhibition after 24 hour (14.53%) by 0.2% ammonium sulfate. The effect of ethanol, methanol and water extracts of pods of *Acacia concinna* (Shikakai), seed pulp of *Terminalia chebula* (harad), *Terminalia belerica* (bahera), *Emblica officinalis* (amla) and seed kernel of *Azadirachta indica* (neem seed) on methane production was evaluated by Patra *et al*.(2006) [14]. He found 95% reduction in methane production at 0.25 ml/30 ml and complete inhibition at 0.50ml/30ml of incubation medium in methanol extract of *T. chebula.* Patra and Yu (2014) [20] studied the effect of saponin (0.6 g/L), nitrate (5 mM) and sulfate (5 mM), alone and in combinations, on *in vitro* methanogenesis and found that combinations of nitrate with saponin and/or sulfate both additively suppressed methane production and maximum reduction (nearly 46%) was observed for the combination of all the three inhibitors.

***In vitro* fermentation**

The effect of different level of urea-calcium sulphate mixture (U-cas @ 0, 3, 6, 9, 12, 15 and 18%) in high-quality feed block (HQFB) on the digestibility, fermentation and gas kinetics in rumen fluid of swamp buffalo was studied by Cherdthong and Wanapat, (2013) [21] by using *in vitro* techniques. Gas production rate was constant for the insoluble fraction but, potential extent of gas and cumulative gas increased linearly with increasing levels of U-cas. The *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, true digestibility and microbial mass were altered by treatments and were highest at 18% U-cas supplementation. Propionate concentration was linearly increased with increasing levels of U-cas and was highest at 18% U-cas supplementation. The NH_3–N concentration tends to reduce with increasing level of U-cas.

Patra *et al.* (2009) [22] reported that addition of 0.50 mL of *S. aromaticum* extracts did not had any effect on concentration of TVFA, but the acetate to propionate ratio increased. IVOMD (%) of the substrate was decreased by the addition of ethanol and methanol extracts of *S. aromaticum* at levels of 0.25 and 0.50 mL. Kumar *et al.* (2016) [23] evaluated the effect of Amla fruit powder (AFP), fengureek seed (FS) and AFP + FS mixture (1: 1) by adding it @ 1, 2, 3% to 60:40 roughage concentrate based diet. Methane production (ml/g DM) was lower ($P<0.01$) when AFP was added at 2 and 3% due to presence of hydrolysable tannins. There was significant ($P<0.05$) decrease (22.84%) in methane production due to 2% or higher level of FS supplementation due to presence of

saponins. Total gas (ml/g DM) production after 24 h incubation varied from 118.49±3.00 to 129.14±2.50 (AFP), 113.26±4.87 to 125.86±2.66 (FS) and from 128.05±0.92 to 139.89±2.84 (AFP-FS mixture) in comparison to the control (111.95±1.13). Digestibility of DM and OM improved (P<0.01) on AFP, FS and AFP+FS supplementation. Chaturvedi *et al.*,(2015) [24] studied the effect of three herbal feed additives ie fruits of Emblica officinalis (Amla), leaves of Azadirachta indica (Neem) and leaves of Tephrosia purpuria (Mokh) were mixed @ 0.5 in mixed substrate and assessed individually under in vitro rumen fermentation using rumen liquor. There was significant increase (P<0.001) in TVFA and a reduction in pH with amla supplementation.

