Effects of 3-nitrooxypropanol on feeding behavior and rumen fermentation in beef cattle fed a growing diet

Research Distinction Thesis

Presented in Partial Fulfillment of the Requirements for Graduation with Research with Distinction

Heather A. Pechtl

Department of Animal Sciences

The Ohio State University

2019

Advisor: Dr. Chanhee Lee, Department of Animal Sciences

Ohio Agricultural Research and Development Center

The Ohio State University

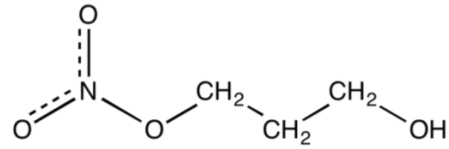
ABSTRACT

Mitigating enteric methane production from ruminants is critical for sustainable livestock operations. Three-nitrooxypropanol (NOP) is an effective feed additive for mitigating enteric methane production in beef cattle. However, feeding NOP to beef cattle often decreases feed intake and alters feed fermentation in the rumen. Therefore, the objective of the study was to determine whether changes in feed intake and rumen fermentation are caused by changes in feeding behavior when supplementing a growing diet with NOP. The experiment used 9 ruminally-cannulated beef steers in a repeated 3 × 3 Latin square design. Steers received one of the three following treatments: a control diet (**CON**); the CON diet supplemented with NOP (**dNOP**; 1g NOP per cow d^{-1}); or the CON diet with an infusion of NOP into the rumen (**NOPinf;** 1g NOP per cow d^{-1}). The CON diet was a typical high forage diet that is fed to growing beef cattle in the US. Rumen content was collected via cannula to examine rumen fermentation. Methane production of individual animals was measured using the Greenfeed system. To observe animal behaviors, cameras were installed to continuously record individual steers for 48 hours. Total duration and frequency of feeding, drinking, activity, and oral manipulation behaviors were collected. Meal time (min/d) and frequency of individual animals were calculated from feeding behavior. Rumen samples were collected via rumen cannula to determine rumen fermentation characteristics (i.e., pH, volatile fatty acid concentration, as well as lactate concentration. Data were analyzed using an analysis of variance model using the repeated measures Mixed Procedure of SAS (SAS Inst. Inc., Cary, NC). Feeding NOP decreased enteric methane production by approximately 18% compared with CON. Rumen fermentation was altered by NOP as expected (i.e., rumen pH and proportion of short chain fatty acids). However, there were no significant differences in feeding, drinking, or activity behaviors between treatments since total duration and frequency of oral manipulation behaviors (chain chewing, biting, and licking) were lower ($P < 0.03$) for dNOP and NOPinf compared to CON. According to the results, NOP changed rumen fermentation, but the changes were not caused by a change in feeding behaviors in beef cattle fed a growing diet.

INTRODUCTION

Agriculture and sustainability are often deemed as contradictions, but in reality, sustainability is a legitimate goal of agriculture. Sustainability contributes to the efficiency of production by maximizing resources. Furthermore, sustainability increases the longevity of the industry by reducing the amount of land required to produce, thereby increasing the amount of farmland available for future agriculture. In other words, if farms aren't being sustainable, they won't survive due to the increasing world population and the pressure from consumers to create more sustainable systems. Methane is a greenhouse gas that ruminants release as an end product of fermentation by microorganisms that break down feed in the rumen, the largest chamber of the ruminant animal stomach (Mitsumori and Sun, 2008; Gerber et al., 2013). Cattle release methane by eructation. Releasing methane increases atmospheric greenhouse gas levels (Tubiello et al., 2014). Livestock production represents 14.5% of global anthropogenic methane emissions, and emissions from beef cattle is 41% of livestock sector emissions, with enteric fermentation as the main source (Gerber et al., 2013). In addition, releasing methane is the loss of feed energy that animals can use for production, as shown by Romero-Perez et al. (2015, 2016). According to Johnson and Johnson in 1995, cattle lose 2-12% of GE intake by methane emission. Therefore, in recent years, animal scientists have attempted to mitigate methane production in multiple forms in order to reduce the amount of the methane entering the atmosphere and to divert the energy from methane production into animals' maintenance and production energy (Haisan et al., 2014). Since enteric methane production is directly caused by digestion, nutritional strategies to lower methane production has been the primary focus of recent research. In the past, nutritional methods have included nitrates, tannins, ionophores, microbials, vaccines, increasing forage digestibility, and decreasing fiber (Hristov et al., 2013). However, these methods present various concerns such as decreasing productivity of ruminant animals.

