# The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets[1]

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**ABSTRACT:** The study objective was to evaluate the combined effects of supplementing monensin (MON) and the methane ($CH_4$) inhibitor 3-nitrooxypropanol (NOP) on enteric $CH_4$ emissions, growth rate, and feed conversion efficiency of backgrounding and finishing beef cattle. Two hundred and forty crossbred steers were used in a 238-d feeding study and fed a backgrounding diet for the first 105 d (backgrounding phase), transition diets for 28 d, followed by a finishing diet for 105 d (finishing phase). Treatments were as follows: 1) control (no additive); 2) MON (monensin supplemented at 33 mg/kg DM; 3) NOP (3-nitrooxypropanol supplemented at 200 mg/kg DM for backgrounding or 125 mg/kg DM for finishing phase); and 4) MONOP (33 mg/kg DM MON supplemented with either 200 mg/kg DM or 125 mg/kg DM NOP). The experiment was a randomized complete block (weight: heavy and light) design with 2 (NOP) × 2 (MON) factorial arrangement of treatments using 24 pens (8 cattle/pen; 6 pens/treatment) at the main feedlot and 8 pens (6 cattle/pen; 2 pens/treatment) at the controlled environment building (CEB) feedlot. Five animals per treatment were moved to chambers for $CH_4$ measurements during both phases. Data were analyzed using a Mixed procedure of SAS with pen as experimental unit (except $CH_4$). Location (Main vs. CEB) had no significant effect and was thus omitted from the final model. Overall, there were few interactions between MON and NOP indicating that the effects of the 2 compounds were independent. When cattle were fed the backgrounding diet, pen DMI was decreased by 7%, whereas gain-to-feed ratio (G:F) was improved by 5% with NOP supplementation ($P < 0.01$). Similarly, MON improved G:F ratio by 4% ($P < 0.01$), but without affecting DMI. During the finishing phase, DMI tended ($P = 0.06$) to decrease by 5% with both MON (5%) and NOP (5%), whereas ADG tended ($P = 0.08$) to decrease by 3% with MON. Gain-to-feed ratio for finishing cattle was improved with NOP by 3% ($P < 0.01$); however, no effects were observed with MON. 3-Nitrooxypropanol decreased $CH_4$ yield (g/kg DMI) by 42% and 37% with backgrounding and finishing diets ($P \leq 0.01$), respectively, whereas MON did not lower $CH_4$ yield. Overall, these results demonstrate efficacy of NOP in reducing enteric $CH_4$ emissions and subsequently improving feed conversion efficiency in cattle fed high-forage and high-grain diets. Furthermore, effects of NOP did not depend on whether MON was included in the diet.

**Key words:** beef, enteric methane, inhibitor, ionophore, 3-nitrooxypropanol

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## INTRODUCTION

The environmental footprint of beef production is under constant scrutiny (Legesse et al., 2016). The beef industry contributes to greenhouse gas emissions, mainly due to methane (**$CH_4$**) produced during enteric fermentation. Furthermore, $CH_4$ production is a loss of potential energy for the animal representing up to 12% of total gross energy intake (Johnson and Johnson, 1995). Hence, strategies to mitigate $CH_4$ production from beef cattle may help lessen environmental concerns and improve feed efficiency, although few studies actually link decreased $CH_4$ production to improved animal performance.

The $CH_4$ inhibitor 3-nitrooxypropanol (**NOP**) is a promising mitigation strategy because it has been shown to consistently decrease $CH_4$ emissions when supplemented to high-forage (Romero-Perez et al., 2014, 2015; Vyas et al., 2016a,b) or high-grain diets (Vyas et al., 2016a,b). Furthermore, no negative effects on feed digestibility have been reported (Romero-Perez et al., 2014), and risks in terms of food safety are low. However, information on the practicalities of using NOP in commercial feedlots is lacking.

The ionophore monensin (**MON**) is routinely used in commercial feedlot diets to improve energy and nitrogen (**N**) utilization (Ruiz et al., 2001; Appuhamy et al., 2013). The effect of MON on efficiency of energy use is attributed to its selective inhibition of gram-positive bacteria resulting in greater propionate proportion. Monensin may be a potential $CH_4$ mitigation strategy due to its effects on increasing ruminal propionate production and G:F (Beauchemin et al., 2008). Although some studies have reported significant reduction (6.5% to 12%) in $CH_4$ production due to feeding MON (Van Vugt, 2005; Odongo et al., 2007), others have found no $CH_4$ reduction (McGinn et al., 2004; Grainger et al., 2008; Waghorn et al., 2008).

The current study explored the effects of NOP, when supplemented alone or in combination with MON, in growing and finishing cattle diets. Because the mode of action of NOP and MON differ, we hypothesized that the efficacy of NOP in decreasing $CH_4$ production would not be affected by MON, regardless of whether a high-forage or high-grain diet was fed.

## MATERIALS AND METHODS

The experiment was approved by Lethbridge Research and Development Center Animal Care and Use Committee under the guidelines of the Canadian Council of Animal Care (2009). The study was conducted with approval from the Veterinary Drugs Directorate of Health Canada (Experimental Studies Certificate; DSTS No. 186831).

### *Animals, Diets, and Experimental Design*

Two hundred and forty crossbred yearling steers were received at the Center, adapted to facilities, and processed according to standard management procedures including ear-tagging for identification and vaccination for infectious bovine rhinotracheitis, bovine viral diarrhea, and parainfluenza-3. Following the receiving phase, steers were stratified by weight (heavy, light) and blocked into 12 heavy and 12 light pens at the main feedlot (8 cattle/pen) and 4 pens each for heavy and light weight groups at a secondary [Controlled Environment Building (**CEB**)] feedlot (6 cattle/pen). Treatments were randomly assigned within each block at the main (6 pens/treatment) and CEB (2 pens/treatment) feedlots. Pens were equipped with fence-line feed bunks and automatic waterers. Eight pens at the main feedlot were equipped with the GrowSafe Feed Intake system (GrowSafe Systems Ltd, Airdrie, AB, Canada), and animals in the GrowSafe pens were fitted with radio frequency ear-tags allowing each meal of individual animals to be recorded.

Steers were used in a 238-d feeding trial and were fed high-forage diets based on barley silage for the first 105 d (backgrounding phase; Table 1). The animals were transitioned to the finishing diets for 28 d by gradually increasing the proportion of barley grain in the diet. The high-grain diets (i.e., finishing phase; Table 1) based on barley grain were fed for the last 105 d. Diets were formulated to provide adequate ME and MP for 300-kg growing beef cattle with an ADG of 1 kg/d during backgrounding phase. Similarly, high-grain diets provided adequate

**Table 1.** Ingredient and chemical composition of the basal diets

| | High forage[1] | | High grain[1] | |
|---|---|---|---|---|
| Item | Control | Monensin | Control | Monensin |
| Ingredients, % of DM | | | | |
| Barley silage | 65.0 | 65.0 | 8.0 | 8.0 |
| Barley, dry rolled | 25.0 | 25.0 | 87.0 | 87.0 |
| Supplement | 10.0 | 10.0 | 5.0 | 5.0 |
| Canola meal | 3.966 | 3.967 | 1.521 | 1.521 |
| Barley, ground | 5.520 | 5.504 | 2.170 | 2.154 |
| Canola oil | 0.051 | 0.051 | 0.052 | 0.052 |
| Limestone | 0.135 | 0.135 | 0.922 | 0.922 |
| Salt | 0.047 | 0.047 | 0.048 | 0.048 |
| Urea | 0.180 | 0.180 | 0.184 | 0.184 |
| Molasses | 0.056 | 0.056 | 0.057 | 0.057 |
| Feedlot premix[2] | 0.045 | 0.045 | 0.046 | 0.046 |
| Rumensin | — | 0.016 | — | 0.016 |
| Chemical composition[3] | | | | |
| DM, % | 50.7 ± 0.31 | 50.7 ± 0.31 | 86.8 ± 1.11 | 86.8 ± 1.10 |
| OM, % | 92.7 ± 0.27 | 92.7 ± 0.31 | 96.4 ± 0.47 | 96.3 ± 0.44 |
| CP, % | 14.2 ± 0.52 | 14.2 ± 0.61 | 13.9 ± 0.86 | 13.9 ± 0.88 |
| NDF, % | 40.5 ± 1.52 | 40.4 ± 1.48 | 18.4 ± 0.63 | 18.4 ± 0.67 |
| ADF, % | 21.5 ± 0.50 | 21.5 ± 0.49 | 7.03 ± 0.7f2 | 7.05 ± 0.74 |
| Starch, % | 31.7 ± 1.81 | 31.8 ± 1.68 | 54.9 ± 2.86 | 54.8 ± 2.88 |

[1]The high-forage diet was fed from d 1 to d 105 (backgrounding phase) followed by sequential adaptation (transition phase) to a high-grain diet (finishing phase, 105 d).

