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Impact of Zootechnical Additive of the Nitrooxypropanol to Minimize Methane Emission in Cattle: A Review

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Abstract

A significant impediment to reducing worldwide enteric CH4 emissions is the problem of adapting mitigation measures to grazing ruminants; this area requires additional investigation. This study aimed to investigate the impact and explore the relationship between raising and lowering levels of 3-NOP in bovine diets in order to impede methanogenesis. The sole CH4 contributor is the end-user process of ruminant fermentation and faeces, especially those of beef and dairy cattle. The amount of carbon dioxide emission from the human body during its lifetime constitutes a small part of the carbon balance in the atmosphere. Their dose-level potential must be assessed whenever feed additives become available in ruminant diets. Feed additives are managed in trace amounts to impact on rumen metabolism. Designed and produced around 2012, 3-Nitrooxypropanol is a synthetic, non-toxic chemical molecule that inhibits the CH4 pathway. Its molecular structure is like that of the methyl coenzyme M. The zootechnical supplement 3-NOP lowers the amount of enteric methane CH4 that cattle release during milk production and reproduction while raising the milk fat concentration. In conclusion, the 3-NOP is established to be a practical CH4 mitigator at elevated and intermediate dosages, recommending that it may have application as an enteric CH4 mitigator.

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Introduction

A major worldwide environmental issue, the greenhouse effect is caused by gases such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and ozone (O_3) that absorb infrared radiation from the atmosphere and contribute to climate change and global warming (Lashof and Ahuja, 1990). Ruminants produce methane, a substantial greenhouse gas (Alvarez-Hess *et al.*, 2019). Methane, the second most major greenhouse gas introduced into the atmosphere, is responsible for 19% of total effective radiative forcing (Thornhill *et al.*, 2021). As in the case of animal digestive tracts, in humans, the primary production of CH_4 occurs through the metabolism of Methanobrevibacter smithii, which is present in the distal colon, and Methanosphaera stadtmanae, a type of methanogenic species (Gaci *et al.*, 2014). To enhance animal performance or lower CH_4 emissions, researchers have tried various treatments targeting the rumen microbiota over the past few decades (Tseten *et al.*, 2022). Diet modification and feed additives lower enteric CH_4 emissions more inexpensively, making them a potentially better option (Honan *et al.,* 2021). Interpretations in feed additive routine are normally presumed to emerge from differences in feed intake, diet composition, roughage quality, and rumen fermentation requirements (Bannink *et al.,* 2023). Feed additives may directly or indirectly suppress methanogenic archaea, reducing CH_4 emissions. As a possible intestinal CH_4 inhibitor, a synthetic molecule called 3-NOP is a structural analogue of methyl-coenzyme M (Duval and Kindermann, 2012; Araújo *et al.,* 2023), (Figure 1). The 3-NOP substance is synthesized by chemo selective reduction to improve the efficacy of calcium channel antagonists (Ogawa *et al.,* 1990). Regarding 3-NOP, the compound has undergone varying phases of experimental testing on several ruminant species, including dairy cows, beef cattle, and sheep (Romero-Pérez *et al.,* 2015). In general, the molecule can reduce enteric CH_4 emissions. A commercial feed additive called 3-NOP has shown promise since it reliably reduces the production of CH_4 in both small and large ruminant trials (Alemu *et al.,* 2021a). Dijkstra *et al.* (2018) have shown that 3-NOP can reduce intestinal CH_4 emissions in dairy cattle by an average of more than 30%. Other studies found that 3-NOP decreases CH_4 emissions in sheep and dairy cattle by up to 60% (Yu *et al.,* 2021). Generally, the rumen molar proportion of acetate has decreased with the administration of 3-NOP and other CH_4 inhibitors, while the molar proportion of propionate has grown and the acetate-to-propionate ratio has decreased simultaneously (Haisan *et al.,* 2014). The 3-NOP exhibits negligible impact on populations of bacteria and protozoa, hence verifying the compound's specificity in targeting Methyl-coenzyme M reductase (MCR) found exclusively in archaea methanogens and not in other rumen microorganisms (Jayanegara *et al.,* 2018). The 3-NOP is added constantly to the diet because it seems to be rapidly metabolized in the rumen (Duin *et al.,* 2016). Despite claims that it reduces enteric CH_4 emissions in dairy and beef cattle, 3NOP has no long-term effect on downstream anaerobic digestion (Nkemka *et al.,* 2019). Nonetheless, longer-term dose-response research with dairy cows at various lactation phases is needed to assess the efficacy of 3-NOP in high-forage diets (Schilde *et al.,* 2021). This review aimed to illustrate the effect and investigate the affinity between extending and reducing levels of 3-NOP in bovine diets to inhibit methanogenesis.

