Nutritional Mitigation of Greenhouse Gases

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Introduction

Methane (**CH_4**), carbon dioxide (**CO_2**), nitrous oxide (**N_2O**), and halocarbons are greenhouse gases (**GHG**) that are able to trap heat in the atmosphere by radiating less heat into the space and increase the effect of solar and thermal radiation on surface and atmospheric temperatures (Knapp et al., 2014). In 2014, total U.S. GHG emissions measured 6,870 million metric tons of CO_2 equivalents. Agricultural activities contributed about 9% of total GHG emissions (U.S. EPA, 2016). Enteric CH_4 generated during feed digestion accounts for most of livestock's direct impact on total GHG emissions representing about 28.6% of U.S. GHG emissions from agricultural activities in 2014 (U.S. EPA, 2016). Although CH_4 constitutes only about 10.6% of total emissions, it has greater impact because it has 28 times the global warming potential of CO_2 over a 100-yr timespan (Myhre et al., 2013). With an energy content of 55.22 MJ/kg (Brouwer, 1965), CH_4 represents a loss of dietary energy from the animal and typically accounts for about 6-12% of the total gross energy consumed by ruminants (Johnson and Johnson, 1995). Thus, CH_4 production by cattle is both an environmental concern and a potential loss in cattle efficiency. Reducing CH_4 losses is an environmentally sound practice with potential to improve production efficiency. Several comprehensive reviews have been published on strategies for CH_4 mitigation (Beauchemin et al., 2008; McAllister and Newbold, 2008, Hristov et al., 2013; Knapp et al., 2014). This paper will focus only on nutritional strategies to reduce enteric CH_4 emissions.

Nutritional strategies to reduce enteric CH_4 emissions

Dietary strategies to reduce CH_4 emissions were initially explored to increase energy efficiency. The first publication appearing in 1948 investigated the effects of dietary fat utilization and energy efficiency in sheep (Swift et al., 1948). However, the database on nutritional strategies to reduce CH_4 emissions has grown exponentially in last two decades after initial publication on the impact of ruminants on GHG emissions (Johnson and Johnson, 1995). Recently, databases generated for quantification of mitigation strategies for enteric CH_4 emission has shown that dietary manipulation by increasing or substituting concentrates in the diet or lipid supplementation has received greater attention to reduce enteric CH_4 emissions because of their effects on energy use efficiency and production (Veneman et al., 2016). Similarly, the efficacy of improved forage quality has been well explored. However, in the last decade research has focused on inhibiting methanogens and targeting rumen fermentation by use of secondary plant metabolites (tannins or saponins), electron acceptors (nitrate), or feed additives (3-nitrooxypropanol) to reduce CH_4 production. This paper aims to summarize

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nutritional strategies for reducing enteric CH_4 emissions relevant to the ruminant production systems and will be primarily focused on highlighting three strategies considered most effective in reducing enteric CH_4 emissions; namely, lipid supplementation, dietary nitrate, and 3-nitrooxypropanol.

Lipid supplementation

Dietary fat is among the most promising strategies for reducing enteric CH_4 emissions (Beauchemin et al., 2008; Grainger and Beauchemin, 2011; Patra, 2013). Dietary fat reduces CH_4 emissions by decreasing organic matter fermentation in the rumen along with reducing the protozoal numbers (Martin et al., 2016) and activity of methanogens (Popova et al., 2011; Guyader et al., 2015). Lipids with greater proportions of polyunsaturated fatty acids (**PUFA**) may help in reducing methanogenesis via channeling hydrogen towards ruminal biohydrogenation of unsaturated fatty acids; however, based on stoichiometric (Czerkawski, 1986) and modeling approaches (Mills et al., 2001), only 1-2% of metabolic hydrogen in the rumen is used for this purpose.

The efficacy of adding dietary lipids to reduce CH_4 emissions is affected by various factors including fat source, fatty acid profile, form in which fat is administered (i.e. either as refined oil or as full-fat oilseeds), level of supplementation, and the type of diet. Grainger and Beauchemin (2008) observed a linear decline in CH_4 production with increasing level of total fat ranging from 1 to 13.1% of dietary DM from 27 studies (**Figure 1**). Similar results were observed when total dietary fat concentration was restricted to < 8% of dietary DM. In another meta-analysis study, Patra (2013) indicated that enteric CH_4 emissions (g/kg of DM or g/kg of milk) declined linearly with increasing dietary lipid concentration when total fat concentration was restricted to < 5% in diets. Based on the results observed from previous meta-analysis studies, it was interpreted that with each percentage unit increase in total fat concentration, CH_4 emissions will be reduced by 0.66 g/kg of DMI (Patra, 2013), 1 g/kg of DMI (Grainger and Beauchemin, 2011), or 0.79 g/kg of DMI (Moate et al., 2011).

