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Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows

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ABSTRACT

An experiment was conducted to study potential interaction between the effects of feeding nitrate and docosahexaenoic acid (DHA; C22:6 n-3) on enteric CH_4 production and performance of lactating dairy cows. Twenty-eight lactating Holstein dairy cows were grouped into 7 blocks of 4 cows. Within blocks, cows were randomly assigned to 1 of 4 treatments: control (CON; urea as alternative nonprotein N source to nitrate), NO_3 [21 g of nitrate/kg of dry matter (DM)], DHA (3 g of DHA/kg of DM and urea as alternative nonprotein N source to nitrate), or NO_3 + DHA (21 g of nitrate/kg of DM and 3 g of DHA/kg of DM, respectively). Cows were fed a total mixed ration consisting of 21% grass silage, 49% corn silage, and 30% concentrates on a DM basis. Feed additives were included in the concentrates. Cows assigned to a treatment including nitrate were gradually adapted to the treatment dose of nitrate over a period of 21 d during which no DHA was fed. The experimental period lasted 17 d, and CH_4 production was measured during the last 5 d in climate respiration chambers. Cows produced on average 363, 263, 369, and 298 g of CH_4/d on CON, NO_3, DHA, and NO_3 + DHA treatments, respectively, and a tendency for a nitrate × DHA interaction effect was found where the CH_4-mitigating effect of nitrate decreased when combined with DHA. This tendency was not obtained for CH_4 production relative to dry matter intake (DMI) or to fat- and protein corrected milk (FPCM). The NO_3 treatment decreased CH_4 production irrespective of the unit in which it was expressed, whereas DHA did not affect CH_4 production per kilogram of DMI, but resulted in a higher CH_4 production per kilogram of fat- and protein-corrected milk (FPCM) production. The FPCM production (27.9, 24.7, 24.2, and 23.8 kg/d for CON, NO_3, DHA, and NO_3 + DHA, respectively) was lower for DHA-fed cows because of decreased milk fat concentration. The proportion of saturated fatty acids in milk fat was decreased by DHA, and the proportion of polyunsaturated fatty acids was increased by both nitrate and DHA. Milk protein concentration was lower for nitrate-fed cows. In conclusion, nitrate but not DHA decreased enteric CH_4 production and no interaction effects were found on CH_4 production per kilogram of DMI or per kilogram of FPCM.
Key words: methane, nitrate, docosahexaenoic acid, milk fatty acid

INTRODUCTION

Enteric CH_4 production in ruminants has received global interest (Hristov et al., 2013), and various feed additives have been suggested as a nutritional mitigation strategy. Feeding nitrate as alternative electron receptor effectively decreases CH_4 production in sheep (van Zijderveld et al., 2010), and a persistent effect was shown in lactating dairy cows (van Zijderveld et al., 2011). A sudden inclusion of high concentrations of nitrate in ruminant diets may result in a condition known as methemoglobinemia, which decreases the oxygen carrying capacity of the blood. Symptoms of nitrate toxicity depend on the level of methemoglobin in the blood and may include reduced intake and performance, brown discoloration of mucosae, and even death (Bruning-Fann and Kaneene, 1993). When animals are gradually adapted to higher concentrations of nitrate in their diets, no signs of (sub)clinical methemoglobinemia were observed (van Zijderveld et al., 2010, 2011; Lee and Beauchemin, 2014).

Supplementation of fat to ruminant diets also lowers CH_4 production (Grainger and Beauchemin, 2011). Specific fatty acids (**FA**) have been evaluated for their effect on rumen fermentation, and docosahexaenoic acid (**DHA**; an n-3 FA; C22:6n-3) has been shown to have a particularly marked effect on microbial metabolism in the rumen (Boeckaert et al., 2008a). Micro-algae enriched in DHA have been shown to decrease CH_4 production in vitro (Fievez et al., 2007), but this could not be confirmed in vivo (Moate et al., 2013).

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The VFA profile in rumen fluid may shift toward more acetate when nitrate is fed, whereas DHA may cause a shift toward a larger relative proportion of propionate (Boeckaert et al., 2008b; Guyader et al., 2015). Propionate production is an H_2-consuming process and can therefore decrease CH_4 production. Because nitrate and DHA have different mechanisms of affecting ruminal methanogenesis, we hypothesize that their effects on CH_4 production are additive.