[2]Feedlot vitamin–mineral premix contained $CaCO_3$, 34.83%; $CuSO_4$, 10.31%; $ZnSO_4$, 28.37%; ethylenediamine dihydriodide (80% concentration), 0.15%; selenium 1% (10,000 mg Se/kg), 5.04%; $CoCO_3$, 0.08%; $MnSO_4$, 14.61%; vitamin A (500,000,000 IU/kg), 1.72%; vitamin D (500,000,000 IU/kg), 0.17%; and vitamin E (500,000 IU/kg), 4.73%.

[3]Determined using samples pooled by diet every 3 wk within each phase; all values except DM are expressed on a DM basis.

ME and MP for 400-kg beef cattle with ADG of 2 kg/d (NRC, 2000). Dietary NOP supplementation was discontinued after the end of the finishing phase. Steers remained on their respective treatment diets, but without NOP, for a minimum of 4 wk prior to slaughter in accordance with the requirement of the Experimental Studies Certificate.

The experiment was conducted as a randomized block (heavy, light) design with 4 treatments arranged in a 2 × 2 factorial layout; 2 levels of NOP (with, without) were combined with 2 levels of MON (with, without). Treatments were as follows: 1) control (no additive); 2) MON (monensin supplemented at 33 mg/kg DM); 3) NOP (3-nitrooxypropanol supplemented at 200 mg/kg DM for backgrounding or 125 mg/kg DM for finishing phase); and 4) MONOP (33 mg/kg DM MON supplemented with either 200 mg/kg DM or 125 mg/kg DM NOP). The MON was incorporated into the supplement at the time of manufacturing, whereas the NOP was homogeneously mixed into the total mixed ration daily. The NOP dose used during the backgrounding phase was based on a previous study (Vyas et al., 2016a), whereas the dose provided during the finishing phase was lowered to 125 mg/kg DM to avoid the excessive reduction in $CH_4$ production observed previously (Vyas et al., 2016a).

### *Measurements*

Cattle were weighed (nonfasted BW) at the start and end of the backgrounding and finishing phases on 2 consecutive days. During the study, cattle were weighed at the end of each 3-wk period (nonfasted BW) before feeding. Average daily gain was calculated by phase as the difference between the initial and final BW divided by the total number of days of feeding. Feed conversion efficiency was measured as G:F (kg gain/kg feed DM) for all pens including GrowSafe pens and determined from ADG and daily DMI calculated by 3-wk period and averaged over the entire phase.

Dry matter intake was determined for the pens weekly as the difference between feed offered and weekly refusals, corrected for DM content. The amounts of feed offered were measured daily, whereas refusals were measured weekly. Samples of the feed offered and the refusals were taken weekly for DM determination. The chemical composition (DM, OM, CP, starch, NDF, and ADF contents)

of the total mixed ration samples was determined on samples pooled by 3-wk periods.

***Enteric gas production.*** Twenty steers from the CEB feedlot were used for enteric $CH_4$, $CO_2$, and $H_2$ gas measurement. Gas emissions were measured from individual steers using open-circuit calorimetry chambers, and each steer was accustomed to the chambers before the study to minimize stress. Animals were trained before entering the chambers to minimize stress. The training protocol involved making animals aware of the chamber surroundings by walking them through the chambers at least once initially, followed by keeping animals in the chambers for 2 to 4 h. Similar steps were repeated multiple times depending on each animal's behavior. Final step of the training involved keeping animals in chambers for 24 h for monitoring intake and behavior. Because only 4 chambers were available at a time, animals were divided into 5 groups of 4 animals each (1 animal/treatment per group). The measurements took place over a 5-wk period. During each week, 4 animals (1 animal/treatment) were randomly allotted to the chambers such that each chamber had an animal from a different treatment. The animals remained in the chamber for 3 consecutive days. During backgrounding phase, measurement period was from 48 to 79 d of the feeding period. Similarly, measurements during finishing phase were taken from 63 to 94 d. Individual daily DMI was measured for all animals during $CH_4$ measurements.

Each chamber measured 4.4 m wide × 3.7 m long × 3.9 m high (63.5 $m^3$; model C1330; Conviron Inc., Winnipeg, MB, Canada). The processes involved in air circulation in the chambers were described by Avila-Stagno et al. (2013). Each chamber housed an animal, and the stall inside the chamber was equipped with a feeder, drinking bowl, and fitted with a rubber mattress. Methane (model Ultramat 5E; Siemens Inc., Karlsruhe, Germany) and $CO_2$ analyzers (model LI-7000, LI-COR Environmental, Lincoln, NE) sequentially monitored the concentrations of $CH_4$ and $CO_2$, respectively, in the intake and exhaust air ducts as described earlier (Romero-Perez et al., 2015). The total quantity of $CH_4$ and $CO_2$ emitted in the chambers was quantified by measuring the gradient of influx and exhausted concentrations and volumes. Methane and $CO_2$ concentrations were recorded every 30 min by a calibrated infrared gas analyzer. The air volume in each chamber was exchanged every 5 min. Concentrations of $H_2$ were monitored using a Breath Tracker Digital Microlyzer (Quintron Instrument Co., Milwaukee, WI) from gas samples taken in vacutainers from exhaust and intake air ducts every 3-h postfeeding when the animals were in chambers.

***Rumen fermentation parameters.*** Thirty-two steers were used from the GrowSafe pens (4 steers/pen) for rumen fluid collection (8 steers/treatment). Rumen fluid was collected orally 3 h after morning feeding using a tube connected to a vacuum pump. Samples were collected every 3 wk of backgrounding and finishing phases. About 100 mL of samples were collected, and care was taken to eliminate saliva contamination by discarding the initial 200 mL of sample and any subsequent sample with signs of saliva contamination.

***Feeding behavior.*** The electronic feed bunk monitoring system (GrowSafe Systems) was used to collect feeding behavior data from day 1 to 105 for the backgrounding phase and day 1 to 105 for the finishing phase. Eight pens with 8 animals per pen were equipped with the GrowSafe systems. Each pen contained 2 feed tubs (0.38 × 0.53 × 0.91 m) suspended on 2 load cells. Each animal was tagged with radio frequency identification (**RFID**) tags and the electronic monitoring system of the GrowSafe pens identified and recorded the presence of animal at the feed bunk by scanning the RFID. Data collection and transmission from the GrowSafe systems were described earlier (Schwartzkopf-Genswein et al., 2011). The bunk attendance data including feeding time and frequency were summarized by establishing a meal criterion of 300 s based on previous studies for similar types of animals (Sowell et al., 1998; Parsons et al., 2004; Schwartzkopf-Genswein et al., 2011). Hence, each meal was defined as a series of feeding events, during which time the animal was actively consuming feed from the feed tub with its head in the down position, separated by short periods of inactivity (head-up position) of ≤300 s. Thus, each meal began and ended with a period of inactivity > 300. The summarized data from the GrowSafe systems allowed us to calculate meal duration (min/d), head-down duration (min/d), and meal frequency (number of meals/d).