Fatty acid composition of dietary fat seems to have an inconsistent effect on CH_4 yield. While Grainger and Beauchemin (2011) observed no relationship between fatty acid composition and CH_4 yield, Martin et al. (2010) observed greater CH_4 reduction with lauric acid (**C12:0**) and myristic acid (**C14:0**). Similarly, Patra (2013) reported C12:0 and PUFA (**C18:3**) as potent inhibitors of methanogenesis while fatty acids C16:0, C18:0, and C18:2 were not effective at reducing CH_4 emissions. Previous studies have proposed that medium chain fatty acids mitigate CH_4 emissions by reducing the abundance and metabolic activity of methanogens (Lillis et al., 2011; Patra and Yu, 2013) while the effects unsaturated fatty acids might be mediated via reducing abundance of methanogens and channeling hydrogen during the biohydrogenation process.

While the effects of dietary lipids on methanogenesis has been well studied, most of the previous studies were short-term and we are still lacking enough literature on the persistence of the anti-methanogenic potential of lipid supplementation (Hristov et al.,

2013). Woodward et al. (2006) reported short-term efficacy of vegetable and fish oil in reducing CH_4 emissions in pasture-fed dairy cows; however, the effects disappeared after 11 wk of feeding lipids. On the contrary, the effects of extruded linseed in reducing CH_4 emissions persisted for one year in dairy cows fed diets based on grazed pasture or grass silage (Martin et al., 2011). Similarly, Grainger and Beauchemin (2011) analyzed 6 long-term studies and reported greater persistence of reduced CH_4 emissions with dietary fat; however, results were inconsistent.

The mitigation strategies using dietary fats should carefully consider its negative impact on DMI, milk yield, and milk fat and protein concentration. Patra (2013) observed increased milk yield in response to fat supplementation; however, while milk yield increased initially, plateau was reached between 3.9-6% total dietary fat concentration and milk yield decreased thereafter. Similarly, DMI levels decreased when dietary fat concentration was > 4.2%. In addition, DM and neutral detergent fiber (**NDF**) digestibility was linearly reduced with increasing fat concentration (Patra, 2013). Previous reviews have also observed negative effects on intake levels with lipid supplementation (Chilliard, 1993; Allen, 2000). While rumen inert fat sources did not affect DMI, oil sources (vegetable oils, medium chain fatty acids) significantly reduced DMI (Knapp et al., 2014). Some lipid sources like vegetable oil, containing unsaturated fatty acids or coconut oil containing medium chain fatty acids might have greater efficacy in reducing CH_4 emissions; however, it might largely be achieved by reduced intake levels, thereby reducing milk yield in the long-term. Lipids causing this kind of production effect cannot be recommended as mitigation agents (Hristov et al., 2013).

Nitrate supplementation.

The CH_4 mitigation potential of supplemental nitrate has received considerable attention recently as nitrate can act as an alternative hydrogen sink in the rumen that competes with CH_4 formation (Lee and Beauchemin, 2014). In the rumen, nitrate is first reduced to nitrite, and is then further reduced to ammonia. Nitrate reduction is considered a thermodynamically more favorable pathway than the reduction of CO_2 to CH_4 and therefore suppresses CH_4 production (Lee and Beauchemin, 2014). Dietary nitrate as a feed additive to mitigate enteric CH_4 emissions is considered an effective strategy based on its consistent and persistent efficacy between studies (Lee et al., 2015). Recently, a meta-analysis from 8 studies including data from sheep, beef cattle, and dairy cattle showed a linear decline in CH_4 production with increasing intake of dietary nitrate per kg of BW (**Figure 2**; Lee and Beauchemin, 2014). Similarly, several studies have reported CH_4 mitigation in the range of 16-25% in CH_4 yield (g/kg of DMI) at nitrate inclusion levels of 2.1% of DMI (van Zijderveld et al., 2011; Lund et al., 2014; Lee et al., 2015; Klop et al., 2016; Olijhoek et al., 2016) in dairy cattle. In addition, long-term persistence of CH_4 mitigation has also been reported with dietary nitrate (Li et al., 2012; El-Zaiat et al., 2014) further confirming the usefulness of feeding nitrate as a potential strategy to mitigate enteric CH_4 emissions from ruminants. The combination of nitrate with other mitigation strategies such as sulfate (van Zijderveld et al., 2010) and linseed oil (Guyader et al., 2015) has been shown to be additive in terms of reducing CH_4 emissions. However, the barrier to the use of nitrate in practical feeding conditions is its potential toxicity. As mentioned earlier, dietary nitrate introduced into the rumen is

