Published May 6, 2016

Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle[1]

D. Vyas,* S. M. McGinn,* S. M. Duval,† M. Kindermann,‡ and K. A. Beauchemin*[2,3]

*Lethbridge Research and Development Center, Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1, Canada; †DSM Nutritional Products France, Research Center for Animal Nutrition, BP170, 68305 Saint Louis Cedex, France; and ‡DSM Nutritional Products, P.O. Box 2676, Bldg. 241/865, CH-4002 Basel, Switzerland

ABSTRACT: The study objective was to evaluate the effects of sustained reduction of enteric methane (CH_4) emissions with dietary supplementation of the inhibitor 3-nitrooxypropanol (NOP) on growth rate and feed conversion efficiency of growing and finishing beef cattle. Eighty-four crossbred steers were used in a 238-d feeding study and fed a backgrounding diet for the first 105 d (backgrounding phase) and transition diets for 28 d followed by a finishing diet for 105 d (finishing phase) with 3 doses of NOP (0, 100, and 200 mg/kg DM). The experiment was a completely randomized design using 21 pens (4 cattle/pen) with 7 pens per treatment. When cattle were fed the backgrounding diet, pen DMI was reduced ($P < 0.01$) whereas G:F tended to improve ($P = 0.06$) with increasing dose of NOP supplementation. During the finishing phase, DMI ($P = 0.06$) and ADG ($P = 0.07$) tended to decrease with increasing dose of NOP supplementation. Although both levels of NOP were effective in reducing CH_4 emissions from the backgrounding diet ($P < 0.01$), only NOP supplemented at the highest dose was effective in reducing total CH_4 emissions from the finishing diet ($P < 0.01$). Methane yield (g/kg DMI) was reduced whereas hydrogen emissions were increased at the highest dose of NOP supplementation with both backgrounding and finishing diets ($P < 0.01$). Overall, these results demonstrate efficacy of NOP in reducing enteric CH_4 emissions from cattle fed backgrounding and finishing diets, and these effects were negated once NOP supplementation was discontinued.

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

J. Anim. Sci. 2016.94:2024–2034
doi:10.2527/jas2015-0268

INTRODUCTION

Agriculture, forestry, and land use activities produce greenhouse gases, with non-CO_2 emissions including methane (**CH_4**) and nitrous oxide (**N_2O**) generated primarily from agriculture (Tubiello et al., 2014). Based on the trends from 2001 to 2011, enteric CH_4 produced in ruminants during the normal process of ruminal fermentation is of primary concern because of its substantial contribution to agricultural emissions (Tubiello et al., 2014).

Many strategies have been proposed to mitigate enteric CH_4 emissions from ruminants (Hristov et al., 2013). In particular, dietary supplementation of 3-nitrooxypropanol (**NOP**) is a promising strategy because of its CH_4 mitigation potential and lack of effects on diet digestibility. It was shown that supplementing the diet of beef cattle (Romero-Perez et al., 2014, 2015), dairy cows (Haisan et al., 2014; Reynolds et al., 2014; Hristov et al., 2015), and sheep (Martinez-Fernandez et al., 2014) with NOP resulted in a significant reduction in enteric CH_4 production. Moreover, NOP was shown to have no negative effect on feed intake or digestibility (Romero-Perez et al., 2014), and no risks in terms of food safety have been reported to date.

[1]This project was supported by Alberta Livestock and Meat Agency, Agriculture and Agri-Food Canada, and DSM Nutritional Products. Authors wish to acknowledge technical staff, postdoctoral fellows, students, and the staff at the Feedlot Unit at the Lethbridge Research and Development Center who helped conduct this research.

[2]Corresponding author: karen.beauchemin@agr.gc.ca

[3]Lethbridge Research and Development Center contribution number 38715085.

Received December 31, 2015.

Accepted March 1, 2016.

Because CH_4 emissions account for up to 12% of GE intake of cattle (Johnson and Johnson, 1995), it is possible that reduced CH_4 emissions might spare ME that could be utilized by the animal to meet its requirements for growth. However, no studies have been published to document the effects of CH_4 mitigation using NOP on the rate of gain and feed efficiency in beef cattle.

We hypothesized that NOP would inhibit CH_4 production and thus enhance the energy status of animals, which would improve growth rate, feed conversion efficiency, or both irrespective of the type of diet offered to animals. The objective of this study was to determine the effects of NOP supplementation on CH_4 production, growth rate, feed intake, and feed conversion efficiency in steers fed backgrounding and finishing diets.

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 on Animal Care (2009). The study was conducted with approval from the Veterinary Drugs Directorate of Health Canada (Experimental Studies Certificate; Drug Submission Tracking System number 177698).

Animals, Diets, and Experimental Design

Eighty-four crossbred yearling steers (319 ± 20 kg) were received at the Lethbridge Research and Development Center (Lethbridge, AB, Canada), adapted to facilities, and processed according to standard management procedures at the center including ear tagging for identification and vaccination for infectious bovine rhinotracheitis, bovine viral diarrhea, and parainfluenza-3. Following the receiving phase, steers were blocked by weight into 4 groups and randomly assigned to 21 pens (4 animals per pen) such that 1 animal from each weight group was assigned to each pen. Pens were equipped with fence-line feed bunks and automatic waterers. Bunk space in each pen was adequate for all 4 animals to eat simultaneously.

Steers were used in a 238-d feeding trial and were fed a backgrounding diet for the first 105 d (i.e., backgrounding phase) followed by transition diets for 28 d with sequential increase in the proportion of barley grain in the total mixed ration (**TMR**; Table 1). A finishing diet was fed for the last 105 d (i.e., finishing phase), and animals were marketed when they reached a live weight of 600 to 630 kg. Initial BW of steers fed backgrounding and finishing diets was 319 ± 20 kg and 475 ± 34 kg, respectively. The backgrounding diet was formulated to provide adequate ME and MP for 300-kg growing beef cattle with an ADG of 1 kg/d, whereas the finishing diet provided adequate ME and MP for 400-kg beef cattle with ADG of 2 kg/d (NRC, 2000). Each phase was divided into five 21-d periods. After the end of the finishing phase, dietary NOP supplementation was discontinued and cattle were fed the control finishing diet for a minimum of 4 wk before slaughter in accordance with the requirement of the experimental studies certificate. This phase constituted the recovery phase in this experiment.

The experiment was conducted as a completely randomized design with 3 treatments: control (no NOP), low NOP (100 mg/kg DM; DSM Nutritional Products AG, Kaiseraugst, Switzerland), and high NOP (200 mg/kg DM). The NOP was homogenously mixed into the TMR daily for the NOP treatments, whereas the placebo was supplemented at similar concentrations in the control diet. The NOP dose used in the present study was based on previous studies (Romero-Perez et al., 2014, 2015) where different doses of NOP were evaluated with backgrounding diets.