***Carcass characteristics.*** All steers were slaughtered at a federally inspected facility at the end of the finishing phase, following the 4-wk withdrawal period. Hot carcass weight, salable meat yield, and marbling were determined by quality graders. Dressing percentage was calculated individually as hot

carcass weight divided by final BW × 100%. Salable meat yield was estimated with consideration for the length, width, and fat cover of the rib eye muscle between the 11th and 12th ribs (Yang et al., 2012).

### *Laboratory Analyses*

Chemical analyses were performed in duplicate, and the analysis was repeated when the coefficient of variation was greater than 5%. The DM content for samples was determined by drying in forced-air oven at 55 °C for 72 h. Dried samples were ground through 1-mm screen in a Wiley mill (A. H. Thomas Co., Philadelphia, PA). Dried ground samples were dried at 135 °C for 2 h (method 930.15; AOAC, 2016), followed by hot weighing for the determination of analytical DM. The OM content was calculated as the difference between 100 and the percentage ash (method 942.05; AOAC, 2005). Both NDF and ADF were determined based on Van Soest et al. (1991) with heat-stable amylase and sodium sulfite used in the NDF procedure. Nitrogen and starch contents were determined on samples ground using a ball grinder (Mixer Mill MM2000; Retsch, Haan, Germany). Starch content was determined by enzymatic hydrolysis of α-linked glucose polymers (Hall, 2015), whereas nitrogen content was determined by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instrumentals, Milan, Italy).

Ruminal VFA were quantified using GC (model 5890, Hewlett Packard, Wilmington, DE) with a capillary column (30 m × 0.32 mm × 1 μm; ZB-FFAP, Phenomenex Inc., Torrance, CA) and flame ionization detector. For VFA, the oven temperature was 170 °C for 4 min, which was then increased by 3.5 °C/min to 190 °C and held at this temperature for 2.5 min. The injector temperature was 225 °C, the detector temperature was 250 °C, and the carrier gas was helium. Crotonic acid was used as internal standard for determination of VFA (Ottenstein and Bartley, 1971). Ruminal ammonia nitrogen ($\mathbf{NH_3}$**-N**) concentration was determined by the salicylate–nitroprusside–hypochlorite method using flow injection analyzer (Sims et al., 1995).

### *Statistical Analysis*

Normality of distribution and homogeneity of variance were determined using the univariate procedure of SAS (SAS Inst., Inc., Cary, NC). The data were subsequently analyzed as 2 × 2 factorial design using a MIXED procedure of SAS. For performance, feeding behavior, rumen fermentation, and carcass characteristics, pen (animal in the case of gas emissions) was the experimental unit. The statistical model included the fixed effects of MON, NOP, and MON × NOP. Day (within period) was used as a repeated measure in the model for the $CH_4$ observations. For $CH_4$ measurements, the data were averaged on days, and animal was the experimental unit ($n$ = 5/treatment). In the case of significant interactions, the PDIFF option was included in the LSMEANS statement to account for multiple comparisons. Time-series covariance structure was modeled using the options of autoregressive order one, compound symmetry, and unstructured order one. The best time-series covariance structure was selected based on the lowest Akaike and Bayesian information criteria. Data are presented as least squares means ± SEM. Statistical significance was declared at $P \leq 0.05$, and a tendency to significance was declared at $0.05 < P \leq 0.10$.

## RESULTS

The study explored the individual and combined effects of MON and NOP using diets typically consumed in western Canadian beef feedlots. Barley silage was the major ingredient in the high-forage diet (650 g/kg DM), whereas the high-grain diet was primarily (870 g/kg DM) based on barley grain (Table 1). The chemical composition of the various ingredients is shown in Table 2.

The lack of interaction ($P \geq 0.12$) between NOP and MON for DMI, ADG, and G:F for cattle fed backgrounding diets indicates that the effect of each compound was independent (Table 3). Dry matter intake was decreased by 7% with NOP ($P < 0.01$); however, intake was not affected by MON ($P = 0.12$). No treatment effects were observed on ADG and final BW ($P \geq 0.21$); however, G:F was increased by 4% with MON ($P < 0.01$) and by 5% with NOP ($P < 0.01$). No treatment effects ($P \geq 0.20$) were observed for head-down eating duration and meal duration with MON and NOP; however, meal frequency tended ($P = 0.07$) to decrease with MON supplementation.

For finishing diets, there were no interactions between the additives ($P \geq 0.44$) for animal performance variables (Table 4). No treatment effects ($P \geq 0.82$) were observed on final BW. Both MON and NOP tended ($P = 0.06$) to decrease DMI by 5% compared with control. Monensin tended ($P = 0.08$) to decrease ADG by 3%, whereas no effects on ADG were observed for NOP. Gain:feed ratio was improved by 3% with NOP ($P < 0.01$),

**Table 2.** Chemical composition of dietary ingredients during backgrounding and finishing phases

| | High forage[1] | | | | High grain[1] | | | |
|---|---|---|---|---|---|---|---|---|
| Chemical composition[2] | Barley silage | Barley grain | Control supplement | Monensin supplement | Barley silage | Barley grain | Control supplement | Monensin supplement |
| DM, % | 28.8 ± 0.47 | 91.2 ± 0.64 | 91.7 ± 0.51 | 91.6 ± 0.44 | 31.7 ± 5.65 | 91.6 ± 0.72 | 91.1 ± 0.72 | 92.0 ± 0.53 |
| OM, % | 90.1 ± 0.30 | 97.8 ± 0.08 | 93.1 ± 0.49 | 93.1 ± 0.93 | 91.3 ± 1.95 | 97.8 ± 0.12 | 77.5 ± 4.23 | 76.3 ± 3.50 |
| CP, % | 12.1 ± 0.38 | 12.8 ± 0.71 | 30.8 ± 0.92 | 30.7 ± 1.86 | 11.6 ± 0.47 | 13.2 ± 0.86 | 30.5 ± 1.52 | 30.6 ± 1.87 |
| NDF, % | 49.9 ± 1.11 | 22.3 ± 2.58 | 24.9 ± 1.57 | 24.4 ± 1.13 | 45.6 ± 1.04 | 15.9 ± 0.46 | 19.3 ± 2.89 | 18.8 ± 3.68 |
| ADF, % | 29.3 ± 0.47 | 5.09 ± 0.50 | 12.1 ± 0.69 | 11.9 ± 0.55 | 25.5 ± 1.83 | 5.17 ± 0.56 | 9.80 ± 1.81 | 10.1 ± 2.15 |
| Starch, % | 21.2 ± 1.84 | 59.7 ± 1.30 | 29.7 ± 2.90 | 31.1 ± 1.60 | 24.1 ± 2.90 | 59.2 ± 2.84 | 28.8 ± 3.23 | 26.7 ± 3.51 |

[1]The high-forage diet was fed from d 1 to d 105 (backgrounding phase) followed by sequential adaptation (transition phase) to a high-grain diet (finishing phase, 105 d).

[2]Determined using ingredient from samples pooled every 3 wk within each phase; all values except DM are expressed on a DM basis.

**Table 3.** Dry matter intake, gain-to-feed ratio, and ADG in feedlot animals fed high-forage diets[1] supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 200 mg/kg DM), and combination of MON and NOP

| | −MON | | +MON | | | Effect, *P* value | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM | MON | NOP | MON × NOP | Period |
| All cattle[1] | | | | | | | | | |
| No. of steers (pens) | 60 (8) | 60 (8) | 60 (8) | 60(8) | — | — | — | — | — |
| Initial BW, kg | 308.3 | 308.0 | 307.5 | 309.6 | 2.22 | 0.86 | 0.69 | 0.58 | — |
| Final BW, kg | 461.8 | 459.3 | 463.9 | 463.9 | 3.32 | 0.31 | 0.71 | 0.71 | — |
| ADG, kg | 1.45 | 1.43 | 1.47 | 1.46 | 0.020 | 0.21 | 0.41 | 0.99 | <0.01 |
| Daily DMI, kg/animal | 8.41 | 7.64 | 8.08 | 7.64 | 0.100 | 0.12 | <0.01 | 0.12 | <0.01 |
| Gain:feed | 0.172 | 0.184 | 0.183 | 0.189 | 0.0020 | <0.01 | <0.01 | 0.12 | <0.01 |
| Behavior cattle[2] | | | | | | | | | |
| No. of steers (pens) | 16 (2) | 16 (2) | 16 (2) | 16 (2) | | | | | |
| Meal duration, min/d | 173.2 | 172.2 | 177.1 | 170.1 | 8.10 | 0.92 | 0.64 | 0.73 | |
| Head-down duration, min/d | 64.0 | 77.7 | 75.8 | 59.6 | 9.70 | 0.77 | 0.91 | 0.20 | <0.01 |
| Meal frequency, events/d | 12.6 | 12.7 | 11.4 | 12.1 | 0.38 | 0.07 | 0.33 | 0.46 | <0.01 |

[1]Data combined from Main and CEB feedlots. Main feedlot had 24 pens, 8 animals per pen, and 6 pens per treatment, and CEB feedlot had 8 pens, 6 animals per pen, and 2 pens per treatment. Location effect was not significant.