reduced to nitrite and ammonia. However, depending on the rate of nitrate reduction, nitrate and nitrite can accumulate in ruminal fluid and absorbed via the rumen wall. While nitrate that appears in blood is not toxic, nitrite binds to red blood cells, gets oxidized to nitrate and changes the ferrous (Fe^{2+}) form of haemoglobin to the ferric (Fe^{3+}) form (methemoglobin) resulting in reduced oxygen carrying capacity of blood causing tissue hypoxia and death (Bruning-Fann and Kaneene, 1993; Leng, 2008). Various factors affect potential toxicity of nitrate in ruminants including the levels and consumption rate of dietary nitrate, along with nitrate and nitrite reducing capacity in the rumen (Lee et al., 2015). The strategy to lower nitrate toxicity includes acclimation by gradual increase in supplemental nitrate thereby increasing the population of ruminal microbes that are able to reduce nitrate and nitrite to ammonia. Shi et al. (2012) confirmed greater nitrate reduction in ruminal fluid from sheep acclimated to nitrate.

From a nutritional perspective, nitrate could potentially replace urea as a non-protein nitrogen (**NPN**) source for microbial protein synthesis as shown by comparable effects on feed intake and production levels in ruminants (Li et al., 2012; El-Zaiat et al., 2014) ensuring that levels of dietary nitrate are below the levels causing potential toxicity and that a proper acclimation period is used. In addition, previous studies have reported either no effects (Nolan et al., 2010; Li et al., 2012) or greater (Lee et al., 2015) total-tract DM digestibility in response to supplemental nitrate. Hence, with no effects on DM digestibility and significant CH_4 mitigation, supplemental nitrate has the potential to increase energy efficiency and productivity in ruminants. However, previous studies conducted over short-term (Lee et al., 2015) and long-term (Li et al., 2012) periods have observed no improvement in production by feeding nitrate. Similarly, Lee and Beauchemin (2014) observed no responses of live weight gain to feeding nitrate in ruminants. Lack of effects on production might be attributed to inefficient energy utilization of hydrogen when used to reduce nitrate to ammonia compared to when used for methanogenesis because 44% of free energy is lost during nitrate reduction compared to a 6% energy loss during CH_4 formation (van Zijderveld, 2011).

3-nitrooxypropanol.

3-Nitrooxypropanol (**3-NOP**) is a novel strategy to reduce CH_4 production by inhibiting methyl-coenzyme M reductase (**MCR**), which catalyzes the biosynthesis of CH_4 (Duin et al., 2016). It has been suggested that 3-NOP, at micromolar concentration, inactivates MCR by oxidation of its active site (Ni^{+1}) required for the CH_4-forming step in rumen fermentation. Also, inhibitory effects of 3-NOP were demonstrated against methanogenic archaea without inducing any effects on growth of non-methanogenic bacteria in the rumen (Duin et al., 2016).

The first in vitro study to investigate the efficacy of 3-NOP reported an 86-96% reduction in CH_4 production without affecting the concentration of volatile fatty acids (**VFA**). This was followed by in vivo experiments with sheep which demonstrated a 26% reduction in CH_4 yield (g/kg of DMI) with 3-NOP provided at 100 g/d (Martinez-Fernandez et al., 2014). While no negative effects were observed on DMI or live weight gain, CH_4 reduction was accompanied by a reduced acetate-to-propionate ratio (Martinez-Fernandez et al., 2014). Similarly, Reynolds et al. (2014) reported a 4.4 and a