A promising product for mitigating methane production in ruminant animals including beef cattle, dairy cattle, and sheep is called 3-nitrooxypropanol (**NOP**) and has been studied in recent years (Haisan et al., 2017; Hristov et al., 2015; Martínez-Fernández et al., 2014; Reynolds et al., 2014; Romero-Perez et al., 2015; Vyas et al., 2016a,b). When supplementing ruminant diets with 3-nitrooxypropanol (NOP), enteric methane emissions have been significantly reduced. NOP is a structural analog of methyl coenzyme M that inhibits methyl coenzyme M reductase during methanogenesis (Romero-Perez et al., 2014). The compound is not yet commercialized, but current studies work towards commercialization (Jayanegara et al., 2018). NOP also may increase feed efficiency by decreasing DMI while maintaining production (Vyas et al., 2018). Furthermore, the compound has no known food safety concerns to date. While studies show that NOP has significantly reduced enteric methane emissions in beef cattle, feeding NOP to beef cattle is also shown to decrease their feed intake and alter feed fermentation in the rumen (Lee et al., 2015; Vyas et al., 2016). The reason for those changes when NOP was fed to beef cattle has not been understood.



3-nitrooxypropanol

Therefore, the objective of the study was to determine whether changes in feed intake and rumen fermentation are caused by changes in feeding behavior when supplementing a growing diet with NOP. This is a logical objective since cattle are selective with their feed and engage in sorting behaviors if there are any unfavorable organoleptic properties of a diet. To our knowledge, there is no research on the effects of NOP on feeding behavior of cattle. Therefore, the hypothesis is that the inclusion of NOP in a diet may alter feed intake and feeding behavior of beef cattle, resulting in altered rumen fermentation. However, changes will not occur when NOP is infused into the rumen, showing evidence of potential changing of organoleptic properties when NOP is included in a diet.

MATERIALS AND METHODS

The study was conducted at the Beef Research Center at the Ohio Agricultural Research and Development Center, The Ohio State University (Wooster, OH). The management and husbandry of the cattle used in the experiment was approved by the university's Institute of Animal Care and Use Committee.

Nine ruminally-cannulated beef steers were used and housed in individual stalls with visual, auditory, tactile, and olfactory access to cattle in adjacent stalls. The experiment was conducted in a replicated 3 × 3 Latin square design and grouped into 3 squares according to body weight (BW). Each of the 3 periods contained a 14-day diet adaptation, a 7-day sample collection, then a 7-day washout between each period to allow for withdrawal of effects from previous treatments.

Table 1. Stall and period assignments for beef steers

			Periods and Period Dates					
Stall	Animals	Group	Pd. 1	Dates	Pd. 2	Dates	Pd. 3	Dates
4	133	3	CON	5/4-5/24	NOPinf	6/1-6/21	dNOP	6/29-7/19
5	122	3	NOPinf	5/4-5/25	dNOP	6/1-6/21	CON	6/29-7/19
6	117	2	dNOP	4/27-5/17	NOPinf	5/25-6/14	CON	6/22-7/12
7	110	3	dNOP	5/4-5/25	CON	6/1-6/21	NOPinf	6/29-7/19
8	105	2	NOPinf	4/27-5/17	CON	5/25-6/14	dNOP	6/22-7/12
9	98	1	dNOP	4/20-5/10	NOPinf	5/18-6/7	CON	6/15-7/5
10	97	1	NOPinf	4/20-5/10	CON	5/18-6/7	dNOP	6/15-7/5
11	95	2	CON	4/27-5/17	dNOP	5/25-6/14	NOPinf	6/22-7/12
12	88	1	CON	4/20-5/10	dNOP	5/18-6/7	NOPinf	6/15-7/5

