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The relationship between milk metabolome and methane emission of Holstein Friesian dairy cows: Metabolic interpretation and prediction potential

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ABSTRACT

This study aimed to quantify the relationship between CH_4 emission and fatty acids, volatile metabolites, and nonvolatile metabolites in milk of dairy cows fed forage-based diets. Data from 6 studies were used, including 27 dietary treatments and 123 individual observations from lactating Holstein-Friesian cows. These dietary treatments covered a large range of forage-based diets, with different qualities and proportions of grass silage and corn silage. Methane emission was measured in climate respiration chambers and expressed as production (g per day), yield (g per kg of dry matter intake; DMI), and intensity (g per kg of fat- and protein-corrected milk; FPCM). Milk samples were analyzed for fatty acids by gas chromatography, for volatile metabolites by gas chromatography-mass spectrometry, and for nonvolatile metabolites by nuclear magnetic resonance. Dry matter intake was 15.9 ± 1.90 kg/d (mean ± SD), FPCM yield was 25.2 ± 4.57 kg/d, CH_4 production was 359 ± 51.1 g/d, CH_4 yield was 22.6 ± 2.31 g/kg of DMI, and CH_4 intensity was 14.5 ± 2.59 g/kg of FPCM. The results show that changes in individual milk metabolite concentrations can be related to the ruminal CH_4 production pathways. Several of these relationships were diet driven, whereas some were partly dependent on FPCM yield. Next, prediction models were developed and subsequently evaluated based on root mean square error of prediction (RMSEP), concordance correlation coefficient (CCC) analysis, and random 10-fold cross-validation. The best models with milk fatty acids (in g/100 g of fatty acids; MFA) alone predicted CH_4 production, yield, and intensity with a RMSEP of 34 g/d, 2.0 g/kg of DMI, and 1.7 g/kg of FPCM, and with a CCC of 0.67, 0.44, and 0.75, respectively. The CH_4 prediction potential of both volatile metabolites alone and nonvolatile metabolites alone was low, regardless of the unit of CH_4 emission, as evidenced by the low CCC values (<0.35). The best models combining the 3 types of metabolites as selection variables resulted in the inclusion of only MFA for CH_4 production and CH_4 yield. For CH_4 intensity, MFA, volatile metabolites, and nonvolatile metabolites were included in the prediction model. This resulted in a small improvement in prediction potential (CCC of 0.80; RMSEP of 1.5 g/kg of FPCM) relative to MFA alone. These results indicate that volatile and nonvolatile metabolites in milk contain some information to increase our understanding of enteric CH_4 production of dairy cows, but that it is not worthwhile to determine the volatile and nonvolatile metabolites in milk to estimate CH_4 emission of dairy cows. We conclude that MFA have moderate potential to predict CH_4 emission of dairy cattle fed forage-based diets, and that the models can aid in the effort to understand and mitigate CH_4 emissions of dairy cows.
Key words: dairy cow, enteric methane production, milk metabolome

INTRODUCTION

Enteric CH_4 production is one of the main targets of greenhouse gas mitigation practices for the dairy industry (Hristov et al., 2013). Quantification of enteric CH_4 production is therefore important. Several CH_4 measuring techniques have been developed, but these are not yet suitable for large scale measurements (Hammond et al., 2016). Proxies (i.e., indirect traits or indicators correlated with CH_4 emission) might, therefore, serve as a good alternative.

Milk fatty acid (**MFA**) concentrations have been suggested as proxy to estimate CH_4 emission in dairy cattle, and many studies have evaluated this proposed relationship between MFA concentrations and CH_4 emission (Chilliard et al., 2009; Mohammed et al., 2011; Rico et al., 2016). However, the results of these studies are inconsistent, with some studies finding a clear and strong relationship between MFA and CH_4 emission (Chilliard et al., 2009; Rico et al., 2016), whereas

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other studies conclude that MFA alone might not be suitable to develop universal CH_4 prediction models (Mohammed et al., 2011). Recently, Castro-Montoya et al. (2017) concluded that MFA are not reliable predictors for specific amounts of CH_4 emitted by a cow. Furthermore, individual MFA selected in optimal models to predict CH_4 emission largely differ between studies, further hampering the applicability of MFA to predict CH_4 emission in various circumstances. Some of these inconsistencies can be explained by dietary composition and lactation stage, both being factors that can influence the relationship between MFA and CH_4 emission (Mohammed et al., 2011; Dijkstra et al., 2016; Vanrobays et al., 2016).

