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Use of Nitrates and Sulphates as Hydrogen Sink in Reducing Enteric Methane Production

***Sandeep Uniyal*[1,]*, *A. K Mishra*[1], *Aswin K.*[1], *J. K. Sahoo*[1], *V.K. Munde*[1], *G. K. Mishra*[2]**
[1]Division of Animal Nutrition, I.V.R.I., Izatnagar, Bareilly, Uttar Pradesh, India
[2]Division of Animal Reproduction, I.V.R.I., Izatnagar, Bareilly, Uttar Pradesh, India

Abstract

Methane (CH_4) and other gases like carbon dioxide (CO_2), nitrous oxide (N_2O), are green house gases (GHGs) that aggravates the effects of solar and thermal radiation on surface and atmospheric temperature. CH_4 is the second largest GHG having 21 times more heat generation potential than CO_2. Global livestock agriculture was responsible for 18% of the anthropogenic GHG emissions annually. CH_4 production in the rumen accounts for 2–12 % loss of gross energy and consequently influence performance of ruminants. So, reducing ruminant CH_4 emissions is an important objective for ensuring the sustainability of ruminant-based livestock farming. CH_4 is formed in the rumen by methanogens (part of the domain Archaea), mainly from H_2 and CO_2. Within the rumen microbial food web, methanogens perform the beneficial task of removing H_2, that allows reduced cofactors to be reoxidized and recycled, thereby enhancing the breakdown and fermentation of plant material. Therefore, rumen CH_4 mitigation strategies need to consider alternative routes of H_2 utilization. Nitrates and sulphates are potent inhibitors of CH_4 in many anaerobic system including rumen; these salts have greater affinity for H_2 as compared to CO_2 resulting in net reduction in CH_4 production. Experimental evidence suggest that nitrate and sulphate inhibit CH_4 production both in vitro and in vivo without any adverse effect on rumen fermentation but the drawback is that nitrate is toxic to the animals; so supplementation at lower level helps the animal to acclimatize and reduces the chances of toxicity

Keywords: *Methane (CH_4), sulphates, hydrogen, methanogens, fermentation*

****Author for Correspondence*** E-mail: Sandeepuniyal18@gmail.com

INTRODUCTION

Methane (CH_4), carbon dioxide (CO_2), nitrous oxide (N_2O), and halocarbons are potent greenhouse gases (GHGs). These gases enhance the effects of solar and thermal radiation on the surface of earth resulting in global warming. CH_4 has several natural sources and man-made sources. Anthropogenic sources account for approximately 58% of total global CH_4 emissions (EPA, 2011) [1]. Global livestock agriculture was responsible for 18% of the anthropogenic GHG emissions annually. On a world-wide basis, dairy animals, including cull cows and beef cattle from dairy breeds, are estimated to contribute only 4% to anthropogenic GHG emissions (FAO, 2010). In many developed countries, the contribution of dairy production to GHG emissions is estimated even lower, due to the higher productivity of livestock agriculture [2]. In developing countries with large cattle populations (e.g., Brazil and India), ruminant livestock can be a very large contributor to the national GHG inventory (FAO, 2010). In India, livestock sector accounts for about 13.9% of the global enteric CH_4 emission (global enteric CH_4 emission of 85.63 Tg/year) and the enteric CH_4 emission has been projected to be 12,848 Gg in 2012, and this could be increased to 14,553 Gg in 2020 [3]. Apart from its global warming potential, CH_4 which is produced in the rumen is an energetically wastage full process which results in loss of up to 2–12% of the gross energy consumed by the animals [4]. Several strategies have been adopted for reducing ruminal CH_4 production but the most effective method is to provide alternative electron acceptors such as nitrate and sulphate. These salts more effectively consume reducing equivalents produced during fermentation so as to redirect electron flow away from the reduction of CO_2 to CH_4. Nitrate and sulphate salts have a higher affinity for H_2 than CO_2 and thus acts as a H_2 sink, diverting H_2 from CH_4 formation to nitrite, ammonia and H_2S, respectively [5].Therefore, nitrate and sulphate salt may act as a potential inhibitor to mitigate CH_4 from animals.