6.7% reduction in CH_4 production with 3-NOP supplemented at 0.5 and 2.5 g/d without affecting DMI, digestibility, or milk yield. However, CH_4 mitigation was accompanied by a reduction in total methanogens, VFA, and molar proportion of acetate while propionate proportion was increased with a higher dose of 3-NOP. Haisan et al. (2014) observed a 60% reduction in CH_4 production with 3-NOP provided at 2.5 g/d. The greater efficacy in reducing CH_4 emissions in this study was attributed to the mode of providing NOP to the cows (mixed with the feed) compared to ruminal dosing in the earlier study (Reynolds et al., 2014). Recently, Hristov et al. (2013) observed an average 30% reduction in CH_4 production when NOP was added to the diet of lactating dairy cows at 40, 60, and 80 mg of NOP/kg of DM over 12 weeks. No adaption to 3-NOP was reported. Both previous studies (Haisan et al., 2013; Hristov et al., 2015) observed increased BW gain suggesting partial redirection of energy from CH_4 to tissue deposition for cows receiving 3-NOP. Likewise, in beef cattle, Romero-Perez et al. (2014) investigated 3 doses of 3-NOP equivalent to 0.75, 2.25 and 4.50 mg/kg of DM and observed a 33% CH_4 reduction at the highest level of supplementation along with a linear reduction in the acetate-to-propionate ratio. No effects were observed on diet digestibility. In another study, Romero-Perez et al. (2015) reported a sustained decrease of methanogenesis for 112 d. Similarly, sustained reduction of enteric CH_4 emissions was demonstrated in beef cattle fed backgrounding and finishing diets for 105 d each. While the extent of CH_4 mitigation with a backgrounding diet (**Figure 3**) was 29% (g/kg of DMI), an 84% reduction in CH_4 emissions was observed with 3-NOP supplemented at 200 mg/kg of DM with high-grain diets (Vyas et al., 2016a). Furthermore, gain-to-feed ratio tended to increase in animals fed high-forage diets supplemented with 3-NOP (**Figure 4**) and it can be speculated that moderate reductions in CH_4 emissions (approximately 30%) appears to be associated with improved performance and energy efficiency, perhaps because the changes in the rumen ecosystem are not drastic (Vyas et al., 2016a). While strong reductions (approximately 80%) in CH_4 emissions might spare energy, negative effects on the rumen ecosystem leading to reduced utilization of the spared energy cannot be overlooked. Vyas et al. (2016b) reported that the optimal dose of 3-NOP supplementation in beef cattle fed high-forage and high-grain diets ranges from 100-200 mg/kg of DM for reducing CH_4 emissions without inducing any negative effects on production parameters.

Hence, based on results from previous studies, dietary supplementation of 3-NOP has consistently reduced enteric CH_4 emission. Moreover, no adaptation to 3-NOP was observed when supplemented over the long-term. Additionally, no negative effects were observed on nutrient digestibility and animal performance and no risk in terms of food safety have been reported to date. However, this product in not available for commercial use as toxicology studies are still being carried out to support registration as a feed additive in the U.S.

Conclusions

Nutritional manipulation is an effective strategy to reduce enteric CH_4 emissions and its impact would be achieved by approaches that would be feasible under practical feeding conditions. Farmers would tend to choose options for CH_4 mitigation that are simple, cost-effective, and without compromising feed efficiency and farm profitability.

Dietary strategies discussed in this paper (lipids, nitrate, and 3-NOP) are promising in persistently reducing CH_4 emissions. While guidelines for addition of fat to TMR diets have been developed to maximize milk production, future studies are still required to establish nitrate and 3-NOP as feed additives.

The demand for animal source food to feed an increasing world population will require more animals, and so total global CH_4 emissions will increase. However, strategies to mitigate CH_4 emissions should focus on reducing the amount of emissions/kg of livestock product. Development of mitigation strategies to reduce CH_4 emissions, while not lowering animal production, is critical to achieving this goal.

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Figure 1. Linear and curvilinear relationships between dietary fat concentration and CH_4 yield. Linear equation: Y = 24.65 (±0.890) - 0.103 (±0.0109)X; curvilinear equation Y = 26.50 (±1.270)− 0.187 (±0.0430)X + 0.0007 (±0.00037)X^2 (Adapted from Grainger and Beauchemin,2011).