[2]DMI was determined for the pens weekly as the difference between feed offered and weekly refusals, corrected for DM content. Daily DMI per animal was estimated by pen DMI divided by number of days and number of animals per pen.

[3]Data were collected from 8 GrowSafe pens (8 animals/pen; 16 animals/treatment).

whereas no effects were observed with MON ($P$ = 0.58). Feeding behavior including meal frequency, head-down duration, and meal duration was lower in the finishing compared with the backgrounding phase; however, none of the indices were altered with MON and NOP treatments ($P \geq 0.22$).

Contrary to the observations for cattle fed the high-forage diets in feedlot conditions, no NOP effects were observed for DMI of animals in the chambers ($P$ = 0.71; Table 5). However, MON tended to decrease chamber DMI ($P$ = 0.09). For cattle fed high-forage diets, daily $CH_4$ production was decreased ($P < 0.01$) by 13% and 42% with MON and NOP, respectively. The interaction between MON and NOP was significant for total $CH_4$ production ($P < 0.01$) because NOP decreased $CH_4$ production to a greater extent when MON was not added to the diet. However, when $CH_4$ production was corrected for intake and expressed as $CH_4$ yield (g/kg DMI), the effect of NOP was consistent regardless of whether MON was supplied as evidenced by the lack of NOP × MON interaction. There was no longer an effect of MON on $CH_4$ ($P$ = 0.65), but feeding NOP decreased $CH_4$ yield by 42% ($P < 0.01$). Correspondingly, $H_2$ production was increased with NOP ($P < 0.01$), whereas no effects were observed with MON ($P$ = 0.77) or MON × NOP ($P$ = 0.77). No treatment effects ($P \geq 0.48$) were observed on total $CO_2$ produced per animal per day.

Hourly total $CH_4$ emissions postfeeding of high-forage diets showed decreased emissions with MON ($P$ = 0.08), NOP ($P < 0.01$), and MON × NOP ($P < 0.01$) treatments (Fig. 1). Total $CH_4$ emissions

**Table 4.** Dry matter intake, gain-to-feed ratio, and ADG in feedlot animals fed high-grain diets[1] supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 125 mg/kg DM), and combination of MON and NOP

| | −MON | | MON | | | Effect, *P* value | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM | MON | NOP | MON × NOP | Period |
| All cattle[1] | | | | | | | | | |
| No. of steers (pens) | 60 (8) | 60 (8) | 60 (8) | 60(8) | — | — | — | — | — |
| Initial BW, kg | 506.9 | 503.9 | 512.2 | 513.3 | 3.80 | 0.06 | 0.81 | 0.60 | — |
| Final BW, kg | 697.5 | 692.2 | 693.6 | 696.5 | 5.24 | 0.97 | 0.82 | 0.44 | — |
| ADG, kg | 1.80 | 1.79 | 1.73 | 1.74 | 0.040 | 0.08 | 0.98 | 0.76 | <0.01 |
| Daily DMI, kg/animal[2] | 12.1 | 11.4 | 11.4 | 11.0 | 0.27 | 0.06 | 0.06 | 0.51 | <0.01 |
| Gain:feed | 0.150 | 0.152 | 0.152 | 0.159 | 0.0020 | 0.58 | <0.01 | 0.66 | <0.01 |
| Behavior cattle[3] | | | | | | | | | |
| No. of steers (pens) | 16 (2) | 16 (2) | 16 (2) | 16 (2) | | | | | |
| Meal duration, min/d | 89.0 | 89.4 | 87.4 | 79.3 | 3.93 | 0.22 | 0.40 | 0.31 | <0.01 |
| Head-down duration, min/d | 35.3 | 41.1 | 38.0 | 26.6 | 3.70 | 0.32 | 0.62 | 0.18 | <0.01 |
| Meal frequency, events/d | 8.52 | 8.49 | 7.92 | 7.40 | 1.820 | 0.29 | 0.75 | 0.87 | |

[1] Data combined from Main and CEB feedlots. Main feedlot had 24 pens, 8 animals per pen, and 6 pens per treatment and CEB feedlot had eight pens, 6 animals per pen and 2 pens per treatment. Location effect was not significant.

[2] DMI was determined for the pens weekly as the difference between feed offered and weekly refusals, corrected for DM content. Daily DMI per animal was estimated by pen DMI divided by number of days and number of animals per pen.

[3]Data were collected from 8 GrowSafe pens (8 animals per pen; 16 animals per treatment).

**Table 5.** Enteric methane ($CH_4$) emissions from feedlot animals fed high-forage diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 200 mg/kg DM), and combination of MON and NOP

| | −MON | | MON | | | Effect, *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM | MON | NOP | MON × NOP |
| No. of steers | 5 | 5 | 5 | 5 | — | — | — | — |
| DMI, kg/d | 6.79 | 6.23 | 5.19 | 6.11 | 0.340 | 0.09 | 0.71 | 0.14 |
| $CH_4$, g/animal per day | 190.3[a] | 87.4[c] | 138.8[b] | 102.6[c] | 10.40 | <0.01 | <0.01 | <0.01 |
| $CH_4$, g/kg of DMI | 28.2 | 15.7 | 28.1 | 17.1 | 1.48 | 0.65 | <0.01 | 0.62 |
| $H_2$, g/animal per day | 0 | 2.26 | 0 | 1.95 | 0.440 | 0.77 | <0.01 | 0.77 |
| $CO_2$, g/animal per day | 7,399 | 6,434 | 6,676 | 6,775 | 605.2 | 0.76 | 0.48 | 0.39 |

[a,b] Values within a row with different letters differ ($P \leq 0.05$).

Days, day × NOP, day × MON, day × NOP × MON were not significant for any of the above mentioned variable.

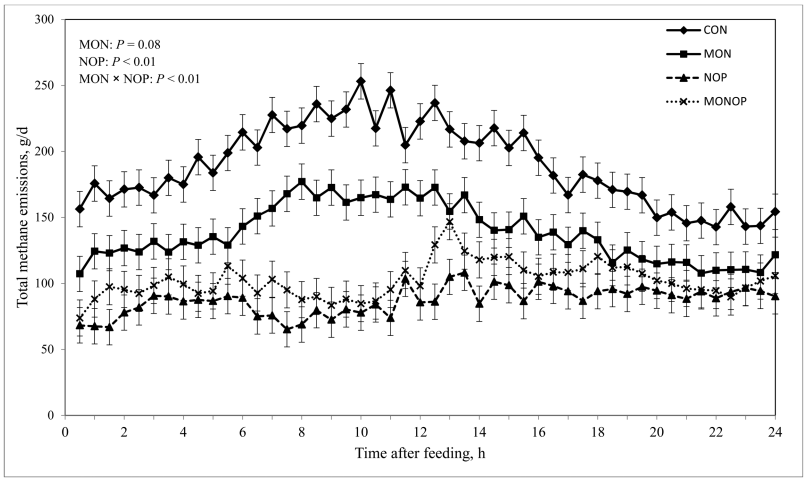


**Figure 1.** Hourly enteric methane ($CH_4$) emissions from feedlot cattle fed high-forage diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 200 mg/kg DM), and combination of MON and NOP ($n$ = 5 steers/treatment).

were consistently lower starting from 0- until 24-h postfeeding for MON, NOP, and MONOP, compared with the control. Correspondingly, $H_2$ emissions were increased with NOP ($P < 0.01$), whereas no effects were observed with MON ($P = 0.73$; Fig. 2). Postprandial variations in $H_2$ emissions showed greater accumulation for NOP with peak response observed 9-h postfeeding ($P < 0.01$).

