J. Dairy Sci. 97:3110–3119
http://dx.doi.org/10.3168/jds.2013-7834

The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation

J. Haisan,* Y. Sun,* L. L. Guan,* K. A. Beauchemin,† A. Iwaasa,‡ S. Duval,§ D. R. Barreda,* and M. Oba*[1]
*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada
†Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1, Canada
‡Agriculture and Agri-Food Canada, Swift Current, Saskatchewan S9H 3X2, Canada
§DSM Nutritional Products Ltd., Centre de Recherche en Nutrition Animale, 1 Bd d'Alsace, Village-Neuf BP 170, France

ABSTRACT

The objective of the current study was to determine the effects of adding 3-nitrooxypropanol to the diet of lactating Holstein cows on methane emissions, rumen fermentation, ruminal microbial profile, and milk production. Twelve ruminally cannulated Holstein cows in midlactation were used in a crossover design study with 28-d periods. Cows were fed a diet containing 38% forage on a dry matter basis with either 2,500 mg/d of 3-nitrooxypropanol (fed as 25 g of 10% 3-nitrooxypropanol on silicon dioxide) or 25 g/d of silicon dioxide (control). After a 21-d diet adaptation period, dry matter intake (DMI) and milk yield were recorded daily. Rumen fluid and digesta were collected on d 22 and 28 for volatile fatty acid analysis and microbial profiling. Enteric methane emissions were measured on d 23 to 27 using the sulfur hexafluoride tracer gas technique. Feeding 3-nitrooxypropanol did not affect DMI; however, methane production was reduced from 17.8 to 7.18 g/kg of DMI. No change in milk or milk component yields was observed, but cows fed 3-nitrooxypropanol gained more body weight than control cows (1.06 vs. 0.39 kg/d). Concentrations of total volatile fatty acids in ruminal fluid were not affected by treatment, but a reduction in acetate proportion and a tendency for an increase in propionate proportion was noted. As such, a reduction in the acetate-to-propionate ratio was observed (2.02 vs. 2.36). Protozoa counts were not affected by treatment; however, a reduction in methanogen copy count number was observed when 3-nitrooxypropanol was fed (0.95 vs. 2.69 $\times$ 10^8/g of rumen digesta). The data showed that feeding 3-nitrooxypropanol to lactating dairy cows at 2,500 mg/d can reduce methane emissions without compromising DMI or milk production.

Key words: 3-nitrooxypropanol, enteric methane emission, milk production, methanogen

INTRODUCTION

Demand exists to reduce greenhouse gas emissions generated by the agricultural sector, in particular, the livestock industry. In recent years, it has been estimated that cattle alone are responsible for 11 to 17% of the methane generated globally (Beauchemin et al., 2009a). As methane has a global warming potential 21 times that of carbon dioxide (United Nations, 2013), the environmental importance of emissions is self-evident. Another consideration is that between 2 and 12% of the ingested gross energy of cattle can be lost to methane (Johnson and Johnson, 1995), a loss of energy that could potentially be used by the animal. Enteric methane emissions from cattle can be reduced through dietary techniques such as improving forage quality, higher inclusion of concentrates in the diet and feeding lipids (Martin et al., 2010; Eckard et al., 2012). Additionally, previous research has shown that natural compounds, such as tannins and saponins, and synthetic dietary compounds, such as ionophores, can reduce methane emissions from ruminants through inhibition of methanogenesis or by shifting fermentation pathways to promote alternative hydrogen sinks, such as propionate production, thus reducing methane emissions (McAllister and Newbold, 2008; Martin et al., 2010).

Recently, several molecules, substituted at various positions with at least one nitrooxy group, were identified as potential inhibitors of enteric methanogenesis (Duval and Kindermann, 2012). One such compound, 3-nitrooxypropanol (**NOP**), was developed from predecessor compounds (ethyl 3-nitrooxy propionate; WO2011/070133 and 3-azido-propionic acid ethyl ester), which were identified from an in vitro rumen simulation screening assay (Soliva et al., 2011). The NOP exhibited a significantly higher potential to reduce methanogenesis in vitro than the well-known model compound bromoethanesulfonate (Soliva et al., 2011). Bromoethanesulfonate is a coenzyme M analog (Gunsalus et al., 1978) with very specific activity against methanogens that inhibits the reduction of

Received December 14, 2013.
Accepted January 26, 2014.
[1]Corresponding author: moba@ualberta.ca

methyl-coenzyme M to methane during the last step of methanogenesis (Immig, 1996).