Methane as a Greenhouse gas

The origins of agriculturally emanated methane emissions are enteric fermentation and, to a more secondary extent, ruminant bovine manure, meaning that the livestock industry donates to climate change (Ripple *et al.,* 2014). Methane is the second most abundant gas on the globe, trapping 28 times more heat than carbon dioxide during 100 years (Almeida *et al.,* 2023). Methane from enteric fermentation accounts for 44% of animal greenhouse gas emissions (Kelly *et al.,* 2022).

Effect of Feeding 3-NOP on Ruminal Fermentation

Ruminal gram-positive bacteria a matter of fact are involved in the fermentation process that produces acetate, butyrate, lactate, hydrogen, and ammonia fermentation products that are coupled with methanogenesis (Ratti *et al.,* 2014; Matthews *et al.*, 2019; Shinkai *et al.*, 2024). Kinley *et al.* (2020) have demonstrated that the direct inhibition of CH_4 synthesis causes an increase in H_2 emissions, depending on the strength of CH_4 suppression and the availability of alternate H_2 metabolic routes. Ruminal methanogens, which account for less than 5% of the rumen microbiota, produce all methane in cows (Pitta *et al.*, 2016). Adding 3-NOP impacted total volatile fatty acid (VFA) concentration, with a minor drop of 3-NOP medium dose (Alemu *et al.,* 2021a). The rumen microbiota primarily converts bovine feed to volatile fatty acids (VFA), releasing CO_2 and H_2, which are then used by methanogenic archaea to produce methane (Knapp *et al.,* 2014). Feeding the 3-NOP delayed the methanogenesis in ruminants by increasing H_2 emissions is an inadequate or unsuccessful formulation (Vyas *et al*., 2018; Alemu *et al.*, 2021b). Simply, because the availability of H_2 is a result of minimizing methanogenesis due to feeding the 3-NOP or any effective methanogenesis inhibitors like monensin and other ionophores. This is confirmed in the current review: Methanogens in the rumen convert CO_2 to CH_4 using H_2 generated during rumen microbial fermentation (Morgavi *et al.*, 2010). Various techniques to decrease CH_4 emissions have been proposed, such as adjusting animal management and breeding, refining their meals, and improving the quality of their feed (Hristov *et al.,* 2013). Recently, Honan *et al.* (2021) and Arndt *et al.* (2022) examined the methods for reducing CH_4, such as feed additives added in small amounts to alter rumen metabolism and decrease methanogenesis. Direct suppression of methanogenesis is the most successful strategy for intestinal CH_4 decrease (Almeida *et al.*, 2021).

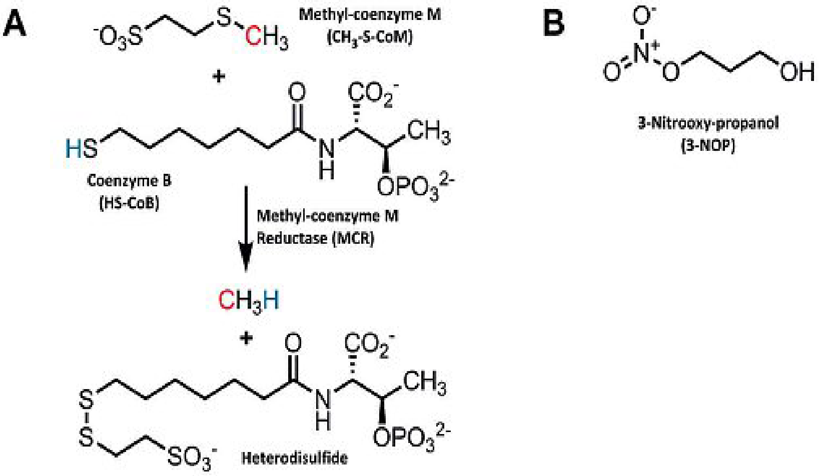

Figure 1. (A) Reaction catalyzed by MCR. (B) Structure of the inhibitor 3-nitrooxypropanol (3-NOP)

Source: Schrickel (2017).