For cattle fed a high-grain diet, MON decreased chamber DMI ($P = 0.04$), whereas no effects were observed with NOP ($P = 0.91$; Table 6). Total $CH_4$ production and $CH_4$ yield were lowered by 41% and 37% with NOP ($P = 0.01$), respectively. No MON ($P = 0.45$) effects were observed on total $CH_4$ production; however, $CH_4$ yield tended to increase for MON ($P = 0.09$). There was no MON × NOP interaction for $CH_4$ production or yield ($P \geq 35$). Hydrogen gas tended (MON × NOP, $P = 0.07$) to accumulate to a lesser extent when NOP was supplemented with MON, whereas MON decreased ($P < 0.01$) hydrogen production. No treatment effects ($P \geq 0.72$) were observed on total $CO_2$ produced per animal per day. Hourly $CH_4$ production postfeeding showed consistent reduction with NOP ($P < 0.01$; Fig. 3). Similarly, $H_2$ production was increased ($P < 0.01$) with NOP with greater accumulation at 12-h postfeeding ($P < 0.01$; Fig. 4).

Rumen fermentation parameters were altered with NOP and MON supplementation in animals fed high-forage diets (Table 7). The interaction between MON and NOP ($P = 0.04$) for total VFA occurred because NOP decreased total VFA only when not supplemented with MON, whereas there were no effects with MON ($P = 0.69$). The molar proportion of acetate was decreased ($P < 0.01$), whereas the proportion of propionate was increased ($P < 0.01$) with NOP. Monensin supplementation had no effects on ruminal acetate proportion ($P = 0.23$); however, molar proportion of propionate was increased ($P < 0.01$). Acetate-to-propionate ratio was decreased with MON ($P < 0.01$) and NOP ($P < 0.01$) with no interaction effects ($P = 0.70$). Ammonia-N concentration was lower with NOP ($P = 0.01$) and MON ($P = 0.04$).

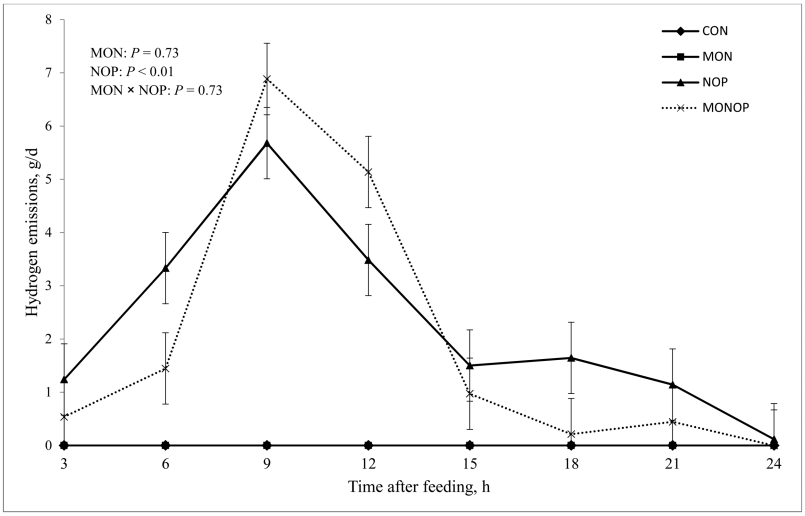


**Figure 2.** Hourly hydrogen emissions from feedlot cattle fed high-forage diet supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 200 mg/kg DM), and combination of MON and NOP ($n = 5$ steers/treatment).

**Table 6.** Enteric methane ($CH_4$) emissions from feedlot animals fed high-grain diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 125 mg/kg DM), and combination of MON and NOP

| | −MON | | MON | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM | MON | NOP | MON × NOP |
| No. of steers | 5 | 5 | 5 | 5 | — | — | — | — |
| DMI, kg/d | 10.2 | 9.90 | 8.12 | 8.57 | 0.690 | 0.04 | 0.91 | 0.64 |
| $CH_4$, g/animal per day | 160.1 | 73.9 | 155.7 | 112.4 | 20.50 | 0.45 | 0.01 | 0.35 |
| $CH_4$, g/kg of DMI | 15.9 | 8.32 | 19.1 | 13.8 | 2.16 | 0.09 | 0.01 | 0.64 |
| $H_2$, g/animal per day | 0.09 | 8.01 | 0.02 | 1.63 | 1.480 | 0.06 | <0.01 | 0.07 |
| $CO_2$, g/animal per day | 11,311 | 11,388 | 11,042 | 10,534 | 1,877.2 | 0.72 | 0.90 | 0.75 |

Days, day × NOP, day × MON, day × NOP × MON were not significant for any of the above mentioned variable.

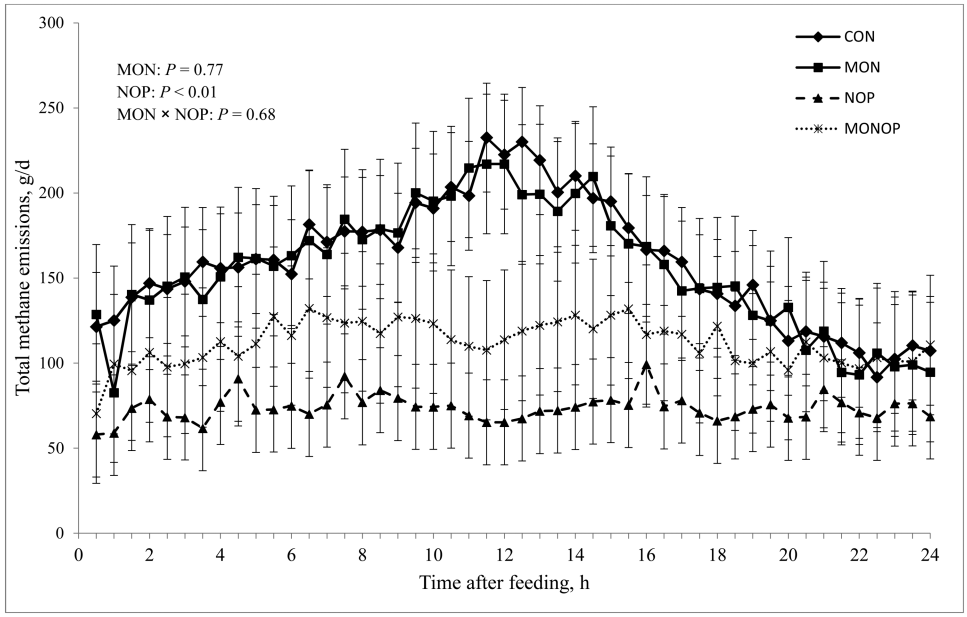


**Figure 3.** Hourly enteric methane ($CH_4$) emissions from feedlot cattle fed high-grain diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 125 mg/kg DM), and combination of MON and NOP ($n$ = 5 steers/treatment).