Table 1. Ingredient and chemical composition of the basal diets

Item	Backgrounding[1]	Finishing[1]
Ingredient, % of DM		
Barley silage	70.0	8.0
Barley, dry rolled	20.0	87.0
Supplement	10.0	5.0
Canola meal	4.0	–
Barley, ground	5.443	3.417
Canola oil	0.057	–
Limestone	0.150	–
Calcium carbonate	–	1.250
Salt	0.050	0.150
Urea	0.200	–
Molasses	–	0.125
Vitamin E (500,000 IU/kg)	–	0.006
Feedlot premix[2]	0.050	0.050
Flavoring agent	–	0.003
Chemical composition[3]		
DM, %	42.8	81.1
OM, %	93.2	95.4
CP, %	13.7	12.2
NDF, %	36.4	19.2
ADF, %	22.4	8.4
Starch, %	26.7	57.0

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

[2]Feedlot vitamin–mineral premix contained 35.01% $CaCO_3$, 10.37% $CuSO_4$, 28.23% $ZnSO_4$, 0.15% ethylenediamine dihydriodide (80% concentration), 5.01% selenium 1% (10,000 mg Se/kg), 0.1% $CoSO_4$, 14.54% $MnSO_4$, 1.71% vitamin A (500,000,000 IU/kg), 0.17% vitamin D (500,000,000 IU/kg), and 4.7% vitamin E (500,000 IU/kg).

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

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.

Dry matter intake was determined for the pens weekly as the difference between feed offered and weekly refusals, corrected for DM content. Samples of the feed offered and the refusals were taken weekly for DM determination. The samples were pooled by 3-wk periods, ground, and analyzed for DM, OM, CP, starch, NDF and ADF contents. Unground fresh ration and ingredients were sampled during each period to determine particle size using sieving techniques (Kononoff et al., 2003). Test weight of the barley grain before and after processing was measured to describe the extent of processing.

Pen feed conversion efficiency was determined from ADG and daily DMI as kilograms gain per kilogram feed DM. This was calculated by 3-wk period and averaged over the entire experiment.

Enteric Gas Production. Fifteen steers (5 steers per treatment) were used for the measurement of CO_2, CH_4, and H_2 production using calorimetry chambers. The cattle were accustomed to the chambers before the study to minimize stress. Because only 4 chambers were available at a time, the animals were divided into 4 groups with 4 animals each in the first 3 groups and 3 animals in the last group. Each treatment was assigned at least once in each group. Methane measurements took place over 4-wk periods during the backgrounding and finishing phases and over 2 wk during the recovery phase. The measurement period coincided with the midpoint of each feeding period, ranging from 63 to 93 d for the backgrounding phase and from 34 to 65 d for the finishing phase. The emissions measured during the recovery phase were from 4 animals, 18 to 25 d after NOP supplementation was discontinued. Animals used in the recovery phase were all fed high levels of NOP (200 mg/kg DM) during the finishing phase. The animals remained in the chamber for 3 consecutive days during each measurement period.

Each chamber measured 4.4 m wide by 3.7 m long by 3.9 m high (63.5 m^3; model C1330; Conviron Inc., Winnipeg, MB, Canada). Processes involved in air circulation in the chambers were described earlier (Avila-Stagno et al., 2013). One animal was housed within each chamber and the stall was equipped with a feeder and drinking bowl and fitted with a rubber mattress. Concentrations of CH_4 in the intake and exhaust air ducts were monitored using a CH_4 analyzer (model Ultramat 5E; Siemens Inc., Karlsruhe, Germany). Standardization and calibration procedures are given by McGinn et al. (2004). Analyzers used for measuring gas fluxes were calibrated; however, between-chamber differences were calculated by releasing the same amount of gas in each chamber and determining whole-chamber flux (McGinn et al., 2004). The average correction factor was 0.99, 1.10, 1.00, and 1.01 for chambers 1, 2, 3, and 4, respectively. Methane concentrations in the intake and exhaust air ducts of the chambers were monitored sequentially as described earlier (Romero-Perez et al., 2015). The total quantity of CH_4 emitted in the chambers was quantified by measuring the gradient of influx and exhausted concentrations and volumes. The air volume in each chamber was exchanged every 5 min. Methane concentration was recorded every 30 min by a calibrated infrared gas analyzer. 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 at 2, 4, 6, 11, 13, and 23 h after feeding during backgrounding phase and every 3 h after feeding in the finishing phase when the animals were in the chambers (Van Zijderveld et al., 2011).

Carcass Characteristics. All steers were commercially slaughtered (Cargill Foods, High River, AB, Canada) at the end of the finishing phase, following a 4-wk withdrawal period (during which time NOP was not fed). All steers were slaughtered on the same day according to industry protocols including stunning with captive bolt before exsanguination. Hot carcass weight was recorded before spray chilling. The carcasses were graded by Canadian Beef Grading Agency graders. Dressing percentage was individually calculated as HCW 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 rib (Yang et al., 2012).

Laboratory Analysis

Analyses were performed on each sample in duplicate; when the CV was greater than 5%, the analysis was repeated. The DM content for all samples was determined by oven drying at 55°C for 72 h. Dried samples were ground in a Wiley mill (A. H. Thomas, Philadelphia, PA) through a 1-mm screen. Analytical DM was determined on ground samples by drying at 135°C for 2 h (method 930.15; AOAC, 2005) followed by hot weighing. The OM content was calculated as the difference between 100 and the percentage ash (method 942.05; AOAC, 2005). Fiber components, NDF and ADF, were determined based on the procedure by Van Soest et al. (1991) with heat stable amylase and sodium sulfite used in the NDF procedure. Gross energy content was determined by adiabatic bomb calorimetry (model 196 E2k; CAL2k,

Table 2. Chemical and particle size analysis of dietary ingredients

Item	Barley silage	Barley grain[1]	Back-grounding supplement	Finishing supplement
Chemical analysis, % DM				
DM, % as fed	33.8 ± 1.50	91.4 ± 0.97	92.2 ± 1.46	93.7 ± 1.01
OM	92.7 ± 0.21	97.1 ± 0.10	92.8	67.6
CP	11.5 ± 0.14	12.2 ± 0.35	28.4	9.0
NDF	51.2 ± 1.48	15.8 ± 1.48	19.6	11.9
ADF	33.8 ± 0.29	5.8 ± 0.71	10.7	4.7
Starch	14.5 ± 1.98	61.9 ± 0.50	24.5	31.4
Penn State Particle Separator,[2] % of DM retained on sieves				
19 mm	5.3 ± 3.61			
8 mm	59.2 ± 5.76			
1.8 mm	34.4 ± 7.95			
Pan	1.2 ± 0.67			

[1]Processing index, defined as the weight of 500 mL of grain after processing divided by the weight of 500 mL of grain before processing multiplied by 100%, was 82%.

