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Effects of repeated subacute ruminal acidosis challenges on the adaptation of the rumen bacterial community in Holstein bulls

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ABSTRACT

We investigated the effect of repeated subacute ruminal acidosis (SARA) challenges on the pH, fermentative function, and bacterial community in the rumen. Four rumen-cannulated Holstein bulls were fed a high-forage diet for 7 d (HF period) followed by a high-grain control diet for 7 d (HG period). Four SARA challenges were carried out consecutively (first, second, third, and fourth challenges). The ruminal pH was measured continuously during the experiment, and rumen fluid samples during the first to fourth challenges were collected at 0800, 1400, and 2000 h on the last days of each feeding period for analysis; volatile fatty acid components, NH_3-N, and lactic acid concentrations were measured. Bacterial community structure was analyzed at 0800 h during the first and fourth challenges on the last days of each period. The 24-h mean ruminal pH was decreased during the transition from high-forage to high-grain diet and tended to differ between the HF and HG periods. During the HG period, ruminal pH <5.6 was maintained for a longer period in the first and second challenges (350 and 405 min/d, respectively) than in the third and fourth challenges (both 120 min/d). A marked increase in total volatile fatty acid and NH_3-N concentrations during the HG period was observed in the later challenges. In addition, lower and higher proportions of acetic and butyric acids, respectively, were observed during the HG period than during the HF period. A total of 37 core bacterial genera were found in all samples; however, the relative abundance of several genera differed significantly between the HF and HG periods (*Prevotella*, *Ruminococcus*, *Eubacterium*, and *Oscillibacter*) and between the first and fourth challenges (*Eubacterium* and unclassified *Clostridiaceae*). During the HG period, lower relative abundances of *Prevotella*, *Eubacterium*, and *Oscillibacter* and higher relative abundance of *Ruminococcus* were detected compared with during the HF period. The relative abundances of *Eubacterium* and unclassified *Clostridiaceae* were lower in the first challenge than in the fourth challenge. Bacterial diversity was greater during the HF period than during the HG period and was greater during the fourth challenge than during the first challenge. Interestingly, diversity indices during the HG period of the fourth challenge were higher than those during the HF period of the first challenge. These results suggested that rumen fermentation in Holstein cattle can adapt to repeated SARA challenges by minimizing the adverse changes in ruminal pH. Moreover, the composition and diversity of the ruminal bacterial community may be affected by ruminal pH and vice versa.

Key words: bacterial community, cattle, repeated subacute ruminal acidosis challenge, rumen

INTRODUCTION

Around the beginning of the lactation period, dairy cows frequently undergo dietary transitions to match the energy requirement for milk production. The transition from a high-forage diet to a high-grain, high-energy diet results in greater VFA or lactate accumulation in the rumen (Owens et al., 1998; Rabelo et al., 2003), which contributes to increased risk of ruminal acidosis or SARA (Nagaraja and Titgemeyer, 2007). A condition characterized by ruminal pH <5.6 (Gozho et al., 2005) or <5.8 (Penner et al., 2007) for an extended period, SARA may cause various health problems in dairy cows, such as feed intake depression, reduced fiber digestion, milk fat depression, diarrhea, laminitis, liver abscesses, increased production of bacterial endotoxins, and inflammation (Plaizier et al., 2008). Recently, high-throughput technologies have been used to evaluate the relationship between the incidence of ruminal acidosis

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or SARA and the rumen microbiota in cattle (Mao et al., 2013; Petri et al., 2013; Sato, 2016).

Repeated ruminal acidosis challenges affect ruminal pH and physiological behaviors, such as feeding, ruminating, and resting (Dohme et al., 2008; DeVries et al., 2009). As a result of the repeated acidosis challenges, depressed ruminal pH, increased feeding and resting times, and decreased rumination times were observed in lactating dairy cows (Dohme et al., 2008; DeVries et al., 2009). During repeated acidosis challenges, less rumination was observed in cows at high risk for ruminal acidosis compared with cows at low risk for ruminal acidosis (DeVries et al., 2009). Furthermore, cows with high risk for acidosis exhibited lower ruminal pH and more severe ruminal acidosis compared with those at low risk for acidosis (Dohme et al., 2008). Therefore, dairy cattle may be able to adapt to repeated ruminal acidosis or SARA challenges by changing digestive behaviors. Furthermore, ruminal pH may be modulated by physiological responses.