The following treatments were randomly assigned to animals in each square. Treatment 1 (CON) was a high forage total mixed ration (TMR) that is typical for growing beef cattle[1]. Treatment 2 (dNOP) was the CON diet supplemented with NOP (DSM Nutritional Products, Animal Nutrition and Health, Basel, Switzerland; 100 mg/kg of dietary dry matter (DM)

[1] Beef cattle in the United States are on two diets throughout their lives. The first is a high forage diet for growing beef cattle (BCNRM, 2016). The second diet is a high grain diet for finishing beef cattle.

equivalent to 1.06 g/d) so that the compound was mixed directly into the feed. Treatment 3 (infNOP) was the CON diet without supplemental NOP, but NOP (1.08 g/d based on dry matter intake (DMI) of each individual steer) was infused into the rumen through a closed cannula attached to an infusion tube that was hung above the individual stall of the cow receiving that treatment. Infusion of NOP allows the compound to go directly into the rumen, thus bypassing any interaction with feeding behavior. Therefore, if infusion of NOP alters rumen fermentation and feed intake, we can conclude that there is a direct effect of NOP on rumen fermentation that is not caused by organoleptic property changes. The amount of NOP used in Treatments 2 and 3 is based on the level of NOP used by previous experiments (Haisan et al., 2017). Diets were prepared every morning and fed once daily for ad libitum intake.

To measure feeding behaviors, the behavior of individual animals was recorded using cameras (Focsam, Model F19805P, Wireless IP cameras, Houston, Texas, USA) stationed on the ceiling between each two stalls so that one camera recorded the activity of two animals in adjacent tie stalls. The video recordings were transferred to external hard drives. The recordings were observed and the behaviors recorded using Noldus Observer (version 5.0.25; Noldus Information Technology B.V., Wageningen, Netherlands). The behaviors were defined and coded using an ethogram.

Table 2. Ethogram

Behavior	Keyboard Code	Description
Feeding	f	Manipulating and/or consuming from feed stations. Cow has head in, above or adjacent to feed trough while actively feeding.
Drinking	d	Manipulating and/or consuming from drinking stations. Head is lowered close to drinker or held over drinker while actively drinking.
Standing	q	Remains in one position with at least 3 legs touching the ground.
Lying	a	Cow is lying fully recumbent or on its sternum with head upright or on the ground.
Oral manipulation	e	Cow is using its mouth to bite, chew or manipulate feed trough, drinker, wooden beams or pen bars.
Tongue rolling	3	Cow is rolling its tongue excessively and repeatedly outside its mouth or across feed trough, drinker, wooden planks, or pen bars.
Other	x	Non-defined behaviors.

The Observer XT, Noldus

Foscam, Model F19805P, Wireless IP cameras, Houston, Texas, USA

From there, behaviors were analyzed using the following categories:

- Standing
- Lying
- Drinking
- Oral manipulation
- Tongue rolling
- Oral and tongue – mixed bouts alternating between tongue rolling and oral manipulation in a rapid fashion
- Frequency feeding (meal frequency)
- Time spent feeding (meal duration)
- Dry matter consumed per meal
- Not in View (NV) – behavior of animal could not be determined because heads were out of view

Meal behaviors were analyzed using the following criteria:

- Meal = the duration of all feeding events occurring within 5 minutes of each other
- Mean DM = $\frac{average\ DMI}{meal\ frequency}$
- Mean meal duration = $\frac{total\ meal\ duration}{meal\ frequency}$
- Duration and frequency are within 24 hours

To measure rumen fermentation, rumen samples were collected from multiple locations in the rumen since the particle consistency varies in different positions in the rumen. From those locations, a mixed subsample was screened and pH, ammonia, VFA, and lactate were measured from the strained liquid.