[2]Particle size distribution was measured using Penn State Particle Separator (Kononoff et. al., 2003).

Johannesburg, South Africa). Samples ground through 1-mm screen were reground using a ball grinder (Mixer Mill MM2000; Retsch GmbH, Haan, Germany) before determination of nitrogen and starch content. The nitrogen content was determined by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instrumentals, Milan, Italy). Starch content was determined by enzymatic hydrolysis of α-linked glucose polymers as described earlier (Chung et al., 2011).

Statistical Analysis

Normality of distribution and homogeneity of variance was determined using the Univariate procedure of SAS (SAS Inst., Inc., Cary, NC). The data were subsequently analyzed using a MIXED procedure of SAS. For all traits, the pen (or the animal, in the case of CH_4) was the experimental unit. The statistical model included the fixed effects of treatment. Period was used as a REPEATED measure in the model except for the CH_4 observations, for which day was the repeated measure. When treatment differences were significant, based on a protected *F*-test, the PDIFF option was included in the LSMEANS statement to conduct multiple comparisons. The time-series covariance structure was modeled using the options of autoregressive order 1, compound symmetry, and unstructured order 1. 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 $0.05 < P \leq 0.10$.

Table 3. Dry matter intake, G:F, and ADG in feedlot animals fed backgrounding and finishing diets supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) doses of 3-nitrooxypropanol

	Treatment				Treatment
Variable	Control	Low	High	SEM	*P*-value
Backgrounding phase[1]					
DMI,[2] kg/d	8.86[a]	8.76[a]	8.15[b]	0.12	<0.01
G:F, g/g	0.143	0.150	0.154	0.003	0.06
ADG, kg/d	1.28	1.31	1.27	0.03	0.62
Finishing phase[3]					
DMI,[2] kg/d	10.87	10.21	9.86	0.28	0.06
G:F, g/g	0.144	0.147	0.143	0.005	0.85
ADG, kg/d	1.55	1.48	1.39	0.05	0.07

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

[1]Backgrounding diets were fed from d 1 to 105.

[2]DMI per animal was calculated by dividing pen DMI by number of animals per pen.

[3]Finishing diets were fed for a total of 105 d following a 28-d transition phase.

RESULTS

Barley silage was the major ingredient in the backgrounding diet (70%) whereas the finishing diet was primarily (87%) barley grain (Table 1). The dietary composition corresponds well with typical diets used by western Canadian feedlots. The chemical composition of various ingredients and particle size distribution for barley silage is shown in Table 2.

Dry Matter Intake and BW

3-Nitrooxypropanol supplemented at a high dose reduced DMI during the backgrounding phase ($P < 0.01$; Table 3), and it tended to reduce intake during the finishing phase ($P = 0.06$). Despite reduced DMI in the backgrounding phase, feeding NOP had no effect ($P = 0.62$) on ADG, resulting in a tendency ($P = 0.06$) to improve G:F. In contrast, ADG tended ($P = 0.07$) to be reduced with NOP in finishing steers resulting in a similar ($P = 0.85$) G:F, irrespective of the dose of NOP fed.

During chamber measurements, no treatment effects were observed on DMI during backgrounding ($P = 0.62$; Table 4) and finishing phases ($P = 0.48$; Table 5); however, the intake levels were reduced in the chambers when compared with levels observed in the feedlot settings.

Gas Emissions

Enteric CH_4 emissions (g/d) were reduced with NOP supplementation of backgrounding diets ($P < 0.01$;

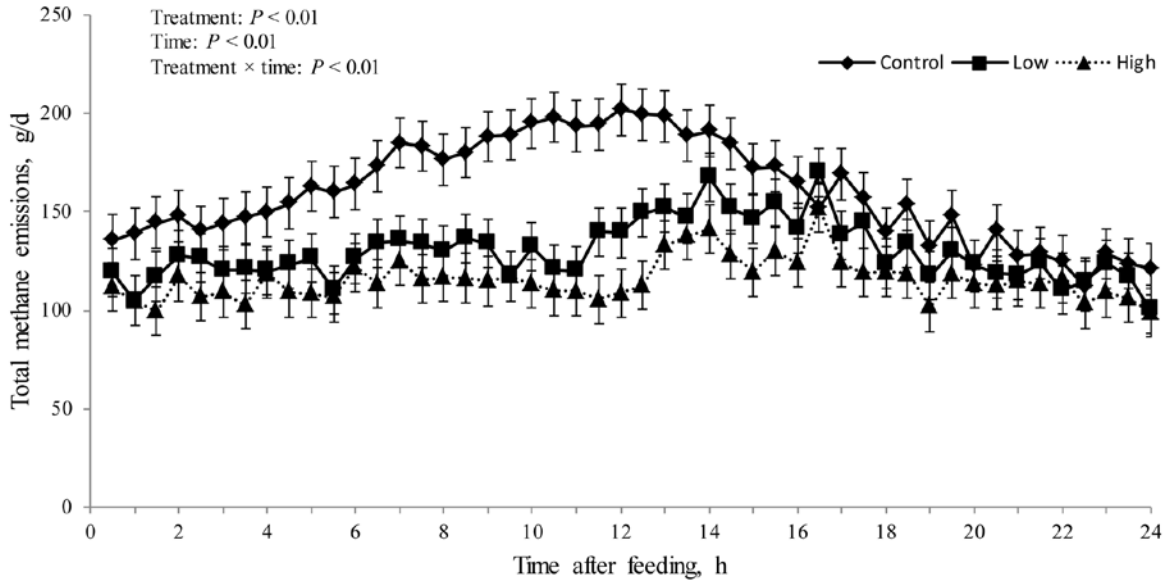

Figure 1. Total methane emissions after feeding in feedlot animals fed a backgrounding diet supplemented with a control and low (100 mg/kg) and high (200 mg/kg) doses of 3-nitrooxypropanol.