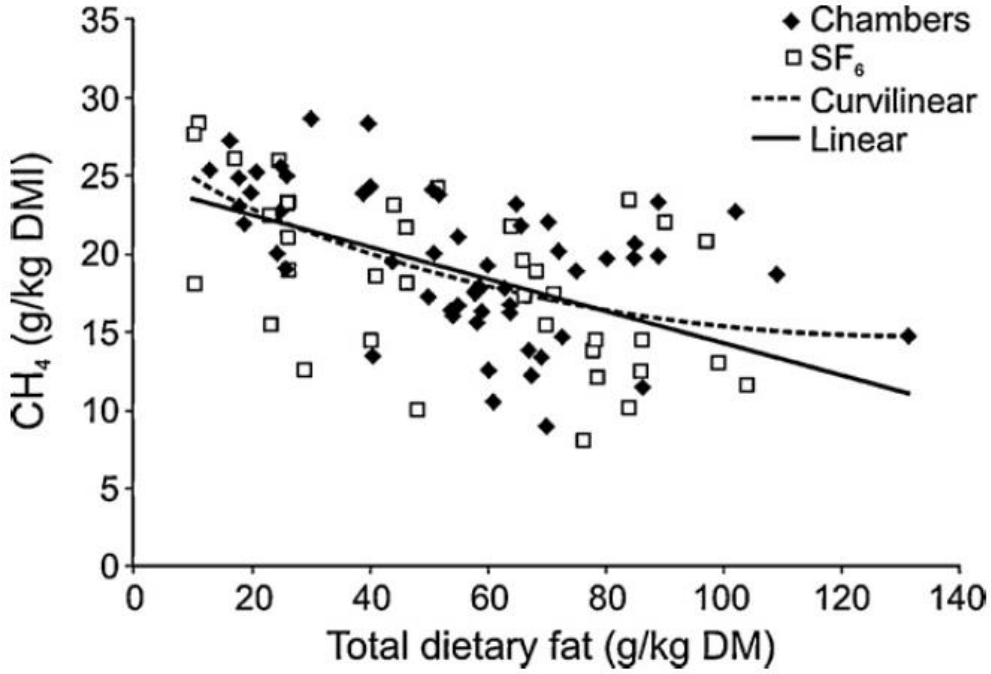


Figure 2. The effects of increasing dietary nitrate in ruminant animals on enteric methane emission responses; Y = 41.3 × nitrate (g kg^{-1} BW d^{-1}) + 1.2; R^2 = 0.76, *P* < 0.001 (Figure adapted from Lee and Beauchemin, 2014).

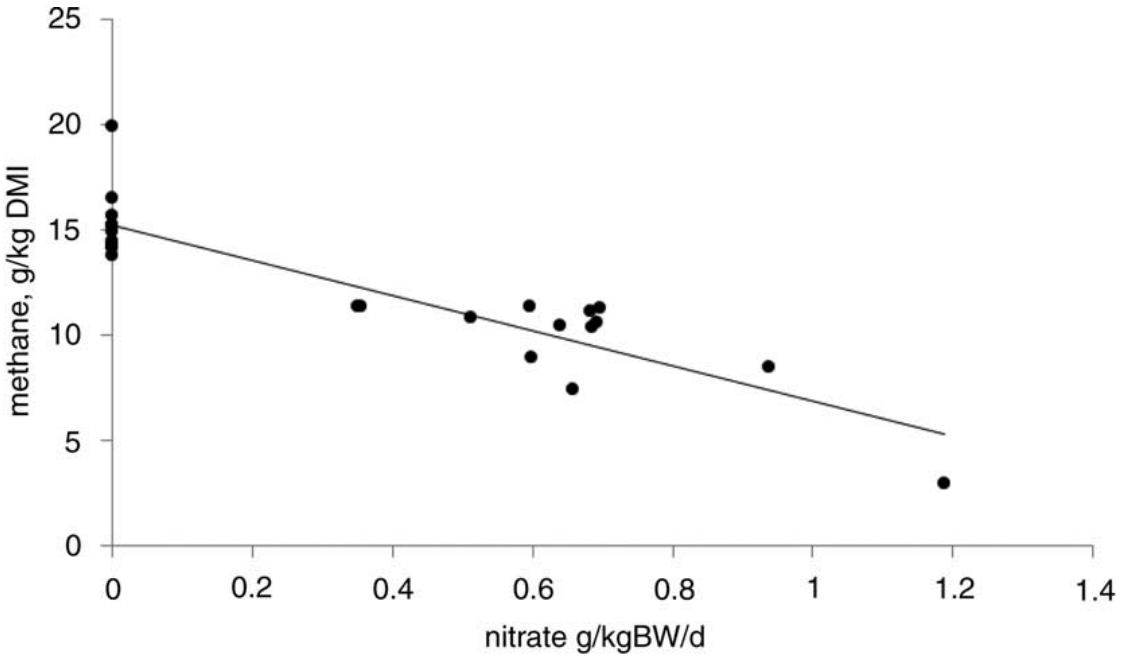


Figure 3. Total CH_4 emissions post-feeding in feedlot animals fed a high-forage diet supplemented with control, low (100 mg/kg), and high (200 mg/kg) doses of 3-nitrooxypropanol; $P < 0.01$ (Adapted from Vyas et al., 2016a).

