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Epimural bacterial community structure in the rumen of Holstein cows with different responses to a long-term subacute ruminal acidosis diet challenge

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ABSTRACT

Subacute ruminal acidosis (SARA) is a prevalent metabolic disorder in cattle, characterized by intermittent drops in ruminal pH. This study investigated the effect of a gradual adaptation and continuously induced long-term SARA challenge diet on the epimural bacterial community structure in the rumen of cows. Eight rumen-cannulated nonlactating Holstein cows were transitioned over 1 wk from a forage-based baseline feeding diet (grass silage-hay mix) to a SARA challenge diet, which they were fed for 4 wk. The SARA challenge diet consisted of 60% concentrates (dry matter basis) and 40% grass silage-hay mix. Rumen papillae biopsies were taken at the baseline, on the last day of the 1-wk adaptation, and on the last day of the 4-wk SARA challenge period; ruminal pH was measured using wireless sensors. We isolated DNA from papillae samples for 16S rRNA gene amplicon sequencing using Illumina MiSeq. Sequencing results of most abundant key phylotypes were confirmed by quantitative PCR. Although they were fed similar amounts of concentrate, cows responded differently in terms of ruminal pH during the SARA feeding challenge. Cows were therefore classified as responders (n = 4) and nonresponders (n = 4): only responders met the SARA criterion of a ruminal pH drop below 5.8 for longer than 330 min/d. Data showed that *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were the most abundant phyla, and at genus level, *Campylobacter* and *Kingella* showed highest relative abundance, at 15.5 and 7.8%, respectively. Diversity analyses revealed a significant increase of diversity after the 1-wk adaptation but a decrease of diversity and species richness after the 4-wk SARA feeding challenge, although without distinction between responders and nonresponders. At the level of the operational taxonomic unit, we detected diet-specific shifts in epimural community structure, but in the overall epimural bacterial community structure, we found no differences between responders and nonresponders. Correlation analysis revealed significant associations between grain intake and operational taxonomic unit abundance. The study revealed major shifts in the 3 dominating phyla and, most importantly, a loss of diversity in the epimural bacterial communities during a long-term SARA diet challenge, in which 60% concentrate supply for 4 wk was instrumental rather than the magnitude of the drop of ruminal pH below 5.8.

Key words: rumen epithelium, subacute rumen acidosis, cattle feeding, amplicon sequencing, bacterial microbiome

INTRODUCTION

Feeding patterns in dairy cattle have changed over the last decades, in favor of energy- and nutrient-rich concentrates fed at the expense of fiber-rich forages. These dietary shifts have supported high milk yields but raised concerns about compromised rumen function (Zebeli et al., 2012; Boerman et al., 2015). Accordingly, SARA, which is characterized as intermittent drops in ruminal pH, has become a prevalent metabolic disorder in intensively reared cattle (Plaizier et al., 2008). In particular, the microbiological changes associated with SARA have attracted the attention of the SARA research (Khafipour et al., 2011; Plaizier et al., 2012). These changes seem to be essential in the modulation of systemic health in cattle, such as the activation of systemic inflammation and increasing susceptibility to

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other diseases (Plaizier et al., 2008; Zebeli and Metzler-Zebeli; 2012; Steele et al., 2016).