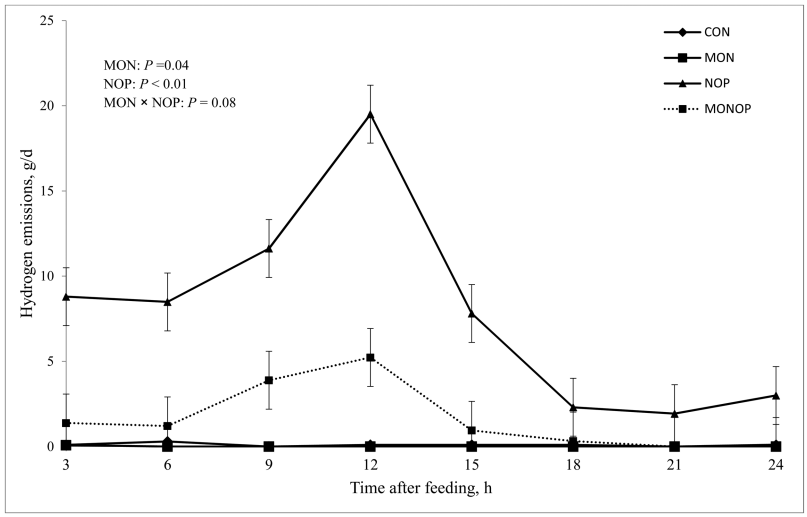


**Figure 4.** Hourly hydrogen emissions from feedlot animals fed high-grain diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 125 mg/kg DM), and combination of MON and NOP ($n$ = 5 steers/treatment).

Neither MON ($P$ = 0.99) nor NOP ($P$ = 0.59) supplementation affected total VFA production in animals fed high-grain diets (Table 8). However, ruminal acetate proportion ($P$ < 0.01) was decreased, whereas ruminal propionate proportion ($P$ < 0.01) was increased with NOP. Correspondingly, acetate-to-propionate ratio was decreased with NOP ($P$ = 0.01). Monensin supplementation decreased caproate proportion ($P$ = 0.04) and tended to decrease valerate proportion ($P$ = 0.09), whereas no effects were observed on other individual VFA proportions. No treatment effects were observed on $NH_3$-N concentration ($P \geq 0.29$).

Neither MON nor NOP affected ($P \geq 0.10$) carcass characteristics including hot carcass weight, grade fat, rib eye area, marbling quality, marbling level, and saleable meat (Table 9). Similarly, liver score and dressing percentage were similar for all treatments.

## DISCUSSION

3-Nitrooxypropanol supplementation is considered a highly effective strategy for mitigating $CH_4$ emissions in beef cattle fed high-forage (Romero-Perez et al., 2014, 2015; Vyas et al., 2016a,b) and high-grain diets (Vyas et al., 2016a,b). To date, it has been shown to have no negative effects on beef cattle performance (Vyas et al., 2016a,b). Ionophores such as MON are commonly used in beef cattle

diets in North America to improve feed efficiency and nutrient utilization (Russell and Strobel, 1989). Additionally, MON has antimethanogenic potential due to its antimicrobial properties and its efficacy in reducing acetate-to-propionate ratio by diverting reducing equivalents toward ruminal propionate synthesis (Beauchemin et al., 2008). Monensin supplementation up to 48 mg/kg has recently been approved by the Canadian Feed Inspection Agency for improving feed efficiency in beef cattle (Canadian Feed Inspection Agency, 2017). Despite widespread use of MON in feedlot cattle diets, previous studies have not explored possible interaction effects of NOP and MON on $CH_4$ mitigation and animal performance. A novel finding of the present research is that the effects of NOP were independent of those of MON in both high-forage and high-grain diets. Most variables examined showed a lack of significant interaction between NOP and MON except total $CH_4$ production (uncorrected for differences in intake). Hence, the discussion is focused on the main effects on MON and NOP considering the responses to these additives were generally independent.

### *Monensin*

Monensin is typically used in dairy and beef cattle diets for improving efficiency of milk and meat production, respectively (McGuffey et al., 2001). The effects of MON on improving energy efficiency are largely attributed to greater ruminal propionate synthesis as a consequence of a shift in the microbial population toward gram-negative bacteria due to selective inhibition of gram-positive bacteria (Ellis et al., 2012). Recently, metagenomic characterization of the ruminal microbiome in response to MON supplementation showed greater abundance of gram-negative bacterial genera including *Megasphaera* and *Selenomonas* that are considered propionic acid producers (Thomas et al., 2017). Although changes in ruminal microbial profiles were not measured in the present study, it is possible that similar changes in the microbiome occurred, at least for the high-forage diet where molar proportion of propionate was increased with MON supplementation. Ruminal propionate is the primary substrate for hepatic gluconeogenesis and glucose synthesized via propionate provides 24% to 61% of total energy production in ruminants (Young, 1977; Bergman, 1990). In the present study, increased propionate proportion in animals fed a MON supplemented high-forage diet was consistent with the observed increase in G:F.

However, the effects of MON on improving feed efficiency are inconsistent (Duffield et al., 2012). Although some studies observed greater feed efficiency with MON (Steen et al., 1978; Horton and Stockdale, 1981), no effects were observed in others (Horton, 1984; Yang et al., 2010). The effects of MON on feed efficiency are influenced by various factors including MON dose, mode of delivery, and dietary composition (Duffield et al., 2012). In the present study, the lack of effect of MON on the proportion of propionate for animals fed the high-grain diet was consistent with the lack of effect on G:F. Duffield et al. (2012) summarized 40 peer-reviewed articles and 24 additional trial reports with MON supplementation in beef cattle and observed greater improvements in feed efficiency when MON was

**Table 7.** Rumen fermentation characteristics from feedlot animals fed high-forage diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 200 mg/kg DM), and combination of MON and NOP

| | −MON | | +MON | | | Effect, *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM | MON | NOP | MON × NOP |
| No. of steers | 8 | 8 | 8 | 8 | — | — | — | — |
| Total VFA, mM | 93.1[a] | 74.5[c] | 84.9[ab] | 80.6[bc] | 2.90 | 0.69 | <0.01 | 0.04 |
| Individual VFA, mol/100 mol | | | | | | | | |
| Acetate (A) | 62.8 | 56.9 | 62.3 | 56.0 | 0.550 | 0.23 | <0.01 | 0.68 |
| Propionate (P) | 18.1 | 20.9 | 20.2 | 23.5 | 0.57 | <0.01 | <0.01 | 0.57 |
| Isobutyrate | 1.16 | 1.25 | 1.15 | 1.30 | 0.060 | 0.71 | 0.12 | 0.64 |
| Butyrate | 13.1 | 15.1 | 12.0 | 13.4 | 0.53 | 0.04 | 0.02 | 0.50 |
| Valerate | 1.80 | 1.89 | 1.58 | 1.66 | 0.060 | 0.02 | 0.22 | 0.99 |
| Isovalerate | 1.98 | 2.97 | 2.06 | 3.40 | 0.170 | 0.16 | <0.01 | 0.31 |
| Caproate | 0.97 | 0.88 | 0.66 | 0.63 | 0.060 | <0.01 | 0.28 | 0.56 |
| A:P ratio | 3.48 | 2.80 | 3.15 | 2.43 | 0.060 | <0.01 | <0.01 | 0.70 |
| $NH_3$-N, mM | 6.98 | 4.88 | 5.52 | 4.12 | 0.380 | 0.04 | 0.01 | 0.37 |

[a,b]Values in a row not bearing a common letter differ ($P \leq 0.05$).

**Table 8.** Rumen fermentation characteristics from feedlot animals fed high-grain diets supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 125 mg/kg DM), and combination of MON and NOP

| | −MON | | +MON | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM | MON | NOP | MON × NOP |
| No. of steers | 8 | 8 | 8 | 8 | — | — | — | — |
| Total VFA, mM | 101.8 | 91.9 | 96.6 | 97.2 | 8.16 | 0.99 | 0.59 | 0.54 |
| Individual VFA, mol/100 mol | | | | | | | | |
| Acetate (A) | 48.7 | 44.1 | 48.6 | 44.8 | 0.62 | 0.70 | <0.01 | 0.59 |
| Propionate (P) | 37.5 | 42.6 | 39.0 | 42.5 | 1.44 | 0.58 | <0.01 | 0.55 |
| Isobutyrate | 1.05 | 1.06 | 1.10 | 1.00 | 0.060 | 0.89 | 0.51 | 0.36 |
| Butyrate | 7.87 | 7.18 | 7.34 | 7.19 | 0.520 | 0.57 | 0.37 | 0.55 |
| Valerate | 2.87 | 3.19 | 2.18 | 2.88 | 0.290 | 0.09 | 0.09 | 0.47 |
| Isovalerate | 1.68 | 1.44 | 1.63 | 1.28 | 0.240 | 0.61 | 0.18 | 0.78 |
| Caproate | 0.38 | 0.39 | 0.27 | 0.31 | 0.030 | 0.04 | 0.43 | 0.63 |
| A:P ratio | 1.42 | 1.06 | 1.33 | 1.07 | 0.090 | 0.61 | 0.01 | 0.52 |
| $NH_3$-N, mM | 4.26 | 2.30 | 2.99 | 2.60 | 0.670 | 0.49 | 0.15 | 0.29 |