Table 4). However, CH_4 emissions corrected for DMI and GE intake were only reduced with a high dose of NOP ($P < 0.01$). The reduction in CH_4 emissions corresponded to an increase in H_2 emissions ($P < 0.01$). Hourly total CH_4 emissions after feeding showed reduced emissions with NOP supplementation, irrespective of the dose supplemented, starting from 30 min until 16 h after feeding in steers fed backgrounding diets ($P < 0.01$; Fig. 1). Concomitant with reduced CH_4 emissions, H_2 emissions were increased particularly at the highest dose of NOP supplementation ($P < 0.01$; Fig. 2) and the peak effect was observed at 11 h after feeding. However, it is possible that the actual peak effect might have occurred between 6 and 11 h because no sample for H_2 measurement was taken between 6 and 11 h after feeding.

In cattle fed the finishing diet, the high dose of NOP reduced total enteric CH_4 emissions (g/d; $P < 0.01$; Table 5) whereas only a numerical reduction was observed for NOP supplemented at a low dose, compared with the control diet. A similar trend was observed for CH_4 emissions corrected for DMI ($P < 0.01$) and GE intake ($P < 0.01$). Hydrogen emissions were increased with supplementation of NOP, with a pronounced response for the high dose ($P < 0.01$). Postprandial variations in hourly total CH_4 emissions showed reduced emissions with the higher dose of NOP compared with the control and the low dose of NOP ($P < 0.01$; Fig. 3). Total CH_4 emissions remained significantly low for 24 h after feeding for cattle consuming the high dose of NOP. Hydrogen emissions after feeding were increased with NOP supplementation and the effects were greater with a higher dose of NOP ($P < 0.01$; Fig. 4), with peak response observed 12 h after feeding. Total CH_4 emissions in steers fed finishing diets, measured 18 to 25 d after discontinuing NOP supplementation, returned to control levels (Fig. 5).

Carcass Characteristics

No treatment effects were observed on HCW (Table 6; $P = 0.50$). Similarly, grade fat ($P = 0.98$), LM area ($P = 0.78$), marbling score ($P = 0.81$), and percent of saleable meat ($P = 0.99$) were not affected by treatments. However, dressing percentage was reduced with both doses of NOP supplementation ($P = 0.02$).

DISCUSSION

3-Nitrooxypropanol is thought to be a structural analog of methyl coenzyme M acting as an inhibitor of the enzyme methyl coenzyme M reductase during the

Table 4. Enteric methane (CH_4) emissions from feedlot animals fed a backgrounding diet supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) doses of 3-nitrooxypropanol

Variable	Treatment			SEM	Treatment *P*-value
	Control	Low	High		
DMI, kg/d	6.68	6.09	6.05	0.50	0.62
CH_4, g/d	176.3[a]	147.1[b]	110.0[c]	8.1	<0.01
Hydrogen, g/d	1.54[a]	3.94[a]	8.51[b]	4.75	<0.01
Methane emission intensity					
CH_4, g/kg of DMI[1]	26.4[a]	24.6[a]	18.7[b]	1.3	<0.01
CH_4, % of GE intake	8.18[a]	7.41[a]	5.88[b]	0.46	0.02

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

[1]Enteric CH_4 production (g of CH_4/d) expressed relative to DMI determined on the days of CH_4 measurement.

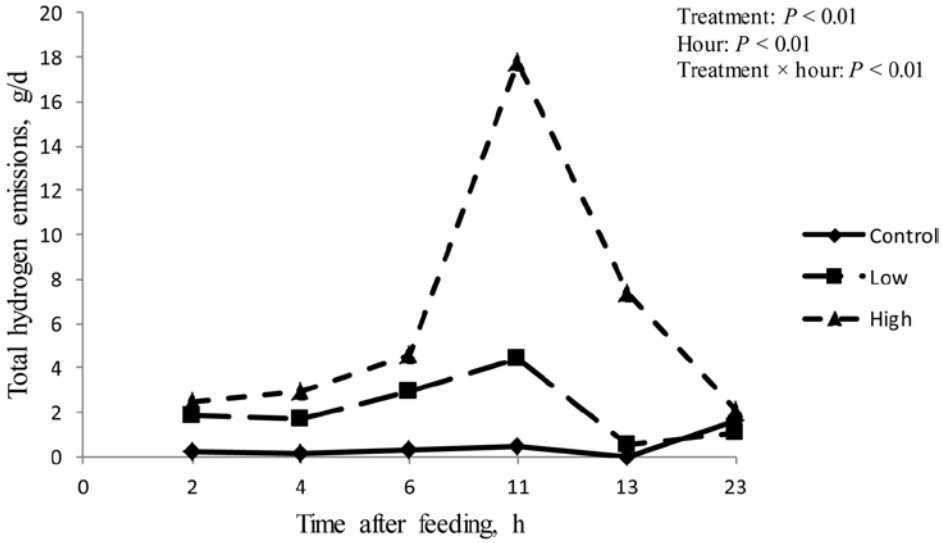

Figure 2. Hydrogen emissions after feeding in feedlot animals fed a backgrounding diet supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) levels of 3-nitrooxypropanol

last step of methanogenesis (Duval and Kindermann, 2012). As expected, NOP was effective in reducing enteric CH_4 emissions in beef cattle fed a backgrounding diet and the results are in agreement with previous studies in beef cattle (Romero-Perez et al., 2014, 2015), dairy cattle (Haisan et al., 2014; Reynolds et al., 2014; Hristov et al., 2015), and sheep (Martinez-Fernandez et al., 2014). The intensity of CH_4 emissions (CH_4 per unit of DMI) was reduced by 29% with the consumption of 1.21 g NOP/d (200 mg/kg DMI). A similar reduction (33%) was previously observed in beef heifers fed backgrounding diets (Romero-Perez et al., 2014); however, in that study, NOP was supplemented at a greater dose (2.7 g/d) than in the present study.

In the present study, NOP was mixed with the diet, allowing NOP to be gradually introduced into the rumen as the animals consumed feed. This synchronization between gradual NOP delivery and feed fermentation is thought to improve the efficacy of NOP in reducing CH_4 emissions. In a previous study with dairy cows, Reynolds et al. (2014) observed only a 7% reduction in CH_4 yield with NOP administered directly into the rumen, in which case synchronization between feed digestion and NOP consumption might not have occurred (Reynolds et al., 2014).

3-Nitrooxypropanol was more effective in reducing enteric CH_4 emissions when mixed with a finishing diet compared with a backgrounding diet. Total enteric CH_4 emissions (g/d) were reduced by 17 and 38% with low and high doses of NOP when supplemented to a backgrounding diet, whereas the reduction was 12 and 84% with similar doses of NOP supplemented to a finishing diet. Similarly, intensity of CH_4 emissions (i.e., CH_4 corrected for DMI and GE) were reduced by 7 and 29% with NOP supplemented at low and high doses to a backgrounding diet, respectively, and 9 and 80% with similar doses of NOP, respectively, supplemented to a finishing diet. This study represents the first evidence in the literature confirming the efficacy of NOP in reducing enteric CH_4 emissions in beef steers fed finishing diets.

