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Effect of 3-nitrooxipropanol on methane and hydrogen emissions, methane isotopic signature, and ruminal fermentation in dairy cows

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ABSTRACT

The objective of this crossover experiment was to investigate the effect of a methane inhibitor, 3-nitrooxypropanol (3NOP), on enteric methane emission, methane isotopic composition, and rumen fermentation and microbial profile in lactating dairy cows. The experiment involved 6 ruminally cannulated late-lactation Holstein cows assigned to 2 treatments: control and 3NOP (60 mg/kg of feed dry matter). Compared with the control, 3NOP decreased methane emission by 31% and increased hydrogen emission from undetectable to 1.33 g/d. Methane emissions per kilogram of dry matter intake and milk yield were also decreased 34% by 3NOP. Milk production and composition were not affected by 3NOP, except milk fat concentration was increased compared with the control. Concentrations of total VFA and propionate in ruminal fluid were not affected by treatment, but acetate concentration tended to be lower and acetate-to-propionate ratio was lower for 3NOP compared with the control. The 3NOP decreased the molar proportion of acetate and increase those of propionate, butyrate, valerate, and isovalerate. Deuterium-to-hydrogen ratios of methane and the abundance of $^{13}CH_3D$ were similar between treatments. Compared with the control, minor (4‰) depletion in the $^{13}C/^{12}C$ ratio was observed for 3NOP. Genus composition of methanogenic archaea (*Methanobrevibacter*, *Methanosphaera*, and *Methanomicrobium*) was not affected by 3NOP, but the proportion of methanogens in the total cell counts tended to be decreased by 3NOP. *Prevotella* spp., the predominant bacterial genus in ruminal contents in this experiment, was also not affected by 3NOP. Compared with the control, *Ruminococcus* and *Clostridium* spp. were decreased and *Butyrivibrio* spp. was increased by 3NOP. This experiment demonstrated that a substantial inhibition of enteric methane emission by 3NOP in dairy cows was accompanied with increased hydrogen emission and decreased acetate-to-propionate ratio; however, neither an effect on rumen archaeal community composition nor a significant change in the isotope composition of methane was observed.

Key words: methane, 3-nitrooxypropanol, rumen fermentation, dairy cow

INTRODUCTION

In the rumen, CH_4 is an end product of microbial fermentation of carbohydrates and AA. Methanogenesis is the major sink for hydrogen in the rumen, but enteric CH_4 represents also a net feed energy loss for the animal (Johnson and Johnson, 1995) and is a major contributor to agricultural greenhouse gas (**GHG**) emissions globally (IPCC, 2014).

Several reviews presented technical options for abatement of livestock GHG emissions (Boadi et al., 2004; McAllister and Newbold, 2008; Hristov et al., 2013). These strategies focus on feeding management practices such as fat supplementation, concentrate inclusion, processing low-quality feeds, and improving overall forage quality, as well as feed additives, such as alternative electron receptors, ionophoric antibiotics, plant bioactive compounds, enzymes, and CH_4 inhibitors.

Among CH_4 inhibitors, bromochloromethane, 2-bromo-ethane sulfonate, and chloroform are the most studied compounds in ruminants (Hristov et al., 2013). Both in vitro and in vivo experiments have demonstrated that these compounds were effective in reducing CH_4 emission without negatively affecting animal productivity (Sawyer et al., 1974; Goel et al., 2009; Tomkins et al., 2009; Abecia et al., 2012). Use of these compounds, however, is limited due to toxicity, rumen adaptation, or environmental regulation issues (Hristov et al., 2013). In response, natural or synthetic compounds with a similar mode of action are being

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developed. The compound tested in this experiment, 3-nitrooxypropanol (**3NOP**), was designed to inhibit the activity of methyl coenzyme-M reductase (Duval and Kindermann, 2012), the enzyme responsible for microbial formation of CH_4 (Ermler et al., 1997). Recent studies showed that 3NOP consistently decreased enteric CH_4 emission in lactating dairy cows (Haisan et al., 2014; Reynolds et al., 2014). In a 12-wk study, Hristov et al. (2015a) reported persistent reduction of enteric CH_4 emission, whereas productive performance of high-producing dairy cows was not affected by 3NOP supplementation. In their study, however, the effect of 3NOP on ruminal fermentation could not be evaluated because the cows used were not ruminally cannulated.