Efficacy and Mechanism of (3-NOP)

The 3-NOP is now approved for use in the European Union, Brazil, and Chile, among other countries (Kebreab, 2022). It is still awaiting registration in the U.S. and Canada (Kebreab, 2023). The initial study conducted on sheep to examine the effectiveness of 3-NOP on generating animals was an in vivo inquiry (Martinez-Fernandez *et al.*, 2014). Because of the chemicals that make up 3-NOP, it is highly soluble and has rapid metabolism in the rumen (Duin *et al.*, 2016). The 3-NOP is a special kind of chemical since it metabolizes into endogenous compounds and possesses two functional groups: an organic nitrate ester group and a primary alcohol (Figure 2) (Thiel *et al.*, 2019a). Three main metabolites are formed when 3NOP is oxidized to 3-nitrooxypropionic acid (NOPA), which is subsequently hydrolyzed to 3-hydroxypropionic acid (HPA) and inorganic nitrate. The trace levels of 3-NOP were detected in plasma two hours after the dose and rapid metabolism (Thiel *et al.,* 2019b). The active ingredient 3-NOP may be dangerous to breathe in, according to the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) conclusion. It is uncomfortable for the skin and irritating to the eyes, but does not affect skin sensitization (FEEDAP, 2021). Kim *et al.* (2019) revealed that the effectiveness of 3-NOP depends on the method of application, dose amount, and nature of the diet. A 3-NOP supplement to ruminants halts the final stage of methanogenesis in the rumen by oxidizing the enzyme Methyl-coenzyme M reductase (MCR) and inhibiting the reduction of CO_2 by dissolved H_2 to generate CH_4 (Duin *et al.*, 2016). The MCR is a crucial enzyme in the process of methanogenesis. This enzyme is saturated by 3-NOP, which lowers the archaea's ability to produce CH_4 (Owens *et al.,* 2020). Feeding 3-NOP to Holstein-Friesian cows in the early stages of lactation is an efficient way to reduce methane emissions while also improving the apparent total tract digestibility of nutrients (Van Gastelen *et al.,* 2020). In high-yielding cows, 3-NOP added at 40–80 mg/kg DM of feed decreased CH_4 emissions by 30% but increased H_2 emissions by 64-fold; the intensity of H_2 emissions declined over time (Hristov *et al.*, 2015).

Sofyan *et al.* (2022) demonstrated that the 3-NOP has a significant inhibiting effect on CH_4 emission and no influence on animal dry matter intake (DMI) in several experiments. Inhibition of ruminal methanogenesis leads to increased molar proportion of propionate at the expense of acetate. Therefore, inhibition of methanogenesis improves the utilization efficiency of metabolizable energy (Melgar *et al.,* 2020). The 3-NOP compound is introduced as a pelleted blend that can be a crucial element of the product in the European dairy market (Van Wesemael *et al.,* 2019). The addition of 3-NOP did not result in changes to the overall numbers of protozoa (Moreno, 2020).