The lower total enteric CH_4 emissions and CH_4 emission intensity of cattle fed a finishing diet compared with a backgrounding diet was expected. Starch fermentation from high-grain diets results in greater production of ruminal propionate, thereby generating an alternative H_2 sink to methanogenesis. The reduced proportion of cellulose with the high-grain diet results in lower acetate production and, subsequently, reduced H_2 production (Murphy et al., 1982). Low ruminal pH with starch fermentation also inhibits rumen methanogens (Kessel and Russell, 1996) and reduces the abundance of rumen protozoa, thereby limiting transfer

Table 5. Enteric methane emissions from feedlot animals fed a finishing diet supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) doses of 3-nitrooxypropanol

	Treatment				Treatment
Variable	Control	Low	High	SEM	*P*-value
DMI, kg/d	7.51	7.55	6.31	0.79	0.48
CH_4, g/d	116.0[a]	101.6[a]	18.2[b]	10.7	<0.01
Hydrogen, g/d	0.02[c]	2.80[b]	12.43[a]	1.90	<0.01
Methane emission intensity					
CH_4, g/kg of DMI[1]	16.1[a]	14.7[a]	3.1[b]	2.1	<0.01
CH_4, % of GE intake	4.45[a]	4.34[a]	0.94[b]	0.63	<0.01

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

[1]Enteric CH_4 production (g of CH_4/animal per day) expressed relative to DMI determined on the days of CH_4 measurement

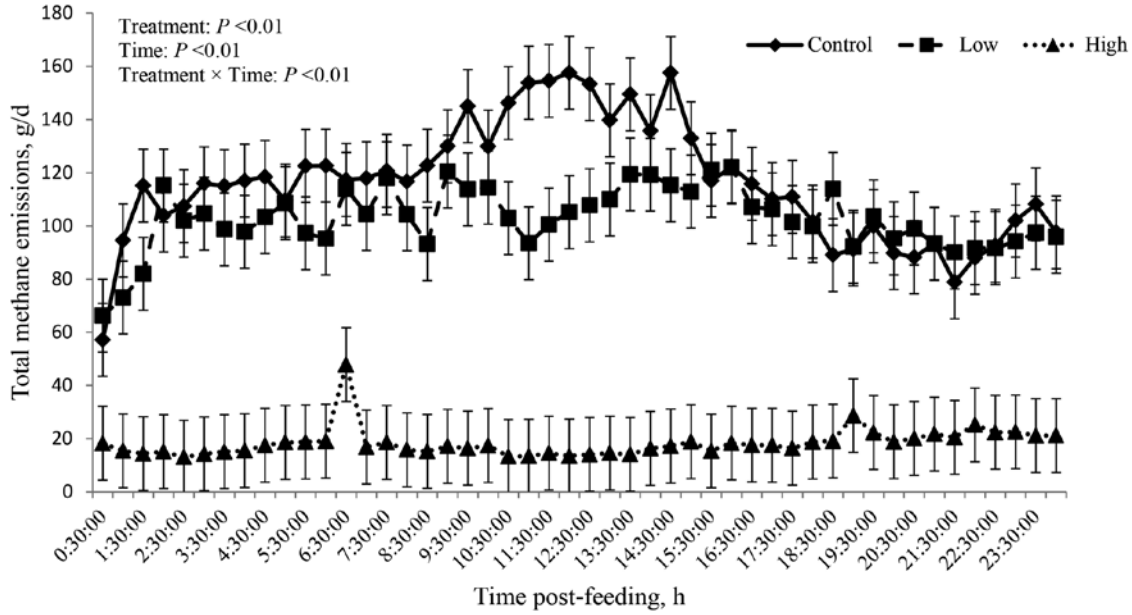

Figure 3. Total methane emissions after feeding in feedlot animals fed a finishing diet supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) levels of 3-nitrooxypropanol.

of H_2 from protozoa to methanogens (Grainger and Beauchemin, 2011). Based on lower CH_4 emissions from cattle fed starch-based diets, we speculate that the concentration of ruminal methyl coenzyme M was less in cattle fed the finishing diet compared with those fed the backgrounding diet. Hence, adding NOP to a high-grain diet might inhibit methyl coenzyme M reductase with greater efficacy due to lower concentration of methyl coenzyme M, possibly explaining the greater inhibitory potential of NOP supplemented to a finishing diet compared with a backgrounding diet.

Although NOP was effective in reducing CH_4 emissions both with backgrounding and finishing diets, the lack of a significant effect observed with NOP supplemented at 100 mg/kg DM in the finishing diet is perplexing. Postprandial variation in CH_4 emissions suggested that NOP supplemented at 100 mg/kg DM lost its efficacy 16 h after feeding whereas NOP supplemented at 200 mg/kg DM remained effective for 24 h. Although feeding behavior was not measured in this study, it is possible that NOP supplemented at higher doses might have reduced meal size and increased meal frequency such that NOP consumption was more gradual throughout the day. Nevertheless, results suggest that the optimum dose for reducing CH_4 emissions with finishing diets lies between 100 and 200 mg/kg DM. This further underscores the need for future studies exploring optimum doses of NOP in diets differing in nutrient composition.

The study indicates that the antimethanogenic potential of NOP varies depending on dietary composition. Although most previous studies with NOP have used backgrounding (beef cattle) and mixed diets (dairy cows), future research needs to be conducted to confirm that NOP is effective in finishing feedlot diets as well as to identify the optimum dose–response for diets differing in nutrient composition.

Average daily gain was increased with reduced CH_4 emissions in sheep fed a hemiacetal of chloral and starch (Trei et al., 1972). Similarly, milk yield was increased with reduced CH_4 production in lactating goats fed a halogenated CH_4 analog (Abecia et al., 2012). Recently, Hristov et al. (2015) observed increased BW gain in high-producing dairy cows fed diets supplemented with NOP. Although previous studies have confirmed the efficacy of NOP in reducing CH_4 emissions, the present study is the first to provide evidence that a reduction in CH_4 emissions due to dietary supplementation with NOP can affect growth rate and feed conversion efficiency in beef steers fed backgrounding diets.

