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Use of *Prevotella bryantii* 25A and a commercial probiotic during subacute acidosis challenge in midlactation dairy cows

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ABSTRACT

The objective of this study was to determine the efficacy of *Prevotella bryantii* 25A as a probiotic during a subacute ruminal acidosis (SARA) challenge using a commercial probiotic as a positive control. Six multiparous ruminally fistulated cows (BW = 685 ± 65 kg; (mean ± SD) in the mid-phase of lactation (70 to 148 DIM) received the following treatments in a replicated 3 × 3 Latin square design: (1) total mixed ration (TMR; control, CON), (2) TMR + 2 g/head per day of a probiotic combination of *Enterococcus faecium* and *Saccharomyces cerevisiae* (EFSC), or (3) TMR + *Prevotella bryantii* 25A. The Latin square consisted of 3 wk of adaptation to the respective treatments during which the animals were fed ad libitum once per day a conventional early-lactation TMR and 1.5 kg of hay. The adaptation was followed by 4 d of SARA (no hay) and 10 d of rest (adaptation diet without probiotics). Dry matter intake and milk production were depressed during SARA (22.0 and 31.8 kg/d, respectively) compared with adaptation (24.4 and 34.0 kg/d, respectively) and did not recover during rest (22.3 and 30.7 kg/d, respectively). During SARA, *P. bryantii* 25A had no effect on rumen pH, whereas EFSC reduced the percentage of time with pH <6.0 (71%) compared with CON (85%) and increased maximum pH. The EFSC treatment tended to increase mean pH over 24 h (5.65) compared with CON (5.45). Proportion of time with pH <5.6 tended to be lower with EFSC (46%) than with CON (62%). Populations of bacteria considered to be the most important cellulose digesters in the rumen (*Ruminococcus flavefaciens, Ruminococcus albus*, and *Fibrobacter succinogenes)* were also monitored during these treatments using culture-independent real-time PCR methods. The population of *R. flavefaciens* was similar between the 2 feeding phases, whereas *F. succinogenes* and *R. albus* were lower during SARA compared with rest. In light of the present study, *P. bryantii* 25A did not prove to be an effective preventative for SARA. The role of EFSC in regulating rumen pH was confirmed, with a possible effect of maintaining *R. flavefaciens* populations during SARA.
Key words: dairy cow, *Enterococcus faecium* and *Saccharomyces cerevisiae*, *Prevotella bryantii* 25A, subacute ruminal acidosis

INTRODUCTION

Subacute ruminal acidosis represents one of the most important metabolic disorders in intensive dairy farms and affects rumen fermentation, animal welfare, productivity, and farm profitability (Morgante et al., 2007). This serious digestive disorder occurs when large quantities of rapidly fermentable carbohydrates that exceed the buffering capacity of the rumen are fed to the animals. As a result, rumen VFA as well as lactate may accumulate, causing a decrease in ruminal pH. If the pH drops below 6.0, fiber digestibility is impaired (Stewart, 1977). When pH values drop between 5.2 and 5.6, animals may show clinical signs of SARA, causing animal discomfort and decreased production performance (Duffield et al., 2004). In the most acute forms, pH decreases to values below 5.2 (Mutsvangwa et al., 2002). The problem is accentuated with the lack of a sufficient adaptation period during which the epithelium papillae surface increases for better absorption of VFA. The transition period following parturition, when a change in diet occurs to meet milk production demand, is most critical, although SARA can occur at any time in high-producing dairy cows (Osborne et al., 2004). The economic consequences resulting from poor performance and animal health have made SARA one of the most prevalent animal welfare issues in intensive ruminant production systems (Stone, 2004).

Several authors have induced SARA using a standard feeding protocol to study the consequences of this digestive disorder on different physiological and productive parameters. To the authors' knowledge, this study is the second one along with Chiquette (2009) to use a

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controlled simulation of SARA to understand the role of probiotics in the regulation of rumen fermentation during this critical period.

Prevotella bryantii 25A was previously selected and isolated for its ability to grow rapidly on starch and to produce end products other than lactate (mainly succinate and propionate; Rodriguez, 2003). In a preliminary study where *P. bryantii* 25A was introduced in the rumen of 3 goats submitted to a lactic acidosis challenge, Rodriguez (2003) observed that ruminal pH values were lower in the control animals following the lactic acidosis challenge. Rumen lactate concentrations peaked at 80 m*M* after 8 h and remained elevated in control animals, whereas a maximal lactate concentration of 15 m*M* was recorded in treated animals during the 4 to 8 h of starch exposure. A rapid decrease to less than 3 m*M* was also observed in treated animals after 12 h.