To measure feed intake, the weight of the orts or feed refusals was subtracted from the weight of the total feed offered.

Methane emissions were also collected to ensure the reliability of the results with previous studies on the effects of NOP on methane emissions in beef cattle. The methane emissions were measured using the Greenfeed system (C-Lock Inc., Rapid City, SD), on which the cattle were trained prior to experimentation. Methane yield was calculated in terms of g/kg DMI on 3 days after the microbial population of the rumen had adjusted to the diets, which occurred in the middle of the treatment period.

Data analysis was conducted using the Mixed Procedures of SAS (SAS 9.4; SAS Institute, Cary, NC) where group and animal within group were random effects and treatment was a fixed effect. The statistical significance level was $P \leq 0.05$, which is the standard to identify significance. A trend toward significance was $0.05 < P < 0.10$. The means were separated by a paired *t*-test because there was a significant main effect of treatment.

RESULTS

The differences in feeding behaviors across the three treatments were not statistically significant (Table 3; Figure 2 and Figure 3). While there were overall no statistically significant differences across treatments, the frequency of oral manipulation behaviors was lower ($P = 0.03$) for dNOP compared to CON.

Table 3. Effects of NOP fed or infused on behaviors of beef steers fed a high forage (Exp. 1) or high grain diet (Exp. 2)

	Treatments[1]				
	CON	dNOP	infNOP	SEM	*P*-value
Exp. 1					
Meal					
Meal criterion, min[3]	5.0	5.0	5.0	-	-
Total meal duration, min/d	226.8	228.5	215.1	17.24	0.71
Meal frequency	18.3	17.9	18.2	0.90	0.95
Mean DM kg/meal	0.59	0.61	0.58	0.039	0.82
Mean duration, min/meal	13.4	12.9	12.0	1.40	0.61
Standing, min/d	494.9	464.5	483.4	21.54	0.39
Lying,[4] min/d	945.2	975.5	956.6		
Drinking, min/d	7.4	6.8	6.9	1.22	0.75
Oral manipulation,[5] min/d	27.5[a]	18.4[b]	21.7[ab]	3.21	0.03
Tongue rolling, min/d	8.1	8.9	10.2	2.35	0.81
Oral + tongue, min/d	35.6	27.4	32.0	4.36	0.14
NV,[6] min/d	16.1	17.6	13.9	3.67	0.84

[1]CON, Control; dNOP, the control diet supplemented with NOP (active compound, 100 mg/kg dietary DM); infNOP, animals were fed the control diet and NOP was ruminally infused.
[2]Average DMI during 2-d behavior observation
[3]Meal criterion of 300 s (5 min) was used for all animals to calculate meal events.
[4]Lying time (min/d) = 1440 - Standing time.
[5]Biting, chain-chewing, and licking
[6]Animal heads were not visible to determine oral manipulation and tongue rolling.

Below are figures based on data from Table 3[2].

