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Variations in methane yield and microbial community profiles in the rumen of dairy cows as they pass through stages of first lactation

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ABSTRACT

Considerable interest exists both from an environmental and economic perspective in reducing methane emissions from agriculture. In ruminants, CH_4 is produced by a complex community of microorganisms that is established in early life but can be influenced by external factors such as feed. Although CH_4 emissions were thought to be constant once an animal reached maturity, recent studies have shown that CH_4 yield significantly increases from early to late lactation in dairy cows. The aim of this study was to test the hypothesis that increases in CH_4 yield over the lactation cycle are related to changes in rumen microbial community structure. Nine cows were monitored throughout their first lactation cycle. Methane and dry matter intake were measured to calculate CH_4 per dry matter intake (CH_4 yield) and ruminal fluid was collected during early, mid, and late lactation. A significant difference in bacterial and archaeal community structure during early and late lactation was observed. Furthermore, when ruminal short-chain fatty acid concentrations were measured, the ratio of acetate and butyrate to propionate was significantly higher in late lactation compared with early lactation. Propionate concentrations were higher in cows with low CH_4 yield during late lactation, but no differences were observed in bacterial or archaeal community structures. *Prevotella* dominated the rumen of cows followed by *Succinclasticum*; *Treponema*, *Fibrobacter*, *Ruminococcus*, and *Bifidobacterium* were also in high abundance relative to other bacterial genera. In general, positive correlations were stronger between the most relatively abundant bacterial genera and acetate and butyrate concentrations in the cows with high CH_4 and weaker between these genera and propionate concentration. This study indicates that increased CH_4 yield in late lactation is reflected in significant changes in microbial community structure.
Key words: dairy cow, methane, rumen microbiome, methanogens, short-chain fatty acids

INTRODUCTION

The microbial ecosystem in the rumen of cattle is highly complex, consisting of many microbial species acting together to convert plant materials into nutrients, primarily short-chain fatty acids (**SCFA**). These fermentation products, predominantly acetate, propionate, and butyrate, are essential for host maintenance and growth (Hobson and Stewart, 1997; Van Houtert, 1993). However, bacterial fermentation also creates by-products, namely hydrogen and carbon dioxide, that cannot be used by the host and are converted to CH_4 by methanogenic archaea (Hobson and Stewart, 1997). The extent of CH_4 produced by archaea is therefore dependent on the level of metabolic by-products formed by other microbial species.

Methane is of no energetic use to the host and is released into the environment through eructation (Dougherty, 1968; Anderson et al., 1987). This is a major environmental problem, as CH_4 is a potent greenhouse gas (**GHG**) that contributes to global warming. Global CH_4 emissions originating from enteric fermentation account for 17% of global CH_4 emissions, whereas dairy cows are estimated to contribute 3.3% to overall anthropogenic GHG emissions (Knapp et al., 2014). In addition, CH_4 represents a 5 to 7% loss of feed energy for dairy cows on commonly fed diets (Arndt et al., 2015), which negatively affects animal productivity. Feed energy loss due to CH_4 is predominantly influenced by the level of feed intake and dietary composition and, to a lesser extent, by feed additives or antimethanogen vaccines (Atakora et al., 2011; Knapp et al., 2014; Sun et al., 2015; Roehe et al., 2016). Implementing strategies to reduce CH_4 emissions from dairy cows would thus be beneficial from both an environmental and economic perspective.

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In the livestock industry, GHG mitigation strategies have largely focused on diet and dietary supplements as a means of reducing CH_4 emissions (de Menezes et al., 2011; Ellison et al., 2014; Cobellis et al., 2016). Although diet has a strong effect on rumen microbial community structure, this effect is often inconsistent and short-lived, making it difficult to create a universal dietary strategy (Yáñez-Ruiz et al., 2015). Furthermore, it is not well understood what type of microbial community profile leads to low CH_4 production. Host genetics have also been shown to influence rumen microbial community structure and CH_4 emissions (Weimer et al., 2010; Goopy et al., 2014). Ranking of sires based on CH_4 yield did not change when they were fed a high concentrate-based diet compared with a medium concentrate-based diet (Roehe et al., 2016), indicating that the influence of host genetics on CH_4 yield did not change with diet.

As microbes in the rumen are responsible for producing the CH_4, information on the factors shaping microbial community composition is needed to inform strategies designed to manipulate these communities to achieve long-term, consistent reduction of enteric CH_4 production. There has been considerable focus on the rumen microbiome of cows in the first year of life, as the rumen microbiome is thought to be relatively stable once mature (Rey et al., 2014; Li et al., 2012; Jami et al., 2013). It has been suggested that early life may represent a window during which the establishing rumen microbiome can be manipulated with long-term effects (Yáñez-Ruiz et al., 2015; Abecia et al., 2014). However, CH_4 emissions vary during different periods of an animal's lifetime; for example, CH_4 levels have been reported to increase by up to 35% from early to late lactation (Bielak et al., 2016; Garnsworthy et al., 2012), but this increase is primarily due to an increase in DMI, the main driver of the CH_4 production. Targeting specific periods of increased CH_4 production may facilitate the development of short-term interventions that can contribute to an overall strategy for the mitigation of CH_4 levels from agriculture. Jewell et al. (2015) reported that bacterial community structure in cows was dynamic over 2 lactation cycles, with specific operational taxonomic units (**OTU**) associated with high and low milk production efficiency; however, those authors did not monitor CH_4 emissions during their study. The aim of the current study was to monitor CH_4 emission per unit of DMI (CH_4/DMI = CH_4 yield), SCFA, and microbial community dynamics of cows during lactation to determine if any observed changes in CH_4 yields across lactation stages were driven by changes in rumen microbiome structure. It was hypothesized that spikes in CH_4 yields would be accompanied by a marked difference in bacterial and archaeal community composition compared with periods when CH_4 yields were lower. Nine cows from the same dairy herd were tracked during their first lactation cycle, with CH_4 production measured in respiration chambers and ruminal fluid samples collected for microbial and SCFA analysis during early, mid, and late lactation (5, 13, and 42 wk postpartum, respectively). In addition, rumen microbial profiles of cows from the dairy herd that were identified as either producing high or low CH_4 yield during the late lactation stage were compared to determine if significant differences in their rumen microbiome could be observed.

MATERIALS AND METHODS

Animals and Feed

A group of 9 German Holstein dairy cows of the same age in first lactation were used for the present study. All animals were treated in accordance with the State Government guidelines for the use of animals as experimental subjects in Mecklenburg-Western Pomerania. All experimental protocols were approved by the local animal ethics committee (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern; approval No. 7221.1–1.-053/13).

Animals were kept in a freestall of the Leibniz Institute for Farm Animal Biology (FBN) in Dummerstorf, Germany, with ad libitum access to fresh water and feed offered as TMR. To investigate whether the microbial community profile changes over the course of lactation and not in response to an altered ration composition, the diet was formulated to ensure a constant nutrient and energy composition over the course of lactation. Individual daily feed intake was recorded via automated weighing troughs and the TMR was sampled at each respiration chamber measurement for the determination of DM and nutrient composition. Feed analysis was performed by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) in Rostock, Germany. Dietary ingredient and nutrient compositions are listed in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-14200).

Methane Measurements in Respiration Chambers

Cows calved between October 2014 and April 2015. In wk 5, 13, and 42 (±0.2 wk; SE) of lactation animals were transferred from the freestall into open-circuit respiration chambers as described previously (Bielak et al., 2016). Before the actual gas exchange measure-