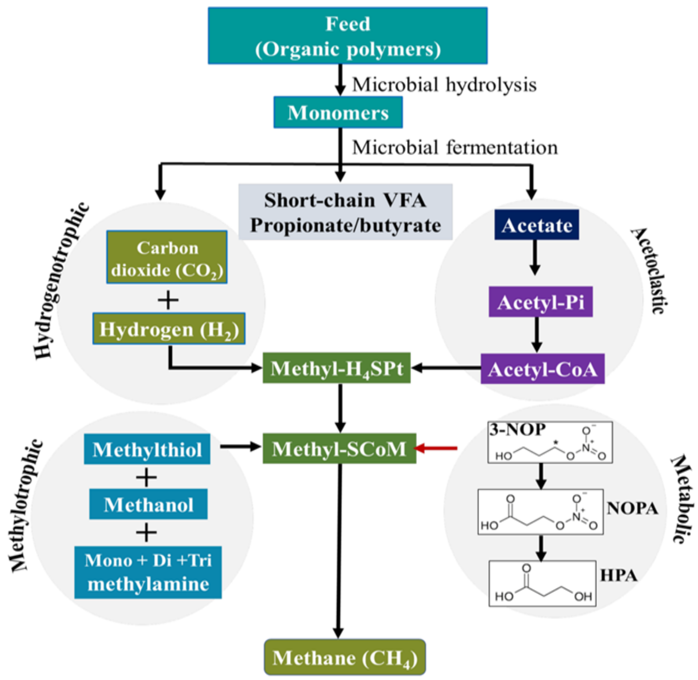

Figure 2. The primary mechanism in the rumen of ruminants that generates CH_4 and how 3-NOP inhibits it. 3-NOP = 3-nitrooxypropanol; NOPA = 3-nitrooxypropionic acid; HPA = 3-hydroxypropionic acid)

Source: Yu *et al.* (2021).

Doses of 3-Nitrooxypropanol

The 3-Nitrooxypropanol, known as Bovaer® 10, is a small-grained white powder that flows smoothly (FEEDAP, 2021). The minimum amount needed for 3-NOP is 10%, with propylene glycol serving as a diluent and dried and precipitated silicic acid as a carrier. For all ruminants to produce milk and reproduce, the supplement should provide a minimum of 52.8 mg 3-NOP and a maximum of 88 mg 3-NOP per kilogram of complete feedstuff (moisture content of 12%), (Schilde *et al.,* 2021). When 3NOP was added (60 mg of feed dry matter), the yield of CH_4 decreased by 21% and 27%, respectively, but the yield of milk remained unchanged, The 200 mg dose reduced ovary size, alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) blood levels (Melgar *et al.,* 2020).

3-Nitrooxypropanol with other feed additives

The combined effects of 3-NOP and monensin (MON) were investigated for the first time with the ionophore monensin (Romero-Pérez *et al.,* 2016). An enzymatic inhibitor called 3-NOP has been shown to reduce enteric (Kononoff, 2024) emissions in ruminants, whereas monensin (MON) has a moderate and occasionally transient effect on CH_4 generation (Romero-Pérez *et al.,* 2016), which means it indirectly reduces it (Gutierrez-Bañuelos *et al.,* 2008). Fatty acid (FA) type also plays a key role by suppressing methanogens, fat and 3-NOP operate differently has a lower capacity for reduction (Ivan *et al.,* 2013). All combinations of fat, nitrate, and 3-NOP did not result in CH_4 reductions that were greater than separate supplementation of the most potent additive within the combination (Maigaard *et al.,* 2024). Implementing 3-NOP and canola oil in animal food rations may be responsible for the CH_4 decrease, but more studies are needed considering these food products' ability to aggravate the digestion of extra substances that lead to worse animal performance (Zhang *et al.,* 2021). Differently from the other inhibitors that act on ruminal bacteria or protozoa entities, 3-NOP is a highly selective inhibitor that targets (Bartzanas *et al.,* 2023) only the guiding rumen methanogens. While canola oil altered the space ecology and its numbers, it also contributed to the lowered count of the rumen protozoa (Zhang *et al.,* 2021). In response to CH_4 inhibition, 3-NOP, and chloroform have demonstrated comparable alterations in rumen metabolism, such as a shift towards increased generation of propionic acid and a decrease in acetate, along with an increase in branched fatty acids (Melgar *et al.,* 2019). Based on Grainger and Beauchemin (2011), the starch content of the diet and subsequent ruminal starch fermentation leads to low ruminal pH, which inhibits the growth of protozoa and methanogens and lowers the concentration of MCR enzymes. Therefore, compared to low-starch diets, adding 3-NOP to high-starch diets may inhibit the MCR enzyme more effectively (Van Gastelen *et al.,* 2022). In commercial beef feedlots, a modest dose of 3-NOP added to corn-based finishing diets resulted in a 76% reduction in CH_4 generation (Alemu *et al.,* 2021b).