Effect on *in vivo* fermentation

Methanogenesis

The effect of sulphate (2.6% of dry matter) on enteric methane emission on crossbred Texel male lambs was studied by Zijderveld *et al.* (2010) [10]. The methane production decreased in sulphate supplement (sulfate:- 16% and nitrate + sulfate: -47% relative to control). He concluded that sulfate had methane suppressing activity over control animals. Inamdar *et al.* (2015) [25] studied the effect of mahua seed cake (*Madhuka longifolia*, M10 @ 100 gm/Kg DMI) or combination of Mahua seed cake with harad seed pulp (*Terminalia chebula*, H20 and H40 @ 20 and 40 gm/Kg DMI) in male buffaloes. They found that Methane production (L/d) was decreased by 11.7%, 12.9%, and 17.6% in M10H0, M10H2 and M10H4 groups, respectively. By feeding harad (source of tannins) seed pulp alone or mixture of harad and garlic (source of essential oils) @ 1% of DMI to sheep resulted in 24% reduction in methane production. Five EOs i.e. cinnamon leaf oil, clove bud oil, thyme oil, origanum oil and rosemary oil were studied for their efficacy towards methane reduction and digestibility in wheat straw based diets by Chaturvedi *et al.* (2015) [24]. Cinnamon oil at a dose of 450mg/l was found to be effective against methanogens and decreased CH4 production slightly as compared to control. Thyme and origanum oil showed significant CH4 reduction (68.8 and 82.5%, respectively).

Rumen Fermentation

Cherdthong *et al.*, (2014) [21] observed the effect of different level of urea calcium sulphate mixture (U-cas @ 120, 150 and 180 g/kg dry matter) on rumen fermentation in Thai native beef cattle. They found that rumen pH and temperature were not affected by U-cas supplementation. At 4 h post feeding, the concentration of ruminal ammonia nitrogen decreased with increasing levels of U-cas (P<0.05). Inclusion of U-cas at 180 g/kg DM increased the ruminal propionic acid concentration at 4 h post feeding while ratio of acetic: propionic acid and acetic plus butyric: propionic acid were lower than those in other groups (P<0.05). Zijderveld *et al.* (2010) [10] studied the effect of sulfate (2.6% of dry matter) on volatile fatty acid concentrations and microbial composition on crossbred Texel male lambs after 4 weeks of adaptation. He found that sulfate had no effects on volatile fatty acid concentrations except for the molar proportion of branched-chain volatile fatty acids, which was higher when sulfate was fed in the diet. The total number of rumen bacteria increased as a result of sulfate inclusion in the diet (Cappenberg, 1974) [22]. Enhanced levels of sulfate in the diet increased the number of sulfate-reducing bacteria. The number of protozoa was not affected by sulfate addition.

Kumar *et al.*, (2016) [23] evaluated the effect of plants mixture (leaves of mango, jamun, guava, seed pulp of harad and fennel in equal proportions @ 40 gm/ 100 kg body wt.) and peppermint oil (@ 2 ml/100 kg body wt.) on rumen fermentation in fistulated buffaloes. They observed that post prandial change in pH, total volatile fatty acids, fibre degrading enzyme activity (CMCase, xylanase and acetylestrase) and protozoa count were similar among the groups. Decreased Propionate production and increased NH_3-N concentration were reported in plant mixture and peppermint oil supplemented group, respectively. Chaturvedi *et al.*, (2015) [24] reported that IVDMD was decreased from 76.19% in case of control diet to 74.78% and 56.87% ($p \leq 0.05$) in origanum and thyme oil treatments. TVFA levels exhibited decline when supplemented with origanum ($p \leq 0.05$) and thyme oil as compared to control diet.

Effect on nutrient utilization and growth performance

Cherdthong *et al.*, (2014) [21] studied the effect of feeding feed blocks containing different levels of urea and calcium sulphate mixture on feed intake, digestibility and rumen fermentation in Thai native beef cattle fed on rice straw. There was highest total intake of DM and energy (ME, MJ/d) in cattle receiving feed block containing urea calcium sulphate at 180 g/kg DM, followed by 150, 120 and 0 g/kg DM. Apparent digestibility of nutrients were enhanced by the increasing levels of urea calcium sulphate supplementation in feed blocks, except acid detergent fiber. Inamdar *et al.*, (2015) [25] found no effect on dry matter intake and digestibility of dry matter, organic matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber in male buffaloes after supplementing mahua seed cake (*Madhuka longifolia*, @ 100 gm/Kg DMI) or combination of mahua seed cake with harad seed pulp @ 20 and 40 gm/Kg DMI). Similar findings were observed by Kumar *et al.* (2016) [22] with leaves mixture (a mixture leaves of mango, jamun, guava, seed pulp of harad and fennel in equal proportions) @ 40 gm/ 100 kg body wt and peppermint oil (@ 2 ml/100 kg body wt. to fistulated buffaloes.