**Table 9.** Carcass characteristics in feedlot animals fed high-grain diet supplemented with no additives (CON), monensin (MON, 33 mg/kg DM), 3-nitrooxypropanol (NOP, 125 mg/kg DM), and combination of MON and NOP

| | −MON | | +MON | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Variable | −NOP | +NOP | −NOP | +NOP | SEM[1] | MON | NOP | MON × NOP |
| No. of steers (pens) | 60 (8) | 60 (8) | 60 (8) | 60 (8) | — | — | — | — |
| BW, kg | 730 | 734 | 732 | 735 | 11.2 | 0.85 | 0.73 | 0.97 |
| Hot carcass weight, kg | 426 | 432 | 434 | 432 | 6.91 | 0.49 | 0.73 | 0.52 |
| Fat cover (1 mm) | 22.2 | 21.5 | 22.6 | 23.7 | 0.90 | 0.14 | 0.82 | 0.32 |
| Fat cover (2 mm) | 22.2 | 21.0 | 21.9 | 22.8 | 0.87 | 0.36 | 0.90 | 0.22 |
| Grade fat[1] | 19.9 | 18.6 | 19.7 | 20.6 | 0.86 | 0.28 | 0.89 | 0.19 |
| Rib eye area, cm | 89.0 | 93.1 | 93.0 | 94.0 | 1.57 | 0.11 | 0.10 | 0.30 |
| Marbling quality | 2.90 | 2.93 | 2.90 | 2.87 | 0.070 | 0.60 | 0.99 | 0.57 |
| Marbling level | 29.4 | 27.5 | 23.9 | 28.0 | 3.58 | 0.45 | 0.73 | 0.37 |
| Saleable meat, % | 49.4 | 50.8 | 50.0 | 49.5 | 0.72 | 0.64 | 0.52 | 0.18 |
| Liver score[2] | 1.70 | 1.58 | 1.57 | 1.74 | 0.140 | 0.95 | 0.89 | 0.44 |
| Dressing percentage[3] | 58.3 | 58.8 | 59.3 | 58.8 | 0.44 | 0.28 | 0.95 | 0.26 |

[1]Grade fat is minimum backfat thickness in the third quarter between the 12th- and 13th-rib interface (Rodas-González et al., 2013).

[2]0 = no abscesses; 1 = small abscesses; 2 = moderate abscesses; 3 = severe abscesses; 4 = adhered (Bauer et al., 2007).

[3]Dressing percentages were calculated individually as hot carcass weight divided by final BW × 100%.

supplemented to corn silage-based diets (high-forage diets) compared with when supplemented to finishing diets.

Increased ruminal propionate with MON supplementation of high-forage diets might have contributed to greater ME availability and subsequently improved G:F. Because high-grain diets contain greater ME content and promote relatively greater ruminal propionate production compared with high-forage diets, supplementation of the high-grain diet with MON might have induced hypophagic effects resulting in a tendency to decrease DMI and ADG (Duffield et al., 2012). The effects of MON supplementation on decreasing $NH_3$-N is consistent with previous in vitro studies (Fuller and Johnson, 1981; Russell and Strobel, 1989) using substrates representing high-forage and high-grain diets. Similar results were observed with in vivo studies using high-forage diets (Lana and Russell, 1997; Guan et al., 2006). The effects of MON supplementation on decreasing $NH_3$-N may be attributed to its inhibitory effects on ammonia producing gram-positive bacteria (Russell and Strobel, 1989).

Feeding behavior and physical activity influence total energy expenditure and contribute to differences in feed efficiency among animals (Susenbeth et al., 1998; Kelly et al., 2010). Previous studies have shown decreased feeding activity with more feed-efficient animals (Golden et al., 2008). Beef

cattle supplemented with MON have been shown to eat smaller and more frequent meals (Fanning et al., 1999), which is the opposite of the observation in the present study where meal frequency tended to decrease with MON supplementation of the high-forage diet. The small decrease in meal frequency, coupled with no changes in meal duration, is consistent with numerically decreased DMI with MON supplementation in backgrounding steers. The effects of MON on meal frequency might be attributed to greater ruminal propionate synthesis, consistent with the observed increase in propionate concentration, as propionate is hypophagic compared with acetate (Allen et al., 2009).

The potential of MON as a $CH_4$ mitigating agent in ruminants is based on its effects on reducing DMI, increasing ruminal propionate synthesis, decreasing acetate-to-propionate ratio, and decreasing the abundance of $H_2$-producing protozoa (Hino, 1981; Russell, 1987). Ruminal propionate is an alternate $H_2$ sink and greater propionate synthesis results in decreased availability of substrates ($H_2$ and formate) for methanogens, subsequently reducing the energy lost as $CH_4$. However, the effects of MON on total $CH_4$ production and $CH_4$ yield are inconsistent (Beauchemin et al., 2008) and are influenced by the dose and duration of MON supplementation, as well as dietary forage content (Guan et al., 2006; Odongo et al., 2007). Although MON supplemented at doses < 15 mg/kg have no effect on $CH_4$ production, 24 to 35 mg/kg has been shown to decrease $CH_4$ production by 4% to 10% and $CH_4$ yield by 3% to 8% (Beauchemin et al., 2008). In the present study, MON numerically decreased DMI and tended to decrease DMI of beef steers in chambers fed high-forage diets. Lower DMI with MON accounted for the decrease in total $CH_4$ production with high-forage diets, as there was no effect of MON on $CH_4$ yield. These results suggest that changes in rumen fermentation parameters might not have influenced $CH_4$ production to a great extent. In agreement with the results of our study, a meta-analysis study by Duffield et al. (2012) observed a 2.3% decline in DMI with MON. Similarly, Appuhamy et al. (2013) summarized 11 studies from 7 peer-reviewed publications in beef steers for a meta-analysis study and observed a 15% decline in total $CH_4$ production with MON supplementation. However, contrary to our results, Appuhamy et al. (2013) observed 9% reduction in $CH_4$ yield with feeding MON at an average dose of 32 mg/kg DM. They also reported that the antimethanogenic efficacy of MON was lost with high-grain diets, despite lower DMI. Variable responses on $CH_4$ production with MON might be attributed to differences in the chemical composition of high-forage and high-grain diets and the duration of feeding MON. Dietary NDF content can influence MON effects on $CH_4$ production in beef steers; a unit increase in NDF content was shown to increase MON induced $CH_4$ mitigation by 0.05 g/d (Appuhamy et al., 2013). The lack of MON effects on $CH_4$ mitigation with the high-grain diet in the present study might be due to its lower dietary NDF content (18.4 vs. 40.5% DM) compared with the high-forage diet.

Additionally, MON induced effects on $CH_4$ production have been shown to be transient with the inhibitory effects dissipating after 4 to 8 wk with high-forage and high-grain diets (Guan et al., 2006). The adaptive response for methanogenesis is reportedly slower for animals fed high-forage diets compared with high-grain diets (Johnson et al., 1997; Guan et al., 2006). The lack of persistence of feeding MON on $CH_4$ production has been attributed to gradual adaptation of ruminal microorganisms. It is possible that the lack of effect of MON on $CH_4$ yield in the present study was because the $CH_4$ measurements occurred 7 to 10 wk and 9 to 13 wk after onset of treatments during backgrounding and finishing phases, respectively.

No influence of MON on carcass characteristics in the present study is in agreement with previous studies (Depenbusch et al., 2008; Yang et al., 2010; Felix and Loerch, 2011).