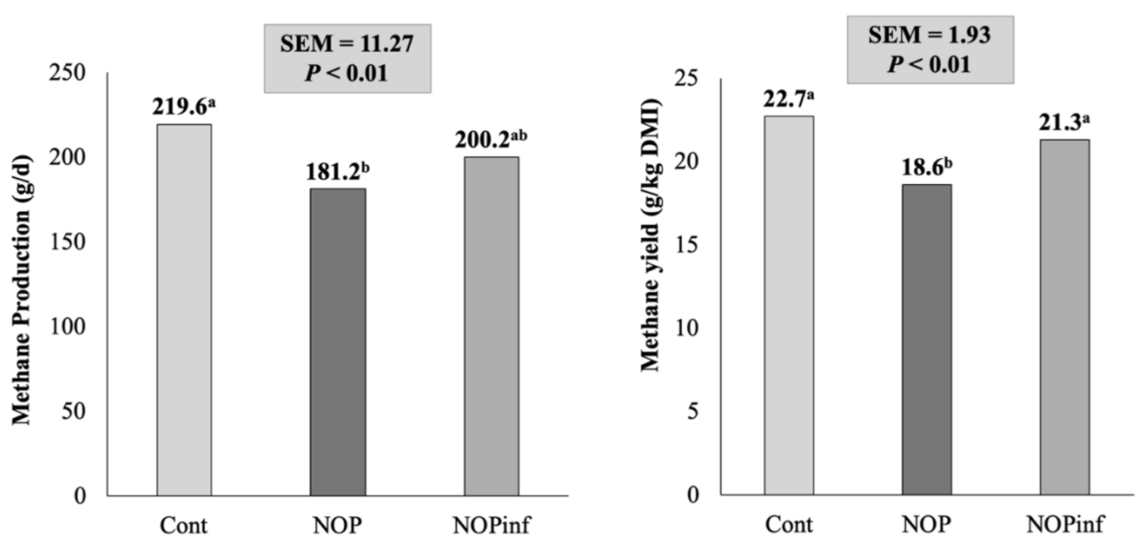


Figure 1. Daily methane production and yield

[2] In the figures, NOP is equivalent to dNOP.