RUMEN METHANOGENS AND THEIR IMPORTANCE

Methanogens belong to the domain Archaea. Most archaea identified in the rumen belong to known methanogen clades with a predominance of *Methanobrevibacter* spp. and accounts for nearly two-thirds of rumen archaea. The remaining one-third was composed of roughly equal parts by phylotypes belonging to *Methanomicrobium* and the rumen cluster C [6]. There are three major substrates used by methanogens to produce CH_4—CO_2, compounds containing a methyl group or acetate [7]. In the rumen, the predominant pathway is the hydrogenotrophic using CO_2 as the carbon source and H_2 as the main electron donor. Formate is also an important electron donor used by many rumen hydrogenotrophic methanogens and may account for up to 18% of the CH_4 produced in the rumen. Methylamines and methanol produced in the rumen can also be used by methylotrophic methanogens of the order *Methanosarcinales* and *Methanosphaera* spp. from the order Methanobacteriales [7]. CH_4 is also produced from acetate via the aceticlastic pathway and this pathway appears to be limited to members of the order Methanosarcinales [7]. Effect of nitrate supplementation on methane emission and production performance of animals is presented in Table. 1 and 2, respectively.

NITRATE AS ALTERNATIVE HYDROGEN SINK IN RUMEN

Nitrates may serve as a terminal electron acceptor and therefore may behave as alternate hydrogen sink and can be converted to ammonia and used in the rumen as a source of nitrogen. The nitrate and nitrite along with CO_2 are the hydrogen acceptors in the rumen. Conversion of nitrate to nitrite and finally to ammonia is carried out by rumen bacteria. Plant materials used for ruminant feeding usually contain nitrate as much as 37% of total nitrogen (N). However, the rate of nitrate reduction is 2.5-fold higher than the rate of nitrite utilization, which brings about temporary nitrite accumulation. The reduction of nitrate to nitrite (Gibbs free energy, $\Delta G^o = -130$ kJ/mol of H_2) and the subsequent reduction of nitrite to ammonia ($\Delta G^o = -124$ kJ/mol of H_2) yield more energy than the reduction of CO_2 to CH_4 ($\Delta G^o = -16.9$ kJ/mol of H_2) which is energetically more favorable than reduction of CO_2 to CH_4 [8]. These processes could be the principal route of hydrogen disposal if sufficient nitrate is available in an actively fermenting rumen ecosystem. The reduction of nitrate to ammonia consumes 8 electrons and each mole of nitrate reduced could thus lower CH_4 production by 1 mole. The ammonia generated will be available for anabolism and would be an important supply of fermentable nitrogen on diets deficient in crude protein (CP) where low rumen ammonia may limit microbial protein synthesis [9]. In animals unadapted to nitrate in their diet, the capacity of the rumen microflora to reduce nitrate to nitrite exceeds the capacity for nitrite reduction [10]. This leads to accumulation of nitrite in the rumen, which is readily absorbed across the rumen wall and converts blood hemoglobin (Hb) from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) form. The ferric form of Hb—methemoglobin (MetHb)—renders the molecule incapable of transporting oxygen to the tissues [11]. The resulting condition, called methemoglobinemia, is a state of general anoxia, which in mild cases may depress animal performance but in severe cases may be fatal [12].