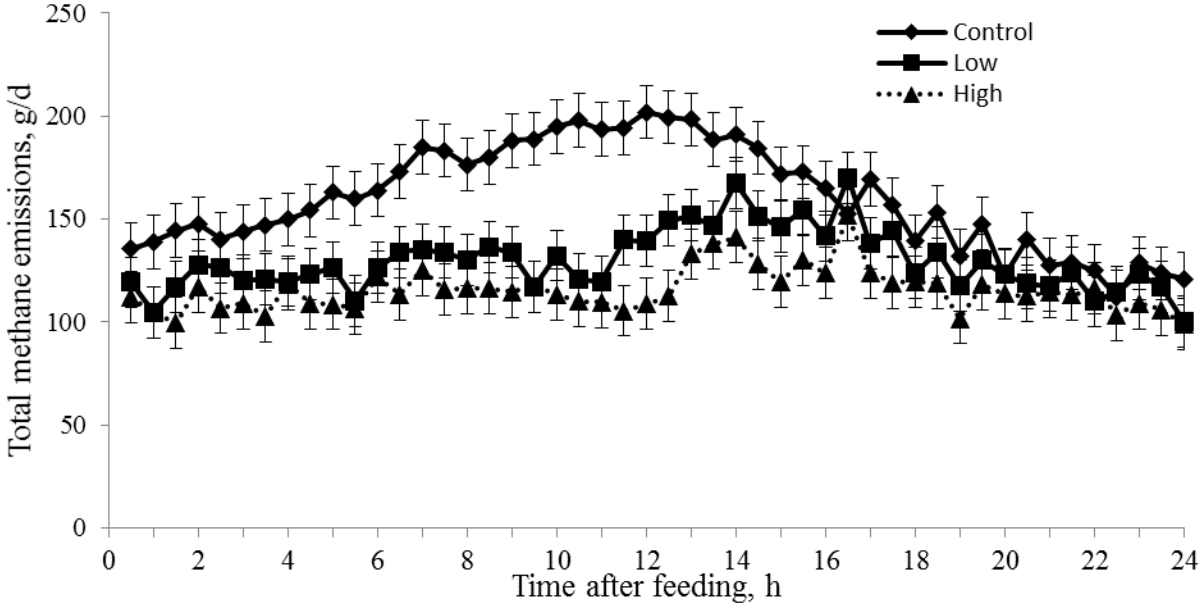
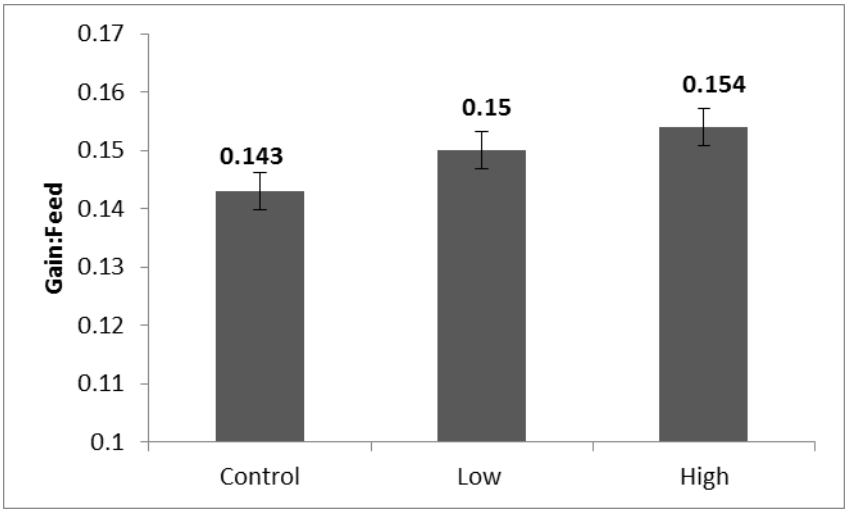


Figure 4. Gain-to-feed ratio and ADG in feedlot animals fed high-forage diets supplemented with control, low (100 mg/kg of DM), and high (200 mg/kg of DM) doses of 3-nitrooxypropanol; $P = 0.06$ (Vyas et al., 2016a).



SESSION NOTES