Mitigation of CH_4 emissions may improve the energy status of animals only if GE intake and digestibility are not altered to an extent that would negate the energy spared by CH_4 mitigation. During the backgrounding phase, intake was reduced when a high dose of NOP was supplemented. Similar results were observed earlier in beef cattle fed NOP at 2.25 mg/kg BW (approximately 1.4 g/d; Romero-Perez et al., 2014). In contrast, NOP supplementation had no effects on DMI in beef cattle fed NOP at 4.5 mg/kg BW (approximately 2.7 g/d; Romero-Perez et al., 2014), in dairy cows (Haisan et al., 2014; Reynolds et al., 2014; Hristov et al., 2015), and in sheep (Martinez-Fernandez et al., 2014). Hypophagic effects of NOP

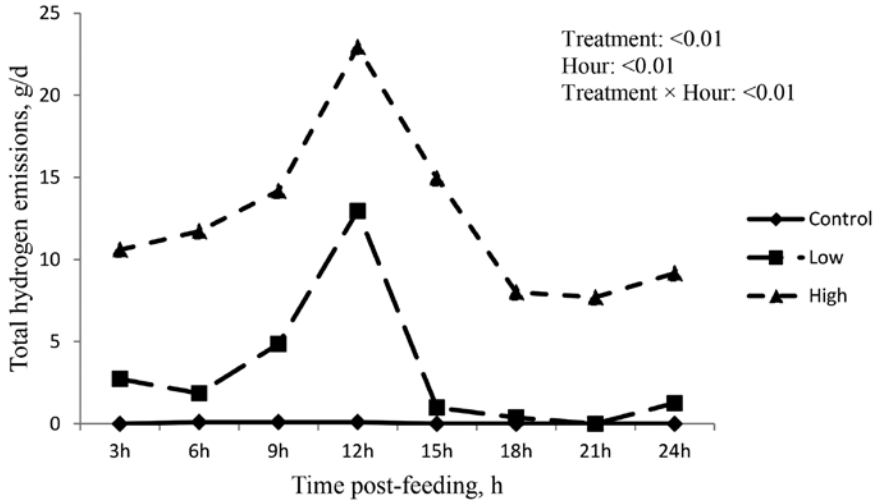

Figure 4. Hydrogen emissions after feeding in feedlot animals fed a finishing diet supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) levels of 3-nitrooxypropanol.

supplementation with backgrounding diets could be attributed to an increased proportion of rumen propionate. Although the rumen fermentation profile was not measured in this study, NOP supplementation increased rumen propionate and thereby reduced the acetate:propionate ratio in previous studies (Martinez-Fernandez et al., 2014; Reynolds et al., 2014; Romero-Perez et al., 2014). Propionate is rapidly metabolized by the liver, thereby producing signals to terminate meals, leading to reduced intake (Allen, 2000; Allen et al., 2009). Despite reduced DMI, steers fed backgrounding diets supplemented with NOP maintained ADG, possibly because reduced CH_4 emissions may have caused ME intake to remain unaffected (i.e., lower CH_4 emission may have increased ME content of the NOP-supplemented diets). Conversely, NOP supplementation of a finishing diet tended to reduce ADG in steers despite a greater reduction in CH_4 emissions relative to the backgrounding diet. The observed effects on ADG may be indicative of inefficient utilization of additional ME spared from methanogenesis and most likely attributed to increased H_2 accumulation, which is energetically inefficient.

The reduction in CH_4 emissions due to NOP supplementation of diets coincided with increased H_2 accumulation, possibly due to incomplete incorporation of H_2 spared from methanogenesis to nutritionally beneficial sinks in the rumen such as propionate. Previous studies have shown that the abundance of methanogens either remains unchanged (Romero-Perez et al., 2014) or decreases (Romero-Perez et al., 2015) in response to NOP; hence, greater H_2 accumulation with reduced methanogenesis is possibly due to overloading the capacity of methanogens to utilize H_2. Methane emissions were reduced by 14 and 98 g/d with low and high doses of NOP in steers fed a finishing diet, respectively. On a molar basis, this corresponds to 4 and 24 g/d of H_2 not utilized for CH_4 synthesis (4 mol of dihydrogen required for 1 mol of CH_4), which is greater than the actual amount of H_2 emitted by the NOP-supplemented steers. Nevertheless, increased H_2 accumulation is energetically inefficient, as H_2 is an energy-dense gas (142 kJ/g of H_2; Afeefy et al., 2011) and increased emissions could offset the energy benefit gained by CH_4 mitigation (Van Zijderveld et al., 2011). Methane mitigation spared 0.8 and 5.4 MJ/d (55.5 kJ/g of CH_4) in beef cattle fed finishing diets, with the energy lost as H_2 corresponding to about 50 and 33% of the observed CH_4 reduction. As dissolved H_2 in ruminal fluid was not measured in this study, increased gaseous H_2 accumulation might not completely explain the fate of energy spared by CH_4 mitigation.

It has been proposed that H_2 accumulation in the rumen might inhibit oxidation of cofactors and inhibit ruminal fermentation (Wolin and Miller, 1988), resulting in reduced diet digestibility. Although diet digestibility was not measured in this study, reduced digestibility with increased H_2 accumulation could explain inefficient utilization of spared ME in steers fed finishing diets. Although diet digestibility was reduced with NOP supplementation in dairy cows (Reynolds et al., 2014), no effects were observed in other studies with NOP supplementation in beef cattle (Romero-Perez et al., 2014) and dairy cows (Hristov et al., 2015) fed backgrounding diets. The inconsistencies between studies might be due to the mode of NOP supplementation. Although Reynolds et al. (2014) administered NOP directly into the rumen, the other studies supplemented NOP either as a single daily dose administered as a "top-dress" to feed (Romero-Perez et al., 2014) or homogenously mixed with the TMR (Hristov et al., 2015). Because NOP was mixed with the TMR in our

