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Effect of dietary nitrate level on enteric methane production, hydrogen emission, rumen fermentation, and nutrient digestibility in dairy cows

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ABSTRACT

Nitrate may lower methane production in ruminants by competing with methanogenesis for available hydrogen in the rumen. This study evaluated the effect of 4 levels of dietary nitrate addition on enteric methane production, hydrogen emission, feed intake, rumen fermentation, nutrient digestibility, microbial protein synthesis, and blood methemoglobin. In a 4 × 4 Latin square design 4 lactating Danish Holstein dairy cows fitted with rumen, duodenal, and ileal cannulas were assigned to 4 calcium ammonium nitrate addition levels: control, low, medium, and high [0, 5.3, 13.6, and 21.1 g of nitrate/kg of dry matter (DM), respectively]. Diets were made isonitrogenous by replacing urea. Cows were fed ad libitum and, after a 6-d period of gradual introduction of nitrate, adapted to the corn-silage-based total mixed ration (forage:concentrate ratio 50:50 on DM basis) for 16 d before sampling. Digesta content from duodenum, ileum, and feces, and rumen liquid were collected, after which methane production and hydrogen emissions were measured in respiration chambers. Methane production [L/kg of dry matter intake (DMI)] linearly decreased with increasing nitrate concentrations compared with the control, corresponding to a reduction of 6, 13, and 23% for the low, medium, and high diets, respectively. Methane production was lowered with apparent efficiencies (measured methane reduction relative to potential methane reduction) of 82.3, 71.9, and 79.4% for the low, medium, and high diets, respectively. Addition of nitrate increased hydrogen emissions (L/kg of DMI) quadratically by a factor of 2.5, 3.4, and 3.0 (as L/kg of DMI) for the low, medium, and high diets, respectively, compared with the control. Blood methemoglobin levels and nitrate concentrations in milk and urine increased with increasing nitrate intake, but did not constitute a threat for animal health and human food safety. Microbial crude protein synthesis and efficiency were unaffected. Total volatile fatty acid concentration and molar proportions of acetate, butyrate, and propionate were unaffected, whereas molar proportions of formate increased. Milk yield, milk composition, DMI and digestibility of DM, organic matter, crude protein, and neutral detergent fiber in rumen, small intestine, hindgut, and total tract were unaffected by addition of nitrate. In conclusion, nitrate lowered methane production linearly with minor effects on rumen fermentation and no effects on nutrient digestibility.
Key words: methane, nitrate, hydrogen, digestibility, dairy cow

INTRODUCTION

Decreasing methane emission from agriculture is globally under investigation. Methane is a potent greenhouse gas having a 28 times higher warming potential over a 100-yr timespan than carbon dioxide (Myhre et al., 2013). Besides the environmental impact, methane eructed by ruminants is considered an energy loss. Methane has a gross energy (**GE**) value of 55 MJ/kg and between 2 to 12% of ingested GE is lost as methane (Johnson and Johnson, 1995).

In ruminants, methanogenic archaea produce methane by reducing principally carbon dioxide with hydrogen ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$). Both carbon dioxide and hydrogen are end products from degradation of plant material (primarily carbohydrates) into VFA. Methanogenesis is considered to be the largest sink in the rumen to remove hydrogen, which is thought to inhibit fermentation (Ellis et al., 2008).

Nitrate (NO_3^-) acts as an alternative hydrogen sink and thereby lowers enteric methane production (Allison and Reddy, 1984). In the rumen, nitrate is first

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reduced to nitrite ($NO_3^- + H_2 \rightarrow NO_2^- + H_2O$) and is then further reduced to ammonium ($NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$). Based on change in Gibbs free energy (ΔG), nitrate reduction to nitrite ($\Delta G = -130$ kJ) followed by nitrite reduction to ammonium ($\Delta G = -371$ kJ) yields more energy compared with methanogenesis ($\Delta G = -67$ kJ; Ungerfeld and Kohn, 2006). Thus, nitrate reduction is highly competitive compared with methanogenesis with respect to available hydrogen and therefore lowers methane production. However, increased hydrogen emissions have been observed when cattle have been fed nitrate (van Zijderveld et al., 2011; Lund et al., 2014; Guyader et al., 2015).

