



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 mM after 8 h and remained elevated in control animals, whereas a maximal lactate concentration of 15 mM was recorded in treated animals during the 4 to 8 h of starch exposure. A rapid decrease to less than 3 mM 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 × 10⁹ cells/dose of 2 lactic acid-producing strains of *Enterococcus faecium* and 2 ×

10⁹ cells/dose of *Saccharomyces cerevisiae* (**EFSC**); or (3) TMR + 25 mL/head per day of *Prevotella bryantii* 25A, 2 × 10¹¹ 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

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

²Vitamin-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).

Table 2. Composition of the diets during the study (% MS)

Diet	Adaptation	SARA	Rest
TMR	95	70	95
Hay	5	—	5
Wheat and barley pellets (50/50)	—	30	—

within 1 h of feeding were placed into the rumen via the fistula. No hay was provided during the week of the challenge. Feed intake was recorded daily, and samples of TMR and WBP were collected weekly and pooled by experimental period.

During the 4 d of SARA induction and on d 7 of rest, 1 L of rumen content was sampled for determination of VFA, lactate, and $\text{NH}_3\text{-N}$, at 0700 h (before feeding) as well as at 1, 2, 3, and 4 h following the first introduction of WBP in the rumen. Rumen content samples were squeezed through 4 layers of cheesecloth to remove large feed particles. Samples for $\text{NH}_3\text{-N}$ were kept at -20°C . Samples for VFA and lactate were acidified before freezing [5 mL of filtered rumen fluid and 1 mL of H_2SO_4 (0.5 M)] and then kept at -20°C until further analyses. Rumen samples (solid + liquid) for bacterial quantification were taken at 0700 h and 3 h post-WBP, on d 3 of SARA, and on d 7 of rest. Those samples were kept unfiltered at -80°C until further analysis.

Analyses of Feed Nutrients

The nutrient composition of the TMR, WBP, and hay (Table 3) was determined as follows: DM content was determined by oven drying at 105°C for 48 h (AOAC, 1990, 930.15), and the ash content was determined by incineration at 550°C overnight. Total N was measured by thermal conductivity (Leco model FP-428 Nitrogen Determinator; Leco Corp., St. Joseph, MI). The concentration of NDF was determined as described by Van Soest et al. (1991), without the use of sodium sulfite and with the inclusion of heat-stable α -amylase. It was expressed inclusive of residual ash.

The NDF procedure was adapted for use in an Ankom²⁰⁰ Fiber Analyzer (Ankom Technology Corp., Fairport, NY). The ether extract content of the TMR, silages, and orts was measured using a Soxtec HT 6 apparatus (Tecator; Fisher Scientific, Montreal, QC, Canada) according to the method of the Association of Official Analytical Chemists (AOAC, 1990, 920.39).

Feed Intake, Milk Production, and Composition

Feed intake and refusals as well as milk production were recorded daily. Milk samples were taken on 4 consecutive milkings during the last week of adapta-

tion, during the challenge, and rest. Milk samples were kept at 4°C using bronopol as preservative and shipped weekly to Valacta Inc. (DHI organization responsible for milk recording in Sainte-Anne-de-Bellevue, QC, Canada). The contents of milk fat, protein, and urea-nitrogen was determined using a near-infrared analyzer (Foss Electric A/S, Hillerød, Denmark) according to AOAC (1990).

Ruminal pH

Ruminal pH was recorded every 10 min over a 24-h period during each of the 3 wk of adaptation, continuously during the 4 d of SARA induction, and over a 24-h period on d 5 of rest. A submersible electrode (Cole Parmer, Mississauga, ON, Canada) was inserted through the rumen fistula and suspended in the ventral sac of the rumen. The electrode was protected by a wire shield and attached to a 0.5-kg weight. Electrodes were connected to a pH meter with an RS-232 recorder output (Oakton 1000; Fisher Scientific, Nepean, ON, Canada), which was connected to a computer with the Balance Talk software program (Labtronics Inc., Guelph, ON, Canada). The electrodes were calibrated with pH 4 and 7 buffer solutions (Fisher Scientific, Nepean, ON, Canada) before insertion into the rumen and were not withdrawn during pH measurement unless questionable readings were recorded. Calibration was checked at the end of data collection. No drifts in calibration were noted.

Table 3. Nutrient composition (% DM)

Nutrient composition	TMR	WBP ¹	Hay
DM	92.4	88.0	90.0
CP	17.2	14.3	7.4
aNDF ²	26.4	18.3	68.4
ADF	17.1	6.4	42.2
Ether extract	4.1	1.9	1.8
NSC ³	46.5	62.3	17.3

¹WBP = Wheat and barley pellets (50/50).

²aNDF = NDF assayed with a heat-stable amylase.

³NSC = $100 - (\text{NDF} + \text{ether extract} + \text{CP} + \text{ash})$.