Prevotella bryantii 25A increased ruminal fermentation products and milk fat concentration in a previous study (Chiquette et al., 2008). Because signs of SARA were not observed in either treated or control cows, no conclusions could be made about protection against acidosis by *P. bryantii* 25A.

The commercial probiotic Probios TC (Chr. Hansen, Milwaukee, WI) is a combination of 2 strains of *Enterococcus faecium* with the yeast *Saccharomyces cerevisiae*. This probiotic mixture controlled ruminal pH decrease in SARA-challenged late-lactating cows (Chiquette, 2009) and more recently in SARA-challenged early-lactating cows (J. Chiquette, unpublished data), as well as in early-lactating cows not challenged with SARA (Nocek et al., 2002). It also increased milk production in early-lactating cows (Nocek et al., 2003; Nocek and Kautz, 2006). The objective of the present project was, therefore, to evaluate the ability of *P. bryantii* 25A to protect against the symptoms of acidosis in SARA-challenged lactating dairy cows, using the commercial Probios TC as a positive control.

MATERIALS AND METHODS

Animals, Feeding, and Sampling Procedure

All animals in this experiment were cared for according to the standards set by the Canadian Council on Animal Care (CCAC, 1993). Six multiparous, ruminally fistulated cows (BW = 685 ± 65 kg, mean ± SD) in the mid-phase of lactation (70 to 148 DIM) received the following treatments in a replicated 3 × 3 Latin square design: (1) TMR (control, **CON**); (2) TMR + 2 g/head per day of a probiotic combination (Probios TC; Chr. Hansen), providing 5×10^9 cells/dose of 2 lactic acid-producing strains of *Enterococcus faecium* and 2×10^9 cells/dose of *Saccharomyces cerevisiae* (**EFSC**); or (3) TMR + 25 mL/head per day of *Prevotella bryantii* 25A, 2×10^{11} cells/dose. Each experimental period of the Latin square consisted of 3 wk of adaptation to the respective treatments during which the animals were fed ad libitum once per day at 0900 h a conventional early-lactation TMR (Table 1) and 1.5 kg of dry hay. The adaptation phase was followed by 4 d of lactic acidosis challenge (as described below), which was followed by 10 d of rest during which the animals were back to the adaptation diet but without probiotic supplementation (Table 2). The TMR was formulated to meet the MP, net energy, mineral, and vitamin requirements for lactating Holstein cows weighing 625 kg and producing 40 kg of 3.9% FCM when consuming 24 kg of DM/d (NRC, 1989).The composition of the diets during the project is given in Table 2. Preparation and handling of *P. bryantii* 25 A before use have been described previously (Chiquette et al., 2008). The probiotic powder EFSC was kept at 4°C, following manufacturer's recommendations, until ready to use. The viability and counts of probiotics were checked at the beginning of the project and at the beginning of each experimental period.

The procedure described by Keunen et al. (2002) was followed for induction of SARA. During the 4 d of induction of SARA, 30% of ad libitum intake of the TMR was replaced with wheat and barley pellets (**WBP**) containing 50% ground wheat and 50% ground barley. The cows were fed 2 kg of the TMR at 0700 h and two-thirds of the WBP at 0900 h. Between 1100 and 1130 h, the cows were given access to their TMR. At 1300 h, they received the remainder of the WBP. From 1500 to 1530 h, the cows again had access to their TMR. At 1700 h, the remainder of the TMR was fed to the animals. Grain pellets that were not consumed

Table 1. Composition of the TMR

Composition	% DM
Grass silage	19.7
Corn silage	19.7
Corn grain	40.8
Soybean meal	15.1
Protein supplement[1]	2.0
Vitamin-mineral mixture[2]	1.7
Calcium carbonate	0.9

[1]Protein supplement contained the following ingredients: corn distillers grains (25%), wheat distillers grains (15%), canola meal (15%), and SoyPLUS (45%; West Central Cooperative, Ralston, IA).

[2]Vitamin-mineral mixture contained the following major minerals (g/kg): Ca (95), P (55), Mg (55), Na (130), Cl (150), K (14), and S (21); the following minor minerals (mg/kg): Fe (2,745), Mn (2,065), Zn (3,000), Cu (495), I (69), Co (33), and Se (20); and the following vitamins (UI/kg): vitamin A (501,859), vitamin D (65,000), and vitamin E (2,600).