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Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows

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ABSTRACT

The objective was to measure effects of 3-nitrooxypropanol (3NP) on methane production of lactating dairy cows and any associated changes in digestion and energy and N metabolism. Six Holstein-Friesian dairy cows in mid-lactation were fed twice daily a total mixed ration with maize silage as the primary forage source. Cows received 1 of 3 treatments using an experimental design based on two 3 × 3 Latin squares with 5-wk periods. Treatments were a control placebo or 500 or 2,500 mg/d of 3NP delivered directly into the rumen, via the rumen fistula, in equal doses before each feeding. Measurements of methane production and energy and N balance were obtained during wk 5 of each period using respiration calorimeters and digestion trials. Measurements of rumen pH (48 h) and postprandial volatile fatty acid and ammonia concentrations were made at the end of wk 4. Daily methane production was reduced by 3NP, but the effects were not dose dependent (reductions of 6.6 and 9.8% for 500 and 2,500 mg/d, respectively). Dosing 3NP had a transitory inhibitory effect on methane production, which may have been due to the product leaving the rumen in liquid outflow or through absorption or metabolism. Changes in rumen concentrations of volatile fatty acids indicated that the pattern of rumen fermentation was affected by both doses of the product, with a decrease in acetate:propionate ratio observed, but that acetate production was inhibited by the higher dose. Dry matter, organic matter, acid detergent fiber, N, and energy digestibility were reduced at the higher dose of the product. The decrease in digestible energy supply was not completely countered by the decrease in methane excretion such that metabolizable energy supply, metabolizable energy concentration of the diet, and net energy balance (milk plus tissue energy) were reduced by the highest dose of 3NP. Similarly, the decrease in N digestibility at the higher dose of the product was associated with a decrease in body N balance that was not observed for the lower dose. Milk yield and milk fat concentration and fatty acid composition were not affected but milk protein concentration was greater for the higher dose of 3NP. Twice-daily rumen dosing of 3NP reduced methane production by lactating dairy cows, but the dose of 2,500 mg/d reduced rumen acetate concentration, diet digestibility, and energy supply. Further research is warranted to determine the optimal dose and delivery method of the product.
Key words: 3-nitrooxypropanol, methane, digestion, rumen, dairy cow

INTRODUCTION

In recent years, a massive global research effort has explored potential nutritional, genetic, and management options for reducing methane emissions from ruminants. Several potential approaches have shown promise (e.g., Blaxter and Czerkawski, 1966; Mills et al., 2001; Beauchemin et al., 2008; McAllister and Newbold, 2008). These include changes in carbohydrate amount and type (e.g., starch or sugar vs. fiber), supplemental fats (through replacement of fermentable substrate and inhibitory effects on methanogenesis), feeding precursors of propionate such as fumaric or malic acid, or feeding bioactive compounds such as ionophores or plant components. Examples of plant bioactive components that are purported to inhibit methanogenesis include compounds from garlic, tannins, or saponins (Beauchemin et al., 2008; McAllister and Newbold, 2008). In addition, hydrogen acceptors such as nitrate or sulfate have been successful in reducing methane production in sheep and dairy cows (Bozic et al., 2009; van Zijderveld, 2011) and inhibitory effects of chloral hydrates on methane production have been observed both in vitro and in vivo (Trei et al., 1972; Clapperton, 1974; Goel et al., 2009; Abecia et al., 2012). In regard to bioactive compounds, supplements that are effective at reducing methane production in sheep have, in some cases, been found to be ineffective

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in lactating dairy cows (McCourt et al., 2008; Foley et al., 2009), which may be due to differences in rumen ecology and dynamics.