In the rumen, bacteria are the predominant microorganisms, being particularly responsible for the fermentation of feeds into short-chain fatty acids (**SCFA**), which serve as an essential energy source for the host animal (Mackie, 2000). Of the ruminal microbial communities, bacteria in the rumen fluid and those attached to feed particles have attracted considerable research interest, but comparatively less is known about bacteria attached to the rumen wall, commonly known as epimural bacteria. In recent years, a few studies have been published describing the bovine epimural bacterial microbiome (**BEBM**) using high-throughput sequencing methods (Mao et al., 2015, Liu et al., 2016, Wetzels et al., 2016), but each have shown low sequence similarity between detected phylotypes and best type strain hits. From the host perspective, the BEBM is the first contact between the rumen environment and the rumen epithelium, competing with adherent and putative pathogenic microorganisms (Kamra, 2005; Khafipour et al., 2011). Therefore, it is reasonable to assume that this microbial community may fulfill an important role in protecting the epithelium from harmful microbes by forming a protective biofilm, in particular during challenging microbial growth conditions such as SARA. Khafipour et al. (2011) showed a burst of potentially pathogenic *Escherichia coli* during SARA, for example. However, the metabolic function of the BEBM is only partially understood, and the overall community structure needs to be evaluated in more detail. Results from earlier studies suggest a role for the epimural bacterial microbiome in the hydrolysis of urea (Fay et al., 1979; Wallace et al., 1979), and direct involvement in oxygen scavenging, responsible for maintaining strict anaerobic conditions (Cheng et al., 1979), as well as tissue recycling (McCowan et al., 1978) and amino acid metabolism (Mao et al., 2015).

Several publications have described microbial changes using different SARA challenge models (Hook et al., 2011; Khafipour et al., 2011; Mao et al., 2013). Current research has shown that dairy cattle respond differently to a concentrate-rich diet. Certain cows do not meet the SARA criteria despite a similar grain-rich diet (Humer et al., 2015), although in this study the effect on microbial communities due to different SARA responses of the animals was not determined. In addition, information is lacking about the effect of a long-term continuously induced SARA challenge on the BEBM, and whether differences in SARA responses can be explained by differences in the composition of cows' BEBM. Our study aimed to evaluate the BEBM during adaptation from a forage-based to a concentrate-based diet and after 4 wk of continuous concentrate-based feeding in cows that did or did not have rumen pH drop in response. We hypothesized that the BEBM composition would shift from the baseline to the adaptation to the SARA challenge, and that it could be distinguished according to the SARA response of the individual cows. We also monitored changes in the BEBM to find putative microbial indicator candidates for SARA.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

A continuous diet- induced SARA challenge experiment was conducted as part of a larger study that investigated long-terms of effects of 2 different models of SARA on the BEBM, with a transient SARA model reported in Wetzels et al. (2016) and a continuous SARA model described here. The experiment was performed with 8 rumen-cannulated (100 mm inner diameter; Bar Diamond, Parma, ID) nonlactating Holstein cows (initial BW 710 ± 118 kg, mean ± SD). Cows were housed together in a freestall barn at the dairy research farm of the University of Veterinary Medicine Vienna in Pottenstein, Austria. The experiment was conducted in 2 separate runs of 7 wk each, with 4 cows tested at the same time in each run. We used a feeding model to induce the continuous and long-term SARA challenge as follows: 2 wk of baseline feeding, followed by 1 wk of gradual adaptation to a 60% concentrate diet, followed by 4 wk of a continuous SARA challenge with 60% concentrate.

During the baseline period, cows were fed a forage mix consisting of 50% grass silage and 50% second-cut meadow hay (DM basis), and containing 54.4% DM, 8.4% ash, 11.3% CP, and 50.0% NDF at a rate of 1.5% of BW. During the adaptation period and SARA challenge, cows were fed a concentrate mixture in separate and controlled feeding troughs (RIC system; Insentec B.V., Marknesse, the Netherlands) in addition to the forage. The concentrate mixture consisted of barley grain (33.0%), wheat (30.0%), corn (15.0%), rapeseed meal (17.0%), dried beet pulp (3.2%), calcium carbonate (0.5%), NaCl (0.3%), and mineral-vitamin premix for cattle (1.0%). During the adaptation period, the concentrate amount was increased by 10% daily up to 60%, where it remained during the 4-wk SARA challenge. The SARA challenge diet contained 74.1% DM, 5.9% ash, 15.4% CP, 31.8% NDF, and 45.3% NFC (all DM basis), and was fed for 2% of BW, meeting cows' voluntary feed intake. Fresh water was provided ad libitum. Daily concentrate and forage intake were recorded electronically. Cows that did not consume their planned concentrate allowance were force-fed the residual concentrate through the rumen cannula to en-