Effect of sulphate and PSM on rumen microbial profile

Cherdthong and Wanapat (2013) [21] found that the populations of rumen bacteria increased quadratically (P<0.05) and that of fungal zoospores linearly (p<0.05), being highest at 180 g urea–calcium sulphate per kg feed block. Supplementation of urea–calcium sulphate increased the population size of total bacteria linearly (P<0.05) and of *F. succinogenes* quadratically (P<0.05), whereas *R. flavefaciens* and *R. albus* were not affected by dietary treatments. Kamra *et al.*, (2006) [25] reported partial removal of protozoa from the rumen of buffaloes by feeding soapnut. The protozoa number was decreased significantly by inclusion of soapnut extract in the incubation medium as counted under the microscope accompanied with *in vitro* methane inhibition and support the previous reports showing the antiprotozoal activity of saponins.

Patra and Yu (2014) [20] studied the effect of saponin (0.6 g/L) and sulfate (5 mM), alone and in combination, on microbial community, and abundance of selected microbial populations. They found that all the inhibitors, either alone or in combinations, did not alter the abundance of total bacteria, *R. albus*, or archaea. However, saponins, alone and together with sulfate, increased the abundance of *F. succinogenes* and *R. flavefaciens*, but decreased that of protozoa. Agarwal *et al.*, (2009) [26] reported that total bacteria (0.23 and 0.49 vs. 1.0),

fungi (0.29 and 0.55 vs 1.0) and methanogens (0.18 and 0.20 vs. 1.0) were adversely affected at higher levels (1.0 and 2.0 µl/ml of incubation mixture) of peppermint oil compared to the control. But at the level 0.33 µl/ml incubation mixture of peppermint oil, the population size of all the microbes increased except *F. succinogenes*.

Effect on blood biochemical parameters and immunity

The effect of sodium sulphate (1.8%) on haematological parameters in male angora goats were studied by Avci *et al.* (2012) [27]. They found that haematological parameters (red blood cell and white blood cell counts), haemoglobinemia and haematocrit glutathione (GSH) concentrations, total antioxidant activity (AOA), glutathione peroxidase (GPx) activity and erythrocyte catalase (CAT) activity were not altered among groups. Inamdar *et al.* (2015) [25] did not find any change in the blood parameters like Hb, PCV, albumin globulin, AST, ALP, LDH etc) by feeding mahua seed cake (saponins source) and harad (tannins source) to buffaloes.

