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

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Abstract
Methane (CH₄) and other gases like carbon dioxide (CO₂), nitrous oxide (N₂O), are greenhouse gases (GHGs) that aggravates the effects of solar and thermal radiation on surface and atmospheric temperature. CH₄ is the second largest GHG having 21 times more heat generation potential than CO₂. Global livestock agriculture was responsible for 18% of the anthropogenic GHG emissions annually. CH₄ production in the rumen accounts for 2–12% loss of gross energy and consequently influence performance of ruminants. So, reducing ruminant CH₄ emissions is an important objective for ensuring the sustainability of ruminant-based livestock farming. CH₄ is formed in the rumen by methanogens (part of the domain Archaea), mainly from H₂ and CO₂. Within the rumen microbial food web, methanogens perform the beneficial task of removing H₂, that allows reduced cofactors to be reoxidized and recycled, thereby enhancing the breakdown and fermentation of plant material. Therefore, rumen CH₄ mitigation strategies need to consider alternative routes of H₂ utilization. Nitrates and sulphates are potent inhibitors of CH₄ in many anaerobic system including rumen; these salts have greater affinity for H₂ as compared to CO₂ resulting in net reduction in CH₄ production. Experimental evidence suggest that nitrate and sulphate inhibit CH₄ 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 (CH₄), sulphates, hydrogen, methanogens, fermentation

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INTRODUCTION
Methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), 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. CH₄ has several natural sources and man-made sources. Anthropogenic sources account for approximately 58% of total global CH₄ 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 CH₄ emission (global enteric CH₄ emission of 85.63 Tg/year) and the enteric CH₄ 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, CH₄ 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 CH₄ 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 CO₂ to CH₄. Nitrate and sulphate salts have a higher affinity for H₂ than CO₂ and thus acts as a H₂ sink, diverting H₂ from CH₄ formation to nitrite, ammonia and H₂S.
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 the rumen cluster C [6]. There are three major substrates used by methanogens to produce CH₄—CO₂, compounds containing a methyl group or acetate [7]. In the rumen, the predominant pathway is the hydrogenotrophic using CO₂ as the carbon source and H₂ 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 CH₄ produced in the rumen. Methylamines and methanol produced in the rumin can also be used by methylotrophic methanogens of the order *Methanosarcinales* and *Methanosphaera* spp. from the order *Methanobacteriales* [7]. CH₄ 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 CO₂ 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, ΔG° = −130 kJ/mol of H₂) and the subsequent reduction of nitrite to ammonia (ΔG° = −124 kJ/mol of H₂) yield more energy than the reduction of CO₂ to CH₄ (ΔG° = −16.9 kJ/mol of H₂) which is energetically more favorable than reduction of CO₂ to CH₄ [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 CH₄ 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⁺) to the ferric (Fe³⁺) 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 CH₄ Production.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Dose of nitrate</th>
<th>Effect on CH₄ production</th>
<th>Reference</th>
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<tbody>
<tr>
<td>In vitro</td>
<td>0–10 mM</td>
<td>Decrease in CH₄ production by 63%</td>
<td>Sakthivel et al., 2012 [14]</td>
</tr>
<tr>
<td>Merino wether</td>
<td>4% KNO₃</td>
<td>Decrease in CH₄ production by 23%</td>
<td>Nolan et al., 2010 [15]</td>
</tr>
<tr>
<td>Cross bred steers</td>
<td>22 g Ca(NO₃)₂/kg DM</td>
<td>Decrease in CH₄ production by 32%</td>
<td>Hulshof et al., 2012 [16]</td>
</tr>
<tr>
<td>Lactating HF</td>
<td>2.6% Calcium nitrate/kg DM</td>
<td>Decrease in CH₄ production by 17%</td>
<td>van Zijdeveld et al., 2011 [5]</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>2% KNO₃/kg DMI</td>
<td>Decrease in CH₄ production by 18%</td>
<td>Sakthivel et al., 2012 [14]</td>
</tr>
</tbody>
</table>

HF: Holstein-Friesian; DM: Dry Matter; DMI: Dry Matter Intake.
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 CH₄ inhibitor in many anaerobic systems including rumen. Reduction of sulphate leads to production of hydrogen sulphide (H₂S) which appears to play a role of electron donor in the reduction of nitrite to ammonia by nitrate-reducing, sulfide-oxidizing bacteria. H₂S 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 CH₄ emission as the energetics of sulphate reduction (G° = −152 kJ/mol) are more favorable than methanogenesis (G° = −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 CH₄ 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* CH₄ production by 43% as compared to control.

**CONCLUSION**

Experimental evidences suggest that both nitrate and sulphate potentially inhibited CH₄ production both *in vitro* and *in vivo* without any adverse effect on rumen fermentation but energy saved in the form of CH₄ was not converted into production in majority of the study.

Nitrites 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 CH₄ is not evident in terms of production performance of the animals.

**REFERENCES**


Cite this Article