The adaptation and recovery of rumen bacterial community structure and diversity were previously reported during single SARA and acidotic challenges (Hook et al., 2011; Petri et al., 2013). For instance, Hook et al. (2011) reported SARA-induced changes in ruminal bacterial density, diversity, and community structure, as analyzed by clone library construction and sequence analysis. Another study using Angus heifers revealed that the rumen core microbiome remains stable regardless of differences in diet or host genetics; several bacterial taxa were identified as part of the rumen core microbiome and were relatively unchanged when cattle were fed forage, mixed forage, or high grain and during acidotic challenges (4 and 12 h postfeeding) and recovery periods (Petri et al., 2013). Therefore, it was suggested that ruminal bacteria can adequately adapt to dietary or pH changes by altering bacterial composition and diversity (Hook et al., 2011; Petri et al., 2013).

Despite adaptation and recovery of rumen bacteria during a single acidosis or SARA challenge following high-grain feeding, it is known that dairy cows are fed concentrated diet every day during the lactation period to maximize milk production; the effect of repeated SARA challenges on the rumen bacterial community is largely unknown. Therefore, the objective of this study was to investigate the changes in ruminal pH and bacterial community adaptation to repeated SARA challenges induced by dietary transition from a high-forage diet to a high-grain diet. We hypothesized that repeated SARA challenges would induce changes in ruminal pH and fermentation ability, which in turn would affect bacterial community and diversity and vice versa.

MATERIALS AND METHODS

Animals and Experimental Design

All animals were cared for according to protocols approved by the Iwate University Laboratory Animal Care and Use Committee (A201401; Morioka, Japan). Four rumen-cannulated Holstein bulls (192 ± 12 kg; 9.0 ± 1.4 mo of age) were used in this study. Following the 7-d high-forage adaptation period, all bulls were fed a high-forage diet for 7 d (**HF** period) and then a high-grain control diet for 7 d (**HG** period). A SARA challenge was defined an HF period followed by a HG period, and each bull was exposed to 4 consecutive SARA challenges (first, second, third, and fourth challenges). It has been demonstrated that the ruminal pH can adapt to different diets within 7 d after dietary change, which prompted us to choose a repeat of a 7-d high-forage diet and 7-d high-grain diet (Sato, 2016). Furthermore, we wanted to investigate ruminal fermentation during repeated SARA in cattle whose fermentative conditions closely resemble those of cattle in the field. Bulls were fed mixed hay (orchard and timothy hay) during the HF period and a high-grain diet with a forage-to-concentrate ratio of 19:81 (DM basis) during the HG period (Table 1). Feed was supplied daily in 2 equal portions at 0800 and 1700 h. Daily DMI was recorded for individual animals throughout the experimental period, and it was confirmed that all feeds offered to animals were consumed. The amount and contents of the high-forage (control) and high-grain (SARA-inducing) diet chosen were based on our previous study (Kimura et al., 2012). The chemical compositions of the mixed hay and high-grain diet fed to the bulls are shown in Table 1.

Table 1. Composition of the high-forage diet and high-grain diet on percentage and DM bases

Item	High-forage diet	High-grain diet
Amount (%)		
Orchard and timothy hay	100	18.8
Concentrate	0	60.9
Corn flakes	0	20.3
DM	87.2	89.1
DM basis (%)		
TDN	60.9	80.1
CP	13	17
Crude fat	1.3	3.6
ADF	40.5	13.8
NDF	68	26.7
Calcium	0.5	0.8
Phosphate	0.3	0.5