Reference

1. IPCC. 2007. Climate change: IPCC Fourth Assessment Report. Cambridge University Press, UK, 2007.
2. Scheehle EZ, Kruger D. Global anthropogenic methane and nitrous oxide emissions. Energy Journal, 2006; 3:33-44.
3. Van Nevel CJ, Demeyer DI. Control of rumen methanogenesis. Environment Monitoring Assesstion, 1996; 42:73-97.
4. Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, De Haan C. Livestock's Long Shadow: Environmental Issues and Options. Rome: Food and Agriculture Organization of the United Nations, 2006.
5. Johnson KA, Johnson DE. Methane emissions from cattle. Journal of Animal Science, 1995; 73: 2483-2492.
6. Moss AR, Jouany JP, Newbold JP. Methane production by ruminants: its contribution to global warming. Annals de Zootechnie, 2000; 49:231–253.
7. Wallace RJ, McEwan NR, McIntosh FM, Teferedegne B, Newbold CJ. Natural products as manipulators of rumen fermentation. Asian Australian Journal of Animal Science, 2002; 15:10-21.
8. Underwood J, Suttle F. The detection and correction of mineral imbalances. The mineral nutrition of livestock. 3rd edition, 1999.
9. Kung Jr L. Metabolism of sulphur in the rumen. *In*: proceeding of sulphur in ruminant diets symposium. Midwestern Section Animal Science Meeting. Des moines. Accessed, 2008.
10. Van Zijderveld SM, Gerrits WJJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA, Perdok HB. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science, 2010a; 93:5856-66.
11. Paul SS, Deb SM, Singh D. Isolation and characterization of novel sulphate-reducing *Fusobacterium* sp. and their effects on *in vitro* methane emission and digestion of wheat straw by rumen fluid from Indian riverine buffaloes. Animal Feed Science and Technology, 2011; 166– 167:132-140.
12. Kroiss H, Wabnegg FP. Sulfide toxicity with anaerobic wastewater treatment. *In* WJ Proceedings of the European Symposium on Anaerobic Wastewater Treatment (AWWT). AWWT Symposium Secretariat, TNO Corporate Communication Department, The Hague, Netherlands, 1983, 72-85.
13. Patra AK, Saxena J. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutrition Research Review, 2009; 22:204-219.
14. Kamra DN, Agarwal N, Chaudhary LC. Inhibition of ruminal methanogenesis by tropical plants containing secondary plant compounds. In: Soliva, CR.; Takahashi J, Kreuzer M. eds. Proceedings of the 2nd International Conference of Greenhouse and Animal Agriculture. ETH Zurich, Zurich, Switzerland, 2005, 102-111.
15. Patra AK, Kamra DN, Agarwal N. Effect of plant extract on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Animal Feed Science and Technology. 2006b; 128:276-291.
16. Patra AK, Kamra DN, Bhar R, Kumar R, Agarwal N. Effect of *Terminalia chebula* and *Allium sativum* on *in vivo* methane emission by sheep. Journal of Animal Physiology and Animal Nutrition. 2011; 95:187-191.
17. Castillejos L, Calsamiglia S, Ferret A. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* system. Journal of Dairy Science, 2006; 89:2649-2658.
18. Sirohi SK, Goel N, Singh N. Utilization of saponins, a plant secondary metabolite in enteric methane mitigation and rumen modulation. Annual Research and Review in Biology. 2013; 4:1-19.
19. Napasirth P, Wachirapakorn C, Saenjan P, Yuangklang C. Effect of sulphate containing compound on methane production by using an *in vitro* gas production technique. Pakistan Journal of Nutrition. 2013; 12:723-729.
20. Patra AK, Yu Z. Combinations of nitrate, saponin, and sulfate additively reduce methane production by rumen cultures *in vitro* while not adversely affecting feed digestion, fermentation or microbial communities. Bioresource Technology. 2014; 155:129-135.
21. Cherdthong A, Wanapata M, Wongwungchuna W, Yeekeng S, Nilthoa T, Rakwongrit D, *et al.* Effect of feeding feed blocks containing different levels of urea calcium sulphate mixture on feed intake, digestibility and rumen fermentation in Thai native beef cattle fed on rice straw. Animal Feed Science and Technology. 2014; 198:151-157.
22. Cappenberg TE. Interrelations between sulphate reducing and methane-producing bacteria in bottom deposits of a fresh water lake II. Inhibition experiments. Antonie Van Leeunhoek JMS, 1974; 40:297-306.
23. Kumar D, Singh P, Chaturvedi VB, Verma AK. In Vitro Digestibility and Rumen Fermentation Parameters of Feeds as Affected by Supplementary Amla Powder and Fenugreek Seeds. Indian Journal of Animal Nutrition, 2016; 33(1):64-69.
24. Chaturvedi I, Dutta TK, Singh PK. Effect of various herbal supplements on in vitro rumen fermentation using goat rumen liquor. Journal of Science. 2015; 1(1).
25. Inamdar AI, Chaudhary LC, Agarwal N, Kamra DN. Effect of *Madhuca longifolia* and *Terminalia chebula* on methane production and nutrient utilization in buffaloes. Animal Feed Science and Technology, 2015.
26. Agarwal N, Shekhar C, Kumar R, Chaudhary LC, Kamra DN. Effect of peppermint (*Menthe piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Animal Feed Science and Technology. 2009; 148:321-327.

27. Avci G, Kucukkurt I, Birdane YO, Eryavuz A, Ozdemir M. Influence of high dietary nitrate intake and sulphur supplementation on oxidant/antioxidant balance and on some hematological parameters in Angora goats. Review Medical and Veterinary. 2012; 163:79-84.