Stable isotope compositions of CH_4 ($^{13}C/^{12}C$, D/H, and $^{13}CH_3D$, where D is a stable isotope of hydrogen with one extra neutron) reflect the isotope compositions of substrate ($^{13}C/^{12}C$ of feeds and D/H of rumen fluids), as well as isotope fractionation associated with microbial methanogenesis. The latter is shown to be a function of pathways, growth phase, and hydrogen levels (Whiticar et al., 1986; Burke, 1993; Valentine et al., 2004; Wang et al., 2015). For the CO_2 reduction pathway, thought to be dominant in the rumen (Hungate, 1966), large ^{13}C-depletion is associated with stationary growth, low metabolic rates, and low H_2 levels, whereas large D-depletion is associated with high metabolic rate and high H_2 levels (Burke, 1993; Zyakun, 1996; Valentine et al., 2004; Wang et al., 2015).

Therefore, one of the objectives of our study was to test if, in addition to decreased CH_4 emission, 3NOP affects CH_4 isotope compositions due to changes in physiology or environmental conditions for methanogenesis in lactating dairy cows. The study also investigated the effect of 3NOP on rumen fermentation, ruminal microbial profile, and production variables. We hypothesized that 3NOP would, similar to previous experiments, decrease acetate-to-propionate ratio in ruminal fluid and, due to the large reduction in CH_4 emission, would also affect the composition of ruminal archaea and the isotopic signature of enteric CH_4.

MATERIALS AND METHODS

Animals involved in these experiments were cared for according to the guidelines of the Pennsylvania State University Animal Care and Use Committee. The committee reviewed and approved the experiment and all procedures carried out in this study.

Animals and Experimental Design

The experiment used 6 ruminally cannulated late-lactation Holstein cows in a 2 × 2 crossover design with 2 experimental periods of 14 d each. A 7-d washout period was allowed between the experimental periods. Cows were grouped by DIM and current milk yield in 2 squares of 3 cows each. Cows were 1.3 (SD = 0.52) lactations, 233 (SD = 45) DIM at the beginning of the experiment, had an average BW during the experiment of 610 (SD = 158) kg, and were fitted with soft plastic ruminal cannulas (10.2 cm internal diameter; Bar Diamond Inc., Parma, ID). Within a period, the first 10 d served as adaptation and the remaining 4 d were used for sample and production data collection. Cows received recombinant bST (Posilac, Elanco Co., Greenfield, IN; 500 mg/cow, i.m.) on d 1 of each experimental period. The following 2 treatments were tested: 0 mg of 3NOP/kg of dietary DM (control) and 60 mg of 3NOP/kg of DM (3NOP; DSM Nutritional Products, Basel, Switzerland). The 60-mg/kg of DM dose was selected based on a previous experiment with 3NOP (Hristov et al., 2015a). The basal diet was formulated to meet or exceed the NE_L and MP requirements of a Holstein cow (according to NRC, 2001) with 610 kg of BW, producing 32 kg of milk/d with 4.10% milk fat and 3.60% true milk protein, and consuming 24 kg/d of DMI (Table 1). Diets were fed as TMR once daily at 0800 h targeting 10% refusals. The 3NOP supplement contained 8.85% 3NOP on SiO_2 and propylene glycol; the placebo supplement contained SiO_2 and propylene glycol only (Hristov et al., 2015a). The supplements were mixed with the TMR to deliver the final 3NOP concentration as indicated above. Cows were milked twice daily at approximately 0600 and 1800 h and had continuous access to a fresh water source.

Sampling and Measurements

During the experiment, TMR offered and refusals were recorded daily. Samples of the forages were collected once weekly and samples of the TMR were collected twice weekly. Samples of the concentrate feeds were collected once per experimental period. Feed samples were dried for 48 h at 65°C in a forced-air oven and ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm sieve for further analysis.

Composite (equal DM weight basis) samples of the forages and concentrate feeds fed during the experiment were submitted to Cumberland Valley Analytical Services (Maugansville, MD) for wet chemistry analyses of CP, NDF, ADF, Ca, P, and estimated NE_L (CVAS, 2014). The analyzed composition of the feed ingredients and their inclusion in the TMR was used to compute the CP, NDF, ADF, Ca, and P concentration of the diets (Table 1). During the last 3 d of each period, CH_4, CO_2, and H_2 emission were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD) 8 times in