Recent studies have established the effects of feeding high nitrate diets on lowering enteric methane production in dairy cattle (van Zijderveld et al., 2011; Lund et al., 2014; Klop et al., 2016). These studies quantified lower methane production of 16 to 25% as grams per kilogram of DMI or liters per kilogram of DMI at a nitrate inclusion level of 21 g of NO_3^-/kg of DM. In a recent meta-analysis including data from 8 studies with sheep, beef cattle, and dairy cattle, Lee and Beauchemin (2014) showed a linear decrease in methane production with increasing levels of dietary nitrate per kilogram of BW. Newbold et al. (2014) and Lee et al. (2015a) observed a linear decrease in methane production with increasing levels of dietary nitrate fed to steers and beef heifers, respectively. The response in methane production to different levels of dietary nitrate intake has not yet been investigated in vivo with lactating dairy cows adapted to dietary nitrate. Furthermore, previous studies (Lund et al., 2014; Guyader et al., 2015) indicate that nitrate or nitrite, or both, may be toxic toward rumen microbes, in particular through inhibition of fibrolytic bacteria and methanogens (Latham et al., 2016). Because of this possible toxicity it is important that microbial protein synthesis and nutrient digestibility in different sections of the gastrointestinal tract, especially rumen digestibility, are not affected by feeding nitrate. The rumen digestibility and microbial protein synthesis have not yet been studied in dairy cattle. Incomplete reduction of nitrate has been reported previously (Newbold et al., 2014) and might result in increased blood methemoglobin (**MetHb**) levels and increased excretion of nitrate and nitrite in milk and urine. The aim of this study was to quantify the methane mitigating effect of 4 different levels of dietary nitrate addition and the effects on hydrogen emission, rumen fermentation, nutrient digestibility (in various sections of the digestive tract), rumen microbial protein synthesis, blood MetHb levels, and nitrate and nitrite concentrations in milk and urine. It was hypothesized that increasing levels of dietary nitrate will result in a linear decrease in methane production as also demonstrated in previous studies (Newbold et al., 2014; Lee et al., 2015a), an increase in hydrogen emission, and have no negative effects on rumen fermentation, nutrient digestibility, and microbial protein synthesis. However, feeding nitrate might increase blood MetHb levels, and nitrate and nitrite concentrations in milk and urine.

MATERIALS AND METHODS

Experimental Design

The experiment complied with the ethical requirements set out in the Danish Ministry of Justice Law No. 726 (September 9, 1993). The experiment was a 4 × 4 Latin square design with 4 lactating Danish Holstein dairy cows (2 in first parity, 1 in third parity, and 1 fourth parity), each fitted with rumen cannulas and duodenal and ileal simple T-cannulas, receiving 1 of 4 experimental diets in each period. Animals were housed in tie-stalls and beds were covered with rubber mats and saw dust. At the start of the experiment, the 2 first parity cows were 72 and 97 DIM, the third parity cow was 139 DIM, and the fourth parity cow was 208 DIM. Average BW ± standard deviations at the start and end of the experiment were 592 ± 28 kg and 598 ± 28 kg, respectively. The treatments were a control diet (control) without nitrate and 3 diets with added nitrate: low, medium, and high, with calculated nitrate levels of 5.3, 13.6, and 21.1 g of NO_3^-/kg of DM, respectively. Period 1 was repeated because the cow receiving the high nitrate diet in period 1 had an abnormally low feed intake (DMI: 12.7 kg/d in period 1 vs. 20.7 kg/d in period 5) and data from this cow were excluded from analysis. Thus, 5 periods had 19 valid observations in total. Each experimental period lasted 28 d and was divided into 4 subperiods for adaption to the diet and sample collection. On d 1 to 6, the level of dietary nitrate was gradually introduced (incremental increase of 3.5 g of NO_3^-/kg of DM per d) until the planned level was reached in 0, 2, 4, or 6 d for the control, low, medium, and high nitrate diets, respectively. From d 7 to 16, the animals were allowed to adapt to the experimental diet. Digesta and urine samples were collected from d 17 to 21. Days 17 to 21 will be referred to as the digesta sampling period. On d 22 and 23, no samples were collected. On d 24, the animals were moved to respiration chambers and gas measurements were taken from d 24 to 28. Days 24 to 28 will be referred to as the gas measurement period.

Diets and Feeding. The rations were formulated using NorFor (Nordic Feed Evaluation System; Volden, 2011) and based on a milk yield of 9,500 kg of ECM per yr. The forage-to-concentrate ratio of the TMR was 50:50 on DM basis. The TMR consisted of corn silage,