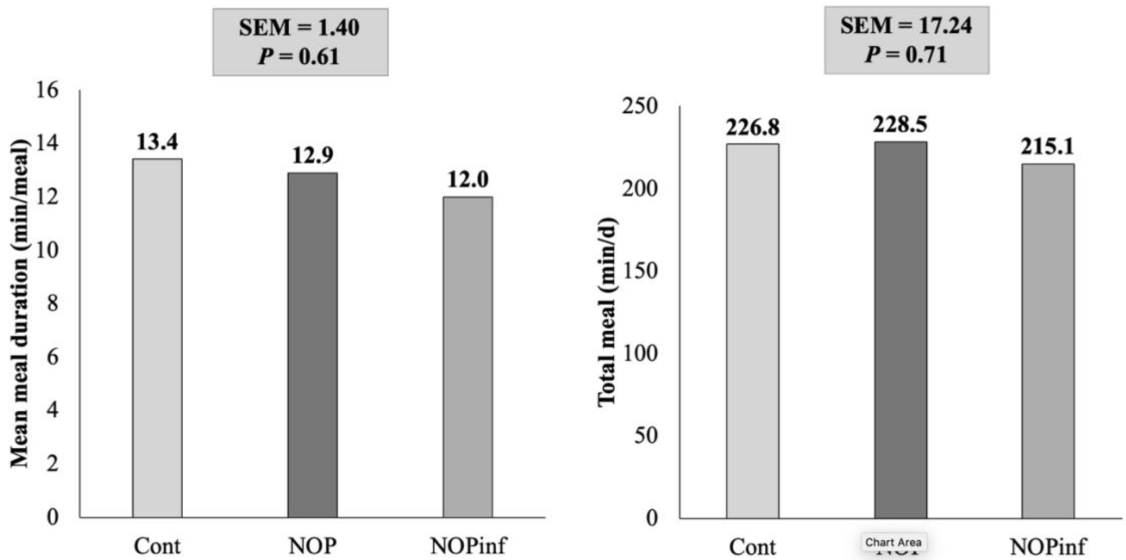


Figure 2. Mean or total meal duration

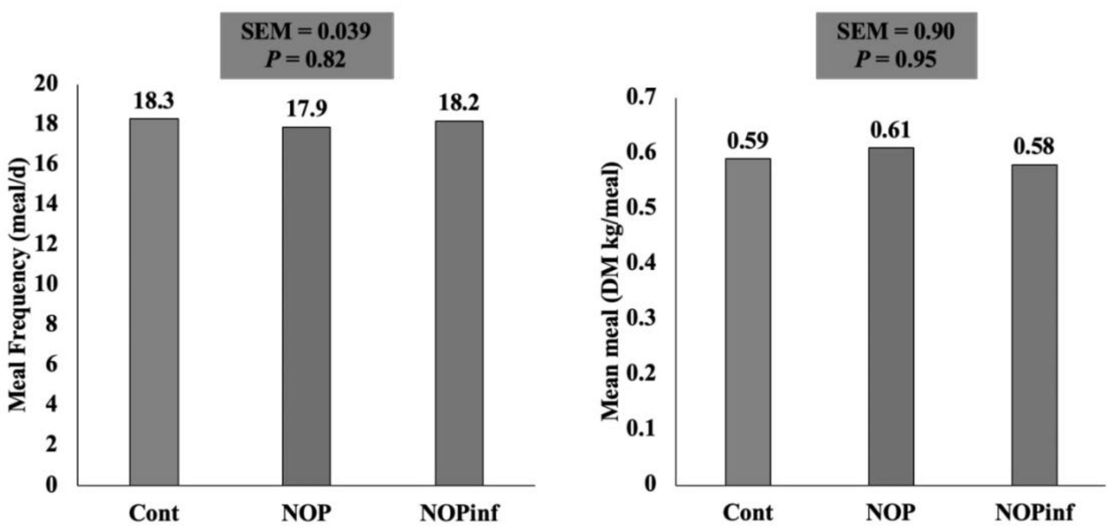


Figure 3. Meal frequency and mean meal amount

Additional results have recently been obtained by Lee et al. (unpublished) from this study. The change in rumen fermentation between dNOP and infNOP was very similar, and the changes in rumen fermentation in dNOP and infNOP were different ($P < 0.01$) from CON (Table 4).

Table 4. Effects of NOP fed or infused on rumen fermentation characteristics in beef steers fed a high forage diet

	Treatments[1]				
	CON	dNOP	infNOP	SEM	*P*-value
Exp. 1					
Overall rumen pH	6.19	6.34	6.27	0.043	0.059
0 h	6.65	6.69	6.67	0.092	0.87
3 h	6.03[c]	6.29[a]	6.15[b]	0.041	< 0.01
6 h	5.91	6.05	5.98	0.056	0.21
NH_3, mg/dL	8.6	7.4	7.2	0.67	0.27
Total VFA, mmol/L	118.6	113.4	111.0	3.53	0.15
Acetate, %	63.7[a]	58.7[c]	61.6[b]	0.87	< 0.01
Propionate, %	20.0[b]	22.7[a]	21.1[b]	0.99	< 0.01
Iso-butyrate, %	0.88	0.99	0.90	0.031	0.052
Butyrate, %	12.2	13.7	12.8	0.48	0.091
Iso-valerate, %	1.63	1.95	1.72	0.092	0.057
Valerate, %	1.23[b]	1.44[a]	1.34[ab]	0.052	< 0.01
C2/C3	3.26[a]	2.65[c]	2.98[b]	0.168	< 0.01
D/L-Lactic acid, mmol/L	0.17	0.15	0.14	0.015	0.14

[1] CON, Control; dNOP, the control diet supplemented with NOP (active compound, 100 mg/kg dietary DM); infNOP, animals were fed the control diet and NOP was ruminally infused.

In addition to data about changes in rumen fermentation, no differences in DMI across treatments were observed (Lee et al., unpublished). Furthermore, methane production and yield were decreased by 18% ($P < 0.01$) for dNOP compared with CON, which was expected based on previous research on the effects of NOP on methane emissions (Figure 1).

DISCUSSION

The steers were cannulated in order to provide a route for Treatment 3, an infusion of NOP into the rumen. Beef steers were used in this study because of known effects of NOP in previous studies (include references). The replicated 3 × 3 Latin square design increases the statistical power of the study since all 9 animals receive each treatment through periods so that animal-to-animal variation can be removed. Therefore, the statistical observation per treatment was 9.