These findings warrant further investigation of other proxies in milk to estimate CH_4 emission of dairy cattle, including volatile metabolites and nonvolatile metabolites. Antunes-Fernandes et al. (2016) and van Gastelen et al. (2017) evaluated the relationship between CH_4 emission and both volatile and nonvolatile metabolites in milk to better understand the biological pathways involved in CH_4 emission in dairy cattle as well as to determine the prediction potential of these milk metabolites. Antunes-Fernandes et al. (2016) concluded that CH_4 intensity (g/kg of fat- and protein-corrected milk; **FPCM**) may be related to lactose synthesis and energy metabolism in the mammary gland, as reflected by the significant relationship between both milk nonvolatile metabolites citrate and uridine diphosphate (**UDP**)-hexose B and CH_4 intensity. Methane yield (g/kg of DMI), on the other hand, may be related to glucogenic nutrient supply, as reflected by the milk nonvolatile metabolite acetone. In a recent review of CH_4 proxies, Negussie et al. (2017) concluded that no single proxy accurately predicts CH_4 emission, and that combining 2 or more proxies is the best way forward for the prediction of CH_4 emission. Van Gastelen et al. (2017) concluded that volatile metabolites and, in particular, nonvolatile metabolites in combination with MFA hold potential to predict CH_4 emission of dairy cows more precisely and accurately compared with MFA alone. The improved prediction potential was relatively small (i.e., the increase in adjusted R^2 and CCC is <0.18 and <0.12, respectively) for CH_4 production (g/d) and CH_4 yield (g/kg of DMI), whereas the prediction potential for CH_4 intensity (g/kg of FPCM) increased considerably (i.e., the adjusted R^2 and CCC increased with 0.36 and 0.24, respectively).

The analysis of both Antunes-Fernandes et al. (2016) and van Gastelen et al. (2017) was based upon a small range of diets (i.e., 4 forage-based diets in which grass silage was replaced partly or fully by corn silage) in one experiment. Therefore, the present study aims to quantify the relationship between CH_4 emission and the milk metabolome in dairy cattle fed a range of forage-based diets with different qualities and proportions of grass silage and corn silage.

MATERIALS AND METHODS

Experiments and Data

Data on individual cows from 6 experiments, all designed as randomized block experiments, from Wageningen University & Research (Wageningen, the Netherlands) were used. These experiments were conducted in accordance with Dutch law, and approved by the Animal Care and Use Committee of Wageningen University & Research. Experiment 1 (Warner et al., 2015) involved 25 Holstein-Friesian dairy cows and 4 grass herbage diets (forage to concentrate ratio of 85:15 based on DM basis). The grass herbage was cut after 3 or 5 wk of regrowth, after receiving either a low (20 kg of N/ha) or a high (90 kg of N/ha) fertilization rate after initial cut. Experiment 2 (van Gastelen et al., 2015) involved 29 Holstein-Friesian dairy cows and 4 diets (forage to concentrate ratio of 80:20 on DM basis). The forage consisted of 1,000 g/kg of DM grass silage, 1,000 g/kg of DM corn silage, or a mixture of both silages (667 g/kg of DM grass silage and 333 g/kg of DM corn silage; 333 g/kg of DM grass silage and 667 g/kg of DM corn silage). Experiment 3 (Warner et al., 2016) involved 52 Holstein-Friesian dairy cows and 6 grass silage-based diets (forage to concentrate ratio of 80:20 on DM basis). The grass silage received low (65 kg of N/ha) or high (150 kg of N/ha) N fertilization level preceding its growth period, and there were 3 regrowth periods (28, 41, and 62 d of regrowth). Experiment 4 (Warner et al., 2017) involved 55 Holstein-Friesian dairy cows and 8 grass silage-based diets (grass silage, corn silage, and concentrate at a ratio of 70:10:20 on a DM basis). The grass silage was cut at 4 growth stages (leafy, boot, early heading, and late heading) and fed at 2 levels of DMI (15.5 and 16.6 kg of DM/d). Experiment 5 (Hatew et al., 2016) involved 25 Holstein-Friesian dairy cows and 4 corn silage-based diets with whole-plant corn harvested at a very early (25% DM), early (28% DM), medium (32% DM), and late (40% DM) stage of maturity, and with corn silage, concentrate, and wheat straw at a ratio of 75:20:5 (DM basis). Experiment 6 (Klop et al., 2017) involved 8 Holstein-Friesian dairy cows and 3 diets containing corn silage, grass silage, and concentrate at a ratio of 40:30:30 (DM basis). The concentrate was either a basal concentrate or contained a blend of essential oils or lauric acid. Repeated measures resulted in 32 observations.

The experimental setup of these experiments was similar. After an adaptation period of 12 d, cows were