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Milk metabolome relates enteric methane emission to milk synthesis and energy metabolism pathways

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ABSTRACT

Methane (CH_4) emission of dairy cows contributes significantly to the carbon footprint of the dairy chain; therefore, a better understanding of CH_4 formation is urgently needed. The present study explored the milk metabolome by gas chromatography-mass spectrometry (milk volatile metabolites) and nuclear magnetic resonance (milk nonvolatile metabolites) to better understand the biological pathways involved in CH_4 emission in dairy cattle. Data were used from a randomized block design experiment with 32 multiparous Holstein-Friesian cows and 4 diets. All diets had a roughage:concentrate ratio of 80:20 (dry matter basis) and the roughage was grass silage (GS), corn silage (CS), or a mixture of both (67% GS, 33% CS; 33% GS, 67% CS). Methane emission was measured in climate respiration chambers and expressed as CH_4 yield (per unit of dry matter intake) and CH_4 intensity (per unit of fat- and protein-corrected milk; FPCM). No volatile or nonvolatile metabolite was positively related to CH_4 yield, and acetone (measured as a volatile and as a nonvolatile metabolite) was negatively related to CH_4 yield. The volatile metabolites 1-heptanol-decanol, 3-nonanone, ethanol, and tetrahydrofuran were positively related to CH_4 intensity. None of the volatile metabolites was negatively related to CH_4 intensity. The nonvolatile metabolites acetoacetate, creatinine, ethanol, formate, methylmalonate, and *N*-acetylsugar A were positively related to CH_4 intensity, and uridine diphosphate (UDP)-hexose B and citrate were negatively related to CH_4 intensity. Several volatile and nonvolatile metabolites that were correlated with CH_4 intensity also were correlated with FPCM and not significantly related to CH_4 intensity anymore when FPCM was included as covariate. This suggests that changes in these milk metabolites may be related to changes in milk yield or metabolic processes involved in milk synthesis. The UDP-hexose B was correlated with FPCM, whereas citrate was not. Both metabolites were still related to CH_4 intensity when FPCM was included as covariate. The UDP-hexose B is an intermediate of lactose metabolism, and citrate is an important intermediate of Krebs cycle–related energy processes. Therefore, the negative correlation of UDP-hexose B and citrate with CH_4 intensity may reflect a decrease in metabolic activity in the mammary gland. Our results suggest that an integrative approach including milk yield and composition, and dietary and animal traits will help to explain the biological metabolism of dairy cows in relation to methane CH_4 emission.
Key words: dairy cow, milk metabolome, enteric methane emission, energy metabolism

INTRODUCTION

Enteric methane (CH_4) production in ruminants mainly occurs in the rumen and is a natural byproduct of microbial feed fermentation and degradation, an essential process to provide nutrients to the animal. An increase of DMI results in a higher CH_4 production because more substrate is available for rumen microbiota to degrade, but diet characteristics, including the type of carbohydrates and fat content, can also have a large effect on CH_4 production (Kirchgebner et al., 1995). Due to the large contribution (approximately 52%) of CH_4 emission to the total greenhouse gas (**GHG**) emissions of the dairy sector (Gerber et al., 2013), mitigation strategies have been widely investigated (Hristov et al., 2013). Dietary changes to influence CH_4 emission are among the most direct CH_4 mitigation strategies (Knapp et al., 2014). Their importance increases because they are also candidates for implementation at dairy farms. According to Dijkstra et al. (2011), evaluating dietary mitigation strategies should be based on CH_4 production relative to feed intake because it avoids confounding effects of DMI on total CH_4 production (CH_4 produced per animal). However, uncertainties in measuring DMI at farm level makes an accurate rela-

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tion of CH_4 to DMI difficult in practice (Bannink et al., 2011). Others have related CH_4 mitigation strategies to their effect on the product (milk) of a dairy farm (Knapp et al., 2014).

To assess GHG emissions by the dairy chain, it is also possible to relate CH_4 production per unit of milk [usually expressed per unit of ECM or per unit of fat- and protein-corrected milk (**FPCM**)]. Higher production levels related to nutritional and nonnutritional management strategies may reduce CH_4 emissions per unit of milk (FAO, 2010). Emissions per unit of animal product reflect the accuracy of management practices on the composite of feed intake, GHG emission, and animal productivity (FAO, 2010). Therefore, evaluating CH_4 production in relation to feed intake and in relation to milk production are complementary.