In addition to specific plant bioactive compounds, there is now interest in the potential development of synthetic compounds as specific inhibitors of methanogenesis (Soliva et al., 2011). In this regard, in silico screening (Halgren et al., 2004) has identified several compounds as potential inhibitors of methyl-coenzyme M reductase, the final step in the reduction of CO_2 to CH_4 by methanogenic archaea (Duval and Kindermann, 2012). Two of these compounds, ethyl-3-nitrooxy propionate and 3-nitrooxypropanol (**3NP**), have recently been shown to be effective at inhibiting methane production in vitro and when fed to sheep for 30 d (Martínez-Fernández et al., 2014). The objective of the present study was to determine the effects of 2 doses of 3NP on methane production by lactating dairy cows and any associated effects on diet digestion and energy and N metabolism. Our hypothesis was that 3NP would reduce methane production in lactating dairy cows.

MATERIALS AND METHODS

Animals and Diet

Six second-parity Holstein-Friesian cows averaging 33.4 kg/d milk yield and 200 DIM at the start of the study were used. Three of the cows were pregnant (92, 97, and 140 d) at the start of the study. An additional cow was confirmed pregnant at the end of the study (55 d). Cows had rumen fistulas established during their first lactation with cannulas (Bar Diamond, Parma, ID) inserted. All procedures were licensed and monitored by the UK Home Office under the Animal Science (1986) Procedures Act. Throughout the study, cows were fed a TMR (Table 1) for ad libitum DMI (5% refusals). Animals were fed twice daily, receiving two-thirds of their daily allocation in the morning and the remaining one-third in the afternoon. Refused food was removed and weighed daily before the morning feeding. Cows were milked twice daily at approximately 0630 and 1630 h. When not restrained for measurements, cows were housed in a cubicle yard with rubber chip-filled mattresses and wood shavings as additional bedding and were milked in a herringbone parlor. While in the cubicle yard, cows were fed individually using an electronic identification controlled pneumatic feed barrier (Insentec, Marknesse, the Netherlands), and drinking water was constantly available from troughs. While restrained in tiestalls or chambers for measurements described below, cows had continuous access to drinking water through drinking bowls and were milked using a pipeline system.

Table 1. Composition of the TMR[1] fed

Ingredient	g/kg of DM
Grass silage[2]	60
Maize silage[3]	420
Chopped straw	32
Ground wheat	110
Ground barley	95
Soybean meal	118
Palm kernel (expeller)	54
Distillers dried grains	45
Sugar beet pulp	40
Megalac[4]	6
Urea	5
Minerals and vitamins	15

[1]Average OM, CP, NDF, ADF, and starch concentrations (g/kg of DM) of 925, 182, 398, 278, and 166, respectively.

[2]Average CP, NDF, and ADF concentrations (g/kg of DM) of 159, 564, and 362, respectively.

[3]Average CP, NDF, ADF, and starch concentrations (g/kg of DM) of 78, 439, 242, and 291, respectively.

[4]Volac International Ltd. (Orwell, UK).

Experimental Design and Treatments

The experimental design was based on 3 treatments and 2 balanced 3 × 3 Latin squares with 5-wk periods. Treatments were a control and 2 doses (500 and 2,500 mg/d) of 3NP. The 3NP was formulated at 50% on silica oxide (SiO_2) and was kept in a sealed container under refrigeration (2 to 4°C) before weighing for administration. Doses were then weighed and wrapped in a single facial tissue and kept in a sealed container before administration through the rumen fistula approximately 10 cm below the surface of the rumen contents. The control treatment was the administration of a single tissue through the rumen fistula.

Measurements

Milk yield was recorded daily using milk meters throughout the 35-d period. Cows were weighed at the beginning of the study and at the end of each period.

Rumen Sampling

During wk 4 of each period (d 24 to 28), animals were transferred to individual tiestalls with rubber mats and wood chip bedding to acclimatize cows to restraint by head yoke and obtain rumen measurements. Rumen fluid samples were taken on d 27, just before and at 0.5, 1.0, 1.5, 2.0, and 4.0 h after both the a.m. and p.m. feedings. Rumen fluid samples (100 mL) were collected from the ventral sac via aspiration through a coarse filtered tube inserted vertically approximately 40 cm into the rumen mat directly below the rumen fistula. The sample was mixed thoroughly and pH measured