### *3-Nitrooxypropanol*

The efficacy of NOP in persistently reducing enteric $CH_4$ emissions without apparent side effects has been observed earlier in beef (Vyas et al., 2016a) and dairy cows (Hristov et al., 2015; Haisan et al., 2017). The antimethanogenic potential of NOP is attributed to inhibition of methyl-coenzyme M reductase (**MCR**), the enzyme required for the last step of methanogenesis (Duin et al., 2016). In the present study, NOP was found to be effective in reducing enteric $CH_4$ emissions during both backgrounding and finishing phases of the study, thereby confirming earlier studies.

During the backgrounding phase, NOP supplemented at 200 mg/kg DMI (1.23 g/d) decreased both total $CH_4$ production and $CH_4$ yield by 42%, respectively, with the results in agreement with previous studies providing either similar (Vyas et al., 2016a) or greater amounts of NOP (2.7 g/d) in beef cattle fed high-forage diets (Romero-Perez et al., 2014). The combination of MON and

NOP significantly decreased total $CH_4$ production compared with MON; however, the extent of inhibition was numerically lower when compared with NOP alone likely due to differences in DMI. The effect of NOP on decreasing DMI during the backgrounding phase is in agreement with previous studies in beef cattle fed high-forage diets (Romero-Perez et al., 2014; Vyas et al., 2016a). Lower DMI with NOP is probably attributed to hypophagic effects of greater ruminal propionate synthesis with NOP (Allen et al., 2009). The dose of NOP used in the finishing phase of this study was considerably lower (125 mg/kg DMI) than levels used during the backgrounding phase. Despite using a considerably lower dose, NOP was equally effective in the finishing phase as total $CH_4$ production and $CH_4$ yield was decreased by 41% and 37%, respectively. Vyas et al. (2016a) showed NOP was highly potent in high-grain diets where a dose of 200 mg/kg decreased total $CH_4$ production and $CH_4$ yield by 84% and 80%, respectively. The effect of NOP on decreasing DMI during the finishing phase was not as pronounced as observed during the backgrounding phase. Similar results were observed earlier despite using a different NOP dose (Vyas et al., 2016a), suggesting diet and not dose-dependent effects of NOP on intake. In contrast, no NOP effect was observed on DMI of animals in chambers perhaps because they only stayed in the chambers for 3 d and intake levels were decreased by 23% and 20% compared with overall DMI during backgrounding and finishing phases, respectively, regardless of the treatments used.

Methane mitigation using NOP increased $H_2$ emissions with both high-forage and high-grain diets. A similar response in $H_2$ emissions to NOP was observed earlier in dairy cows (Hristov et al., 2015) and beef cattle (Vyas et al., 2016a,b). Hydrogen emissions peaked at 9-h postfeeding and this peak corresponded to peak CH4 inhibition by NOP. The observed decrease in $H_2$ emissions with the combination of MON and NOP compared with NOP alone might be attributed to the numerically lower extent of $CH_4$ mitigation with MONOP.

The variability in the efficacy of NOP with changes in dietary composition might be due to lower $CH_4$ emissions from cattle fed high-grain diets. The lower $CH_4$ yield with high-grain diets is attributed to greater starch fermentation and ruminal propionate synthesis thereby decreasing $H_2$ availability for methanogenic archaea for $CH_4$ synthesis. Moreover, the abundance of MCR might be lower in the rumen of cattle fed high-grain diets thereby accounting for the greater effectiveness of NOP in inhibiting MCR.

This is the first study to demonstrate feeding behavior in response to NOP supplementation during backgrounding and finishing phases of beef cattle production. Despite improvements in G:F with high-forage and high-grain diets, NOP supplementation did not alter feeding behavior of the animals.

Methane represents a loss of about 3% to 12% of the total gross energy consumed by ruminants and $CH_4$ mitigation has potential to improve production efficiency. Producers may be more willing to adopt $CH_4$ mitigation practices if associated with improvement in G:F or ADG. The present study demonstrates that feed efficiency, measured as G:F, was improved in beef cattle fed high-forage and high-grain diets when supplemented with NOP. Improved G:F in the high-grain diet is in contrast to Vyas et al. (2016a), where no effects on G:F were observed with high-grain diets when NOP was supplemented at 200 mg/kg DM and $CH_4$ decreased by >80%. The contrasting results between these 2 studies indicate that the optimum dose rate of NOP for improving cattle performance is less in high-grain diets than in high-forage diets (Vyas et al., 2016a).

In the present study, NOP decreased $CH_4$ production in finishing beef cattle by 65 g/d which is equivalent (e.g., in terms of reducing equivalents) to releasing 16 g/d $H_2$. However, only 31% (5 g/d) of expected $H_2$ was released; thus, it appears that the remaining spared $H_2$ was diverted toward alternate $H_2$ sinks or toward dissolved $H_2$, which is a larger pool compared with gaseous $H_2$ emissions (Janssen, 2010). However, Guyader et al. (2017) observed greater increase in gaseous $H_2$ (+81%) compared with dissolved $H_2$ (+24%) during NOP induced $CH_4$ mitigation using a rumen simulation technique. Moreover, Lopes et al. (2016) observed no changes in dissolved $H_2$ concentration with NOP supplemented at 60 mg/kg DM. Vyas et al. (2016a) observed greater effects on $CH_4$ mitigation when NOP was added to a high-grain diet at 200 mg/kg; total $CH_4$ production decreased by 98 g/d compared with control. However, the efficiency of $H_2$ capture was lower in that previous study (Vyas et al., 2016a) compared with the present study with 52% of spared $H_2$ released as gas leaving only 48% to be diverted to alternate $H_2$ sinks such as formate, propionate, valerate, caproate, heptanoate, unsaturated fatty acids, nitrate and sulfate reduction, and microbial protein synthesis. Using a rumen simulation technique to evaluate NOP, Guyader et al. (2017) accounted for only 54.3% of the spared $H_2$

in fermentation end products, demonstrating that future studies are needed to understand the modifications in metabolic pathways resulting from dietary supplementation of NOP. Hydrogen is an energy-dense gas (142 kJ/g $H_2$; Afeefy et al., 2011) and greater accumulation of $H_2$ might offset the advantage of energy spared by $CH_4$ mitigation. Methane mitigation with high-grain diets spared 3.63 MJ of energy (55 kJ/g $CH_4$); however, 20% of spared energy was lost as gaseous $H_2$ and the net gain in energy was 2.90 MJ. Although mitigating $CH_4$ emissions has potential to improve energy efficiency, it is important that spared $H_2$ is efficiently diverted toward nutritionally beneficial sinks in the rumen such as propionate.

The effects of NOP supplementation on decreasing $NH_3$-N during the backgrounding phase are not in agreement with previous studies in beef cattle (Romero-Perez et al., 2014). However, Lopes et al. (2016) observed lower $NH_3$-N with NOP (60 mg/kg DM), and results were attributed to lower abundance of rumen microbes with proteolytic and deaminative activities (e.g., *Clostridium* spp.). Although bacterial abundance was not measured this study, it is possible that the lower $NH_3$-N with NOP was due to changes in the rumen microbial population.

Vyas et al. (2016a) observed no changes in the carcass characteristics with NOP supplementation. Similar findings were observed in the present study further underscoring no negative effects of NOP supplementation on carcass characteristics.

## CONCLUSIONS

Overall, the results demonstrate efficacy of NOP in reducing enteric $CH_4$ emissions and subsequently improving feed conversion in cattle fed backgrounding and finishing diets. In both phases of the study, NOP lowered $CH_4$ yield by approximately 40%, and the reduction in $CH_4$ was not affected by whether MON was included in the diets. Both compounds improved feed conversion efficiency in the backgrounding phase, but only NOP improved feed conversion in the finishing phase. We conclude that NOP is a potent $CH_4$ inhibitor that can be added to conventional feedlot diets containing MON without incurring negative effects on performance or carcass characteristics. Furthermore, the study suggests a possible link between sustained reduction in $CH_4$ and improved feed conversion efficiency, which may encourage producers to adopt this $CH_4$ mitigation approach if the product becomes commercially available.

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