Effect of 3-NOP on Animals' Performance

One example of an in vivo chemical inhibitor is 3-NOP (Martins *et al.,* 2024), which can lower intestinal CH_4 emissions in dairy cows by up to 30% (FAO, 2023) without negatively impacting the animals' performance (i.e., milk fat concentration) (Hristov *et al.,* 2022; Martins *et al.,* 2024).

The effectiveness of 3-NOP in decreasing intestinal CH_4 emissions and consequently enhancing feed conversion efficiency in cattle (Moreno, 2020). that are fed diets rich in forages and grains (Vyas *et al.*, 2018). Enteric CH_4 production was proven to be decreased by around 50% when pharmacologic inhibitors were added (Hristov *et al.*, 2013). The findings have shown that dietary supplementation with 3-NOP had a negligible effect on milk yield (Baumont, 2018) DM intake, or milk for fat and protein (Van Gastelen *et al.*, 2019). The 3-NOP therapy validates the encouraging rise in body weight depending on concentration and metabolism (Martinez-Fernandez *et al.*, 2018). The 3-NOP methane mitigation effect decreases before feeding and peaks right after feeding (Hristov and Melgar, 2019; Moreno, 2020).

Table 1. Results of studies on Additive of the 3-nitrooxypropanol (3-NOP) to Minimize Methane in Cattle

Addition amount	Aims	Results	References
The 3-NOP applied at 60 mg/kg feed dry matter	Impact of 3-nitrooxypropanol (3-NOP) on the content of emission enteric methane in from Holstein dairy cows, and production of milk.	The 3-NOP reduced daily methane emission, emission yield, and emission intensity by 26, 27, and 29%, respectively, when treated at 60 mg/kg feed dry matter. Carbon dioxide emissions from the mouth were unaffected, while 3-NOP increased hydrogen emissions six times.	(Melgar *et al.*, 2021)
The 3-NOP applied at 60 mg/kg feed dry matter	Impact of 3-nitrooxypropanol (3-NOP), a forceful methane inhibitor and metabolically.	The 3-NOP applied at 60 mg/kg feed dry matter decreasing in *Methanobrevibacter ruminantium* by 3-NOP feeding.	(Pitta *et al.*, 2021).
The 3-NOP applied at 52 mg /kg DM (3-NOP) and a diet	Impact of 3-nitrooxypropanol (3-NOP) on enteric methane emission in dairy cows in early lactation.	3-NOP applied at 52 mg /kg DM enteric emission of carbon dioxide was not affected, and hydrogen emission was increased.	(Van Gastelen *et al.*, 2019).
The 3-NOP applied at 60 mg/kg feed DM	Impact of 3-nitrooxypropanol (3-NOP) on enteric methane emission, in lactating dairy cows.	The 3-NOP applied at 60 mg/kg feed DM is a peak level of methane relief after feeding and underneath before feeding.	(Hristovand Melgar 2019; Moreno, 2020).

The 3-NOP applied at 2.5 g animal/day	Impact of 3-nitrooxypropanol (3-NOP) on methane (CH_4) and H_2 production.	The 3-NOP applied at 2.5 g animal/day) is an inhibitor of methanogenesis and fermentation process.	(Martinez-Fernandez *et al.*, 2018).

Conclusions

In conclusion, 3-NOP affected dairy animals more antimethanogenic than it did beef cattle. More data induced by this line of research backs the vision that 3-NOP could safely mitigate methane production in cattle. The 3-NOP was proven to be an effective CH_4 mitigator at both high and moderate dosages, suggesting that it may have application as an enteric CH_4 mitigator. Prospective studies should probe the potential additive or synergistic influences of combining dietary mitigation techniques with distinct steps to maximize mitigation potential.

Conflict of Interest

The writers express no conflicts of interest about the journal of this manuscript.

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