While there is no effect of NOP on feeding behavior or feed consumption, changes in rumen fermentation were observed. Therefore, we reject the hypothesis that feeding behaviors from a diet supplemented with NOP alters rumen fermentation because supplementation and infusion both showed similar changes in proportions of volatile fatty acids (VFAs) in the rumen regardless of the presence of NOP in the feed ingested by the cattle. The change in rumen fermentation between dNOP and infNOP was very similar, and the changes in rumen fermentation in dNOP and infNOP were different ($P < 0.01$) from CON (Table 4). Therefore, we can conclude that changes in rumen fermentation are not due to changes in feeding behavior, inferring that there are no effects of NOP on the organoleptic property of a growing diet when NOP is supplemented at 100 mg/kg dietary DM.

Furthermore, previous research has shown that NOP decreases DMI but has no effect on production (i.e., increased feed efficiency; Vyas et al., 2018). Therefore, since NOP is a promising feed additive to mitigate enteric methane production as well as increasing feed efficiency, more studies should be conducted to investigate the effects of NOP on rumen fermentation because our results indicate that the presence of NOP in the rumen alters feed fermentation.

In addition to observing feeding behavior, changes in organoleptic properties of a diet can be observed indirectly via feed consumption rate and feed sorting (during which animals pick and choose feed particles to consume based on texture or palatability preference). Feed consumption rates are usually decreased and animals sort against concentrate if a feed additive added to the diet negatively alters organoleptic properties of the diet. However, the changes in feed consumption rates and feed sorting by the diet with NOP were not observed in this study.

The frequency of oral manipulation behaviors was lower ($P = 0.03$) for dNOP compared to CON. This may suggest that supplementation of NOP in a diet may stimulate the cattle by providing oral enrichment during the feeding process, thereby reducing the oral manipulation behaviors performed by the animals. However, if this was true, differences in feeding behaviors would also be observed. Therefore, changes in oral manipulation are likely not due to altered oral enrichment.

Another explanation for the changes in oral manipulation is the possibility that during NV activity (animal head was not visible to monitor feeding behavior) the cattle performed oral manipulation behaviors that were thus not recorded. Furthermore, while behavior observation training occurred at the beginning of data collection, researchers for future experiments should implement a standard training protocol entailing periodic assessments of data collection protocol, particularly as new members join the research project in order to maintain consistent behavior logging. It is possible that due to inconsistencies in the operationalizations of behaviors among researchers, challenges may have been posed with interrater reliability, thereby impacting the prominence of recorded oral manipulation behaviors.

In previous studies, there was a negative relationship between DMI of beef cattle and the supplementation of NOP in a diet (Vyas et al., 2016) where the dosage level of NOP was twice as high as the dosage level used in our study. Therefore, to further understand the impacts of NOP supplementation on feeding behaviors, we need to use a larger dosage of NOP since the amount used in this experiment (100 mg/kg DM) was relatively small. We used a smaller dosage since this level still reduces enteric methane production of cattle and a small dosage rate is more practically feasible for producers to use at farms. In future experiments, we might increase the dosage level of NOP to see if there are significant differences in feeding behavior. If the effects of NOP can be understood, the appropriate dosage level of NOP could be determined for beef cattle as a commercial product to reduce the environmental impact of ruminant animal production, as well as increase the efficiency of production in ruminants without compromising the well-being of the animals.

CONCLUSIONS

A high-forage diet supplemented with NOP decreased the methane yield of beef steers by 18%. Feeding behavior was not affected by supplementation or infusion of NOP. However, NOP in the diet altered rumen fermentation. These results lead us to conclude that NOP did not affect the organoleptic properties of a growing diet. To further observe the effects of NOP on the feeding behavior of cattle, future experiments should implement a higher dosage of NOP. Furthermore, animal scientists and agriculturalists need to continue to research concerns posed by consumers regarding subjects surrounding sustainability. Researching these concerns will maintain objectivity in making decisions about animal agriculture, in addition to improving the efficiency of animal production.

ACKNOWLEDGEMENTS

Research funding was provided by the Animal Science Undergraduate Research Experience (ASURE) Program through the Department of Animal Sciences at The Ohio State University in Columbus, Ohio. Funding was also provided by DSM Nutritional Products through the Research Center for Animal Nutrition in Cedex, France.

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