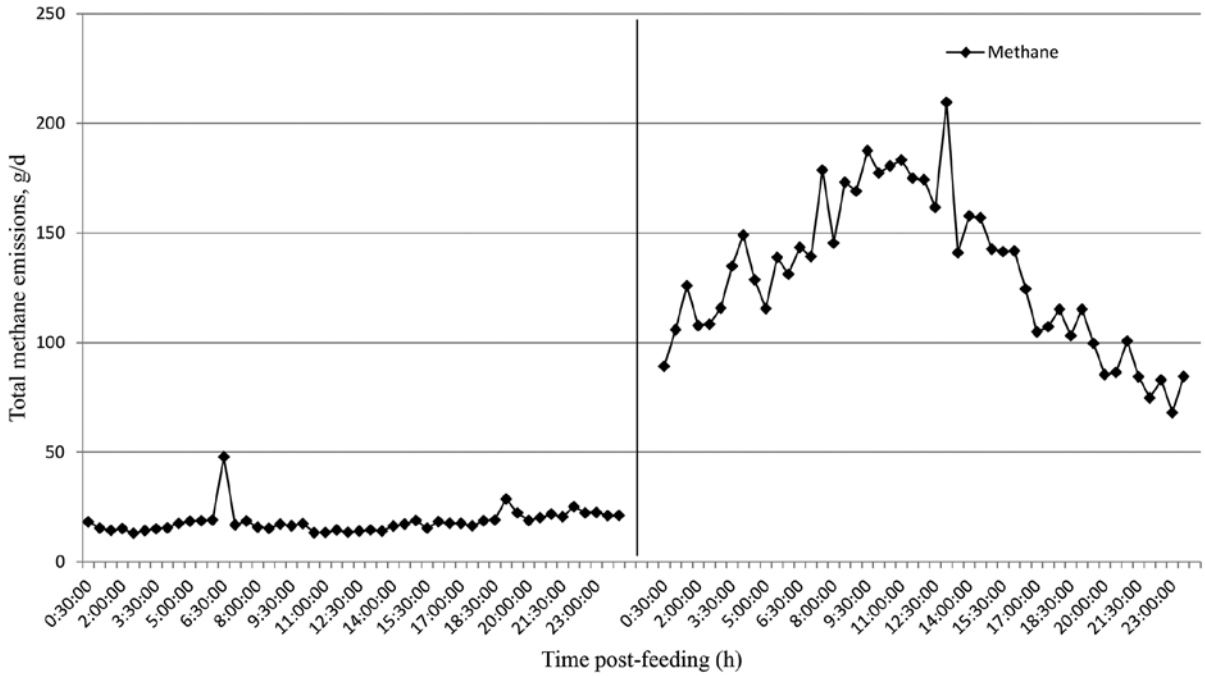

Figure 5. A comparison of average total methane (CH_4) emissions in finishing feedlot cattle before (left panel) and after discontinuing 3-nitrooxypropanol (NOP) for 18 to 25 d (right panel) over time after feeding. Average CH_4 emissions in feedlot animals fed high (200 mg/kg) levels of NOP = 18.2 g/d; DMI = 6.31 kg/d; CH_4 emission intensity = 3.1 g/kg DMI (left panel). Average CH_4 emissions in feedlot animals fed the control diet for 18 to 25 d following discontinuing inclusion of a high (200 mg/kg) level of NOP = 124.9 g/d; DMI = 8.35 kg/d; CH_4 emission intensity = 15.1 g/kg DMI (right panel).

study and the doses did not exceed the maximum doses used in previous studies, it is unlikely that NOP supplementation reduced diet digestibility. Nevertheless, future research should investigate effects of NOP supplementation on nutrient digestibility in animals fed finishing diets because all other beef studies with NOP were performed in cattle fed backgrounding diets.

Previous studies have explored the effect of reduced CH_4 emissions on growth and energy metabolism using bromochloromethane in sheep fed a pelleted grower ration (Sawyer et al., 1974) or steers fed a finishing diet (Tomkins et al., 2009). Results were consistent with the present study, where no effects were observed on live weight gain despite a reduction in CH_4 emissions. Lack of effect on animal performance in studies using bromochloromethane was attributed to gradual adaptation of the rumen ecosystem resulting in reduced antimethanogenic efficacy over the long-term supplementation along with the toxic effects to the animals resulting in reduced growth performance (Sawyer et al., 1974; Tomkins et al., 2009). However, CH_4 mitigation potential of NOP was studied for 112 d in beef cattle fed backgrounding diets and antimethanogenic efficacy remained unaltered, suggesting no adaptation of animals to NOP over time (Romero-Perez et al., 2015). Results from previous studies also suggest a nontoxic nature of NOP, as no negative effects have been observed on BW (Romero-Perez et al., 2015) and nutrient digestibility (Romero-Perez et al., 2014).

Methane emissions recovered rapidly when NOP was removed from the diets during the recovery period. Previous in vitro continuous culture studies (A. Romero-Perez, Lethbridge Research and Development Center; E. K. Okine, University of Alberta; L. L. Guan, University of Alberta; S. M. Duval, M. Kindermann, and K. A. Beauchemin, personal communication.) indicate that CH_4 production is restored to baseline within a week of withdrawal of NOP. Similarly, CH_4 emissions returned to baseline after discontinuing NOP supplementation for 16 d in beef cattle fed backgrounding diets (Romero-Perez et al., 2015). These results suggest that the inhibitory effect of NOP is dependent on its presence in the rumen ecosystem and that full recovery occurs soon after its withdrawal from the diet, be it due to its degradation by rumen microbes or its rapid elution out of the rumen (S. Duval, personal communication).

Based on the results observed in the present study, we can speculate that there may be a relationship between the level of CH_4 mitigation and the potential benefit in terms of energy retention and improvement in animal performance. Moderate reduction 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. However, strong reduction (approximately 80%) in CH_4 emissions might spare energy, but negative effects on the rumen ecosystem leading to reduced utilization of the spared energy cannot be overlooked.

Table 6. Carcass characteristics in feedlot animals fed finishing diet supplemented with a control and low (100 mg/kg DM) and high (200 mg/kg DM) levels of 3-nitrooxypropanol

	Treatment[1]				
Item	Control	Low	High	SEM	Treatment *P*-value
BW, kg	681.6	688.4	682.3	8.3	0.82
HCW, kg	412.6	410.1	404.3	5.1	0.50
Dressing percentage,[2] %	60.6[a]	59.6[b]	59.3[b]	0.3	0.02
Grade fat,[3] mm	21.3	21.6	21.5	1.1	0.98
LM area, cm^2	88.1	86.9	86.6	1.7	0.78
Marbling score[4]	40.0	47.3	42.7	6.3	0.81
Saleable meat, %	48.1	47.7	48.2	0.9	0.99

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

[1]$n = 7$ for each treatment.

[2]Dressing percentages were calculated individually as HCW divided by final BW × 100%.

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

[4]Marbling score of 60.0 to 69.9 = modest, 50.0 to 59.9 = small, 40.0 to 49.9 = slight, and 30.0 to 39.9 = traces.

Future research is needed to explore the relationship between the extent of CH_4 mitigation and animal performance with respect to changes in the rumen ecosystem.

Carcass characteristics in response to NOP supplementation have not been previously documented. In the present study, dressing percentage was reduced with NOP supplementation and the reasons for this are unclear; however, treatment differences were small and might not be of biological significance. Nevertheless, the effect of NOP supplementation on dressing percentage needs further investigation. Supplementation with NOP had no effects on other carcass characteristics including marbling score, saleable meat, HCW, and grade fat, which suggests that NOP supplementation does not reduce the quality of meat.