Table 1: *Effect of Supplementation of Nitrates on* CH_4 *Production.*

Animals	Dose of nitrate	Effect on CH_4 production	Reference
In vitro	0–10 mM	Decrease in CH_4 productiion by 63%	Sakthivel *et al*., 2012 [14]
Merino wether	4% KNO_3	Decrease in CH_4 productiion by 23%	Nolan *et al*., 2010 [15]
Cross bred steers	22 g $Ca(NO_3)_2$/kg DM	Decrease in CH_4 productiion by 32%	Hulshof *et al*., 2012 [16]
Lactating HF	2.6% Calcium nitrate/kg DM	Decrease in CH_4 productiion by 17%	van Zijderveld *et al*., 2011 [5]
Buffaloes	2% KNO_3/kg DMI	Decrease in CH_4 production by 18%	Sakthivel *et al*., 2012 [14]

HF: Holstein-Friesian; DM: Dry Matter; DMI: Dry Matter Intake.

Table 2: *Effect of Supplementation of Nitrates on Performances of Animals.*

Animals	Dose of nitrate	Effect on methane production	Effect on production performance	References
36 Holstein Steers (288±25 kg) adapted for 25 days	0–3% $CaNO_3$ of dietary DM	Decrease with dose (49%)	-DMI decrease -ADG not affected -Blood MetHb increases	Newbold *et al*., 2014 [17]
44 Merino lambs (22.7±0.17 kg) Experimental duration: 69 days	0–1.88% $CaNO_3$+ 0–0.40% S of dietary DM	Decrease (24%)	-No effect on DMI, ADG, feed conversion efficiency -Increase in clean wool growth by 37%	Li et al. 2013[21]
18 Hiefers Experimental duration: 28 days	0–3% EN of dietary DM	Decrease (19%)	-DMI tend to decrease, no effect on growth -ME intake increases	Lee *et al*., 2015 [18]

ADG: Average daily gain; DM: Dry Matter; DMI: Dry Matter Intake; EN: encapsulated nitrate; ME: metabolizable energy

Careful stepwise introduction of nitrate in the diet of sheep allows the rumen microflora to adapt and increase their capacity to reduce both nitrate and nitrite. Sheep gradually adapted over a period of 10 weeks to high nitrate diets (1.5 g of nitrate/kg of body weight per day) exhibited no clinical signs of methemoglobinemia [13].

SULPHATE AS ALTERNATIVE HYDROGEN SINK

Sulphates also act as potent CH_4 inhibitor in many anaerobic systems including rumen. Reduction of sulphate leads to production of hydrogen sulphide (H_2S) which appears to play a role of electron donor in the reduction of nitrite to ammonia by nitrate-reducing, sulfide-oxidizing bacteria. H_2S acts as a source of sulphide for the rumen microorganisms which are unable to directly utilize sulphate; it also stimulates cellulose degrading bacteria and fungi which requires sulphur in the form of sulphide [19]. Since methanogens and dissimilatory sulphate-reducing bacteria requires hydrogen, encouraging competition between these two groups or rumen microorganisms may reduce ruminal CH_4 emission as the energetics of sulphate reduction (G^o = −152 kJ/mol) are more favorable than methanogenesis (G^o = −131 kJ/mol).

EFFECT OF SUPPLEMENTATION OF SULPHATES ON METHANE PRODUCTION

van Zijderveld *et al*., (2010) [20] supplemented 2.6% sulphate and nitrate to the diet of sheep and found that CH_4 production significantly reduced up to 47% in the group fed sulphate and nitrate in combination as compared to nitrate (37%), sulphate (16%), and control groups. Patra and Yu [3] also observed that supplementation of nitrate and sulphate in combination decreased the *in vitro* CH_4 production by 43% as compared to control.

CONCLUSION

Experimental evidences suggest that both nitrate and sulphate potentially inhibited CH_4 production both *in vitro* and *in vivo* without any adverse effect on rumen fermentation but energy saved in the form of CH_4 was not converted into production in majority of the study.

Nitrates are toxic to the animals so supplementation at lower level may help the animals to acclimatize that may reduce the chances of toxicity. Future research should be focused on energy partitioning when dietary nitrate and sulphate are being used as hydrogen sink to know why dietary energy conserved by reduction of CH_4 is not evident in terms of production performance of the animals.

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