Research using a rumen simulation technique showed that NOP is capable of reducing methane production (Romero Perez et al., 2013b). Furthermore, when NOP was directly dosed into the rumen of sheep (Martinez-Fernandez et al., 2013) and lactating dairy cows (Reynolds et al., 2013) or fed once daily to beef cattle (Romero Perez et al., 2013a), methane emissions were reduced and an increase in propionate concentration was observed, suggesting a shift in rumen fermentation. However, the effect of NOP on methanogen numbers or microbial profile was not reported or consistently demonstrated in those previous studies.

Although use of NOP is a promising approach to reduce enteric methane emissions from ruminants, further studies are required to confirm its efficacy in reducing methane emissions while evaluating its effects on rumen fermentation and animal productivity. It was hypothesized that lactating dairy cows fed NOP would have reduced methane emissions; hence, more energy would be available for milk production. The objectives of the present study were to determine the effects of NOP on methane emissions, animal performance, rumen fermentation, and rumen microbial profile of lactating dairy cows.

MATERIALS AND METHODS

All procedures were preapproved by the Animal Care and Use Committee for Livestock at the University of Alberta and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada). The use of the biochemical compound [10% NOP on silicon dioxide (SiO_2)], developed by DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland) in the diet of lactating dairy cows at 25 g/d was preapproved by the Veterinary Drugs Directorate Division (Health Canada, Ottawa, ON, Canada) for research. According to their instructions, milk was discarded for the duration of the study and an additional 14-d milk withdrawal period was implemented upon completion of the study.

Experimental Design, Diet, and Treatment

Twelve lactating Holstein cows with ruminal cannulas (Bar Diamond Inc., Parma, ID) were used in a crossover design study with 28-d periods consisting of 21 d of adaptation and 7 d of data and sample collection. Cows were separated into 2 groups based on the pre-experiment DIM (means ± SD: Group 1 = 100 ± 5.4, Group 2 = 76 ± 10.1). Group 1 had 4 multiparous and 4 primiparous cows and group 2 had 2 multiparous and 2 primiparous cows, and they were randomly assigned to the treatment sequence. Pre-experiment BW (means ± SD) were 591.5 ± 58.9 and 567.5 ± 93.5 kg for groups 1 and 2, respectively. The study was conducted using 2 groups of cows to facilitate methane measurement; however, we did not have sufficient supplies to measure methane emission for all cows at once, thus the whole study protocol was staggered by 7 d between the 2 groups. Cows were housed individually in tiestalls and milked twice daily in their stalls at 0400 and 1600 h.

All cows were fed the same diet as a TMR (Table 1), ad libitum, once daily at 0900 h, allowing for 5% refusals throughout the study, and had free access to water. The diet was formulated to provide adequate ME and MP for a 650-kg cow producing 40 kg of milk per day (NRC, 2001) and to ensure ME and MP intake did not limit milk production of all cows. Cows were fed either SiO_2 as a control (**CON**) or NOP at 25 g/d, resulting in 0 and 2,500 mg/d of 3-nitrooxypropanol, respectively. Both NOP and SiO_2, in powder form, were hand-mixed with 80 g of ground barley grain, 50 g of wet molasses, and 40 g of canola oil to improve adhesion to feed particles and palatability. Cows were assigned to either treatment on d 1 of each period without incremental adaptation. To avoid contamination of feeding equipment at the farm, each treatment mixture was applied by hand-mixing into the TMR once daily, within 30 min of feeding. This protocol also simulated an on-farm feeding scenario, allowing for consumption of NOP throughout the day as feed is consumed.

Data and Sample Collection

The amount of feed offered and refused was recorded for individual cows at the time of feeding, and the amount of TMR fed was adjusted daily to maintain 5% refusals. The NOP content in refusals was not determined. Dietary ingredients were sampled (approximately 500 g) on d 25 to 27 and composited for each period to determine the chemical composition of the diet. All samples were dried for 72 h at 55°C in a forced-air oven and stored at 4°C until further analysis. Additionally, diets were adjusted weekly to maintain the same concentrate-to-forage ratio on a DM basis. Body weight was measured at the beginning and end of each period. Milk yield was recorded at every milking, and milk samples (approximately 50 mL) were taken from 6 consecutive milkings from d 25 to 27 and stored at 4°C with 2-bromo-2-nitropropane-1,3-diol until milk composition analysis.

Rumen digesta were collected through a rumen cannula, from 5 locations in the rumen (cranial dorsal, cranial ventral, central rumen, caudal dorsal, and caudal