Many studies have focused on the effect of CH_4 mitigation strategies on milk composition, but mainly on the macro constituents level (Mohammed et al., 2011; Hart et al., 2015). Less attention has been paid to individual metabolites of milk, with the exception of milk fatty acids (**MFA**; Odongo et al., 2007; Chilliard et al., 2009). This focus on MFA is because of the relation between MFA and ruminal activity with respect to microbial metabolism and type of VFA formed (Vlaeminck and Fievez, 2005). Changes in feeding can result in clear changes in MFA, which are partly related to how feed is degraded in the rumen (Halmemies-Beauchet-Filleau et al., 2014). Although MFA may predict CH_4 emission accurately within a limited range of dietary variation (e.g., variation in lipid source only; Chilliard et al., 2009), MFA cannot accurately predict the differences in CH_4 emission on a wider range of diets (van Lingen et al., 2014; Williams et al., 2014).

Milk volatile metabolite and nonvolatile metabolite profiles can be used to monitor animal health, feeding regimens, and metabolism in dairy cows. Based on different feeding regimens, indole and skatole present in the volatile fraction of milk were pointed out as indicative of the feeding regimen of dairy cows (Toso et al., 2002; Croissant et al., 2007). Further, Hettinga et al. (2008) used the milk volatile metabolite profile to detect and differentiate mastitis caused by different pathogens. Also, Klein et al. (2012) indicated the ratio of the nonvolatiles glycerophosphocholine and choline as possible predictor for developing ketosis in dairy cows and Lu et al. (2013), showed that phosphate sugars can be related to energy balance of the cow, due to a different organization of the epithelial membrane in relation to energy balance. These authors also showed that determining milk components using different techniques simultaneously can be useful for a more integrated understanding of the metabolism of cows (Klein et al., 2010; Lu et al., 2013).

Many fields of research analyze the same bio-matrix with different methods and integrate the resulting information to better monitor, predict, and interpret biological processes. Although milk volatile metabolite and nonvolatile metabolite profiles have been used to monitor digestion and metabolism in dairy cows, to the best of our knowledge these profiles have not been related to CH_4 emission. The present study explores the milk metabolome by GC-MS (GC-MS metabolomics; milk volatile metabolites) and proton nuclear magnetic resonance (**^{1}H-NMR** metabolomics; milk nonvolatile metabolites) to better understand the biological pathways involved in CH_4 emission.

MATERIALS AND METHODS

Experimental Design

Data from a completely randomized block design experiment were used with a total of 32 multiparous lactating Holstein-Friesian cows fed 4 diets that differed in grass silage (**GS**) and corn silage (**CS**) content. The experiment was fully described by van Gastelen et al. (2015). The experiment was conducted in 2012 in accordance with Dutch law and approved by the Animal Care and Use Committee of Wageningen University (Wageningen, the Netherlands).

The 4 diets had a roughage:concentrate ratio of 80:20 based on DM content. The composition of the concentrate was similar for all diets, whereas the roughage consisted of 100% GS, 67% GS and 33% CS, 33% GS and 67% CS, and 100% CS (ingredient as percentage of the total amount of roughage in the diet, all DM basis). Feed intake was restricted (95% of ad libitum DMI) to avoid confounding effects of DMI on CH_4 production. After an adaptation period of 12 d, on d 13, cows were housed in climate respiration chambers (**CRC**) for a 5-d period. Cows were milked and fed twice daily. Production of CH_4 was determined in 10 min intervals during 3 full 24-h periods in the CRC. The details of the CRC used in this experiment are extensively described by van Gastelen et al. (2015).

Milk Yield and Composition

Milk yield was recorded during each milking, and a milk sample (10 mL) was collected for analyses of fat, protein, and lactose content by mid-infrared spectroscopy by Qlip (Zutphen, the Netherlands). In addition, a representative milk sample (5 g/kg of milk production) was obtained at each milking from each cow. The first milk sample was collected on d 13 in the afternoon and the last milk sample was collected on d 17 in the morning, whereas cows were housed in the