In conclusion, the present study demonstrates the efficacy of NOP in reducing enteric CH_4 emissions in beef cattle fed both backgrounding and finishing diets. This is the first study to provide evidence of a tendency to improve G:F with sustained reduction of CH_4 emissions in steers fed backgrounding diets supplemented with NOP. Despite a substantial reduction in CH_4 emissions from cattle fed finishing diets, animal performance was not improved, and optimal dose rates in grain-based diets needs further investigation.

LITERATURE CITED

Abecia, L., P. G. Toral, A. I. Martín-García, G. Martínez, N. W. Tomkins, E. Molina-Alcaide, C. J. Newhold, and D. R. Yáñez-Ruiz. 2012. Effect of bromochloromethane on methane emission, rumen fermentation pattern, milk yield, and fatty acid profile in lactating dairy goats. J. Dairy Sci. 95:2027–2036. doi:10.3168/jds.2011-4831

Afeefy, H. Y., J. F. Liebman, and S. E. Stein. 2011. Neutral thermochemical data. NIST Chemistry WebBook, NIST Standard Reference Database Number 69. Vol. 2011. P. J. Linstrom and W. G. Mallard, ed. National Institute of Standards and Technology, Gaithersburg, MD.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. doi:10.2527/jas.2009-1779

AOAC. 2005. Official methods of analysis. 18th ed. AOAC Int., Gaithersburg, MD.

Avila-Stagno, J., A. V. Chaves, M. L. He, O. M. Harstad, K. A. Beauchemin, S. M. McGinn, and T. A. McAllister. 2013. Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles, and carcass traits of lambs. J. Anim. Sci. 91:829–837. doi:10.2527/jas.2012-5215

Canadian Council on Animal Care. 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON, Canada.

Chung, Y. H., N. D. Walker, S. M. McGinn, and K. A. Beauchemin. 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J. Dairy Sci. 94:2431–2439. doi:10.3168/jds.2010-3277

Duval, S., and M. Kindermann. 2012. Use of nitooxy organic molecules in feed for reducing enteric methane emissions in ruminants, and/or to improve ruminant performance. International Patent Application WO 2012/084629 A1. World Intellectual Property Organization, Geneva, Switzerland.

Grainger, C., and K. Beauchemin. 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol. 166–167:308–320. doi:10.1016/j.anifeedsci.2011.04.021

Haisan, J., Y. Sun, L. L. Guan, K. A. Beauchemin, A. Iwaasa, S. Duval, D. R. Barreda, and M. Oba. 2014. The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. J. Dairy Sci. 97:3110–3119. doi:10.3168/jds.2013-7834

Hristov, A. N., O. Joonpyo, G. Fabio, Y. W. Frederick, M. T. Harper, H. L. Weeks, A. F. Branco, P. J. Moate, M. H. Deighton, S. R. O. Williams, M. Kindermann, and S. Duval. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Natl. Acad. Sci. USA 112:10663–10668. doi:10.1073/pnas.1504124112

Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013. Special Topics — Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069. doi:10.2527/jas.2013-6583

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483–2492.

Kessel, J. A. S., and J. B. Russell. 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiol. Ecol. 20:205–210. doi:10.1111/j.1574-6941.1996.tb00319.x

Kononoff, P., A. Heinrichs, and D. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86:1858–1863. doi:10.3168/jds.S0022-0302(03)73773-4

Martinez-Fernandez, G., L. Abecia, A. Arco, G. Cantalapiedra-Hijar, A. I. Martín-García, E. Molina-Alcaide, M. Kindermann, S. Duval, and D. R. Yáñez-Ruiz. 2014. Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. J. Dairy Sci. 97:3790–3799. doi:10.3168/jds.2013-7398

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 8:3346–3356.

Murphy, M., R. Baldwin, and L. Koong. 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. J. Anim. Sci. 55:411–421.

NRC. 2000. Nutrient requirements of beef cattle: 7th rev. ed. Natl. Acad. Press, Washington, DC.

Reynolds, C. K., D. J. Humphries, P. Kirton, M. Kindermann, S. Duval, and W. Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. J. Dairy Sci. 97:3777–3789. doi:10.3168/jds.2013-7397

Rodas-Gonzàlez, A., M. Juàrez, W. M. Robertson, I. L. Larsen, and J. L. Aalhus. 2013. Characterization of Canadian grade standards and lean yield prediction for cows. Can. J. Anim. Sci. 93:99–107. doi:10.4141/cjas2012-091

Romero-Perez, A., E. K. Okine, S. M. McGinn, L. L. Guan, M. Oba, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. J. Anim. Sci. 92:4682–4693. doi:10.2527/jas.2014-7573

Romero-Perez, A., E. K. Okine, S. M. McGinn, L. L. Guan, M. Oba, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2015. Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. J. Anim. Sci. 93:1780–1791. doi:10.2527/jas.2014-8726

Sawyer, M., W. Hoover, and C. Sniffen. 1974. Effects of a ruminal methane inhibitor on growth and energy metabolism in the ovine. J. Anim. Sci. 38:908–914.

Tomkins, N., S. Colegate, and R. Hunter. 2009. A bromochloromethane formulation reduces enteric methanogenesis in cattle fed grain-based diets. Anim. Prod. Sci. 49:1053–1058. doi:10.1071/EA08223

Trei, J., G. Scott, and R. Parish. 1972. Influence of methane inhibition on energetic efficiency of lambs. J. Anim. Sci. 34:510–515.

Tubiello, F. N., M. Salvatore, R. D. Condor Golec, A. Ferrara, S. Rossi, R. Biancalani, S. Frederici, H. Jacobs, and A. Flammini. 2014. Agriculture, forestry and other land use emissions by sources and removals by sinks. ESS working Paper No. 2. Food and Agriculture Organization of the United Nations, Rome, Italy.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Van Zijderveld, S. M., W. J. J. Gerrits, J. Dijkstra, J. R. Newbold, R. B. A. Hulshof, and H. B. Perdok. 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J. Dairy Sci. 94:4028–4038. doi:10.3168/jds.2011-4236

Wolin, M. J., and T. L. Miller. 1988. Microbe-microbe interactions. In: P. N. Hobson, editor, The rumen microbial ecosystem. Elsevier, New York, NY. p. 343–359.

Yang, W., Y. Li, T. McAllister, J. McKinnon, and K. Beauchemin. 2012. Wheat distillers grains in feedlot cattle diets: Feeding behavior, growth performance, carcass characteristics, and blood metabolites. J. Anim. Sci. 90:1301–1310. doi:10.2527/jas.2011-4372