An additive, or positive, interaction effect of the 2 additives would be of interest because it would allow for a similar decrease in CH_4 emissions using lower doses of the separate additives. The latter would alleviate the risk of negative effects of the additives on cow health and performance. Moreover, feeding DHA to lactating dairy cows has been reported to increase the proportions of CLA and DHA in milk fat and decrease the SFA proportion (Boeckaert et al., 2008b). From a human health perspective, such an alteration in milk FA composition is of interest (Shingfield et al., 2013). To the best of our knowledge, the effect of feeding nitrate on milk FA profile is unknown.

The main objective of this study was to investigate whether the effects of nitrate and DHA on CH_4 production and animal performance in lactating dairy cows are additive or not. Milk FA profile is a potential indicator of CH_4 production (van Lingen et al., 2014), and therefore the effects of nitrate and DHA fed alone or in combination on milk FA composition were also evaluated.

MATERIALS AND METHODS

Experimental Design, Animals, and Housing

All experimental procedures were approved by the Animal Care and Use Committee of Wageningen University (Wageningen, the Netherlands). The experiment was set up as a completely randomized block design with 4 treatments. Eight primiparous and 20 multiparous lactating Holstein cows (125 ± 16 DIM at the start of the experimental period; mean ± SD) were blocked according to parity, lactation stage, milk production and presence or absence of a previously fitted rumen cannula. Within blocks, animals were randomly assigned to 1 of the 4 experimental diets. One of the 8 cows with a rumen cannula had to be culled because of foot injuries and was replaced by a nonfistulated reserve animal already adapted to the same experimental diet (NO_3).

Animals were housed in a freestall barn from which blocks of 4 cows consecutively entered a 17-d experimental period. This 17-d period consisted of 12 d in tie-stalls, and from 1500 h on d 13 until 0900 h on d 17, cows were housed individually in climate respiration chambers (**CRC**).

Diets and Feeding

The experimental diets consisted of 49% corn silage, 21% grass silage, and 30% concentrates on a DM basis. Treatments consisted of a control treatment (**CON**; no nitrate or DHA added), a nitrate treatment (**NO_3**; 21 g of nitrate/kg of total DM), a DHA treatment (**DHA**; 3 g of DHA/kg of total DM), and a treatment including both nitrate and DHA in the diet (**NO_3 + DHA**; 21 g of nitrate/kg of total DM and 3 g of DHA/kg of total DM). Nitrate, DHA, or both were included in the concentrates (Table 1). Diets were balanced for N content by isonitrogenous exchange of nitrate and urea. Cellulose and limestone were added to balance DM and Ca content of the concentrate mixtures. DHA gold (DSM Nutritional Products, Columbia, MD) was exchanged against wheat because of the similar CP content. The chemical composition of DHA gold was described by Boeckaert et al. (2007) where the DHA content was 198 g/kg of DM. In the present study, DHA content of DHA gold was 254 g/kg of DM. Chromium oxide (1.7 g/kg of DM) was included in all concentrates to estimate total-tract diet digestibility of energy and nutrients. Diets were offered to the cows as TMR (Table 2). Drinking water was continuously available during the entire experiment.

All animals that were assigned to either the NO_3 or the NO_3 + DHA treatment, including 2 reserve animals, were gradually adapted to the experimental level of dietary nitrate (21 g/kg of DM) over a period of 21 d. Cows were group-fed once daily around 0900 h and received 25% of the experimental dose of dietary nitrate during the first week, followed by incremental steps of 25% per week and thereafter all cows received the full experimental dose of dietary nitrate. No DHA was fed during this period of adaptation to increasing levels of dietary nitrate.

During the experimental periods, cows were fed individually with 2 equal portions offered twice daily (at 0600 and 1600 h). A mixture of grass silage and corn silage was prepared twice weekly and weighed into crates that were stored in a cooling room (±7°C). The concentrates were in meal form and weighed separately into buckets and manually mixed into the roughage mixture at the moment of feeding. Until d 9 of the tie-stall period, each block of cows had free access to feed. Thereafter, DMI within a block was restricted to 95% of that of the animal with the lowest voluntary DMI between d 5 and 8, while ensuring that none of the animals in the block was restricted to less than 80% of its voluntary DMI.