Ruminal VFA, Lactate, and Ammonia-N Determination

Upon thawing, VFA samples were centrifuged ($29,000 \times g$ for 20 min at 4°C). Approximately 1 mL of an ion-exchange resin (Dowex 50 WX8-100; Sigma-Aldrich, St. Louis, MO) was added for purification, incubated for 10 min, and then samples were filtered through a 0.22- μm syringe filter. Subsamples (0.5 μL) were analyzed using a Hewlett-Packard model 6890 gas chromatograph (Hewlett-Packard, Agilent Technology Canada Inc., Mississauga, ON, Canada). The column used was a DB-FFAP (25 m \times 0.32 mm \times 0.50 μm ; Agilent Technologies Inc., Santa Clara, CA). The temperatures of the injector and detector were 250 and 300°C , respectively.

D,L-Lactate was analyzed using the colorimetric assay of Taylor (1996). For the determination of $\text{NH}_3\text{-N}$ concentration, filtered rumen content was centrifuged ($17,750 \times g$ for 5 min at 4°C), and the supernatant was analyzed using the phenol-hypochlorite reaction (Weatherburn, 1967) with volumes adapted for microplates.

Quantification of Bacteria

Bacterial Strains and Growth. Strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The following strains of rumen bacteria were used as reference strains: *Ruminococcus flavefaciens* (strain C52, ATCC 49949), *Ruminococcus albus* (strain 7, ATCC 27210), *Fibrobacter succinogenes* (strain GC5, ATCC 51216), and *Megasphaera elsdenii* (ATCC 25940). They were cultured in a rumen fluid-based medium as previously reported (Klieve et al., 1989). For *M. elsdenii*, the medium used was reinforced clostridial medium BD218081 (Fisher Scientific Ltd., Ottawa, ON, Canada), as recommended by ATCC, and did not contain rumen fluid.

The procedure for DNA extraction and purification from pure bacterial cultures and mixed ruminal content is described in Chiquette et al. (2007). The only modification is that DNA from rumen fluid samples and from bacterial cultures was extracted in duplicate instead of triplicate.

Real-Time Primers, Probes, and Operating Conditions. The development and application of a real-time Taq nuclease assay for the enumeration of *R. flavefaciens*, *R. albus*, *F. succinogenes*, and *M. elsdenii* have been reported previously (Ouwerkerk et al., 2002; Chiquette et al., 2007). Quantification of DNA of each bacterial species in total rumen DNA was performed using an ABI 7500 Fast Real-Time PCR System (PE Applied BioSystems, Foster City, CA). Optimal

PCR reactions (10- μL total volume) contained 5 μL of TaqMan Universal PCR Master Mix (PE Applied Biosystems), 2 μL of diluted DNA, forward primers, reverse primers, and probes at the following concentrations: 300, 300, and 200 nM; 900, 900, and 200 nM; 300, 300, and 200 nM; and 300, 300, and 200 nM for *R. flavefaciens*, *R. albus*, *F. succinogenes*, and *M. elsdenii*, respectively. Analyses were performed in triplicate. The cycling parameters were 20 s at 95°C , followed by 40 cycles of 3 s at 95°C and 30 s at 60°C . Real-time TaqMan assays were calibrated by the cycle threshold method (Heid et al., 1996). Serial dilutions of purified genomic DNA from reference strains cited previously were used to construct species-specific calibration curves. The standard curves for each primer/probe set were linear (correlation coefficients >0.998), with target cell concentrations ranging from 7.6×10^3 to 7.6×10^7 , 8.6×10^2 to 8.6×10^6 , 1.3×10^2 to 1.3×10^6 , and 1.1×10^4 to 1.1×10^8 cells/mL for *R. flavefaciens*, *R. albus*, *F. succinogenes*, and *M. elsdenii*, respectively. Amplification efficiency, calculated from the slopes, ranged from 77 to 93%. A standard curve was made for each analysis run. The number of cells in fresh overnight cultures was determined by microscopic enumeration using a Petroff-Hausser counting chamber, at $\times 400$ magnification and diluted in the Norris-Powell solution (Norris and Powell, 1961), as described by Koch (1994). Counts were repeated 4 times for each bacterial group. The quantity of bacterial groups was expressed as cells per milliliter of ruminal content. Possible PCR inhibitors such as reagents used during nucleic acid extraction or co-purified components from rumen content were tested by serially diluting several unknown samples and assessing the efficiency of amplification (Nolan et al., 2006).

Statistics

Variables were summarized to 1 data point per cow per period and statistically analyzed according to a 3×3 Latin square design balanced for residual effects, with cow, period, and treatment as fixed factors. Phases, days within SARA phase, and pre- and postfeeding times were analyzed as repeated measurements. The MIXED procedure of SAS (SAS Institute, 2002) was used for all analyses. For all variables, adaptation and rest phases were compared with the SARA phase. Within SARA, data from d 1 to 4 were analyzed for linear and quadratic effects using polynomial contrasts. When the effect of probiotics was significant, both *P. bryantii* 25A and EFSC were further compared with CON, using Dunnett's correction. For bacterial populations, the rumen was only sampled on d 3 of SARA; results from pre- and postfeeding were compared dur-

ing both SARA and rest. Lactate concentration and bacterial population data were log transformed to obtain a normal distribution and homogeneous residual error and reconverted to millimolar concentration and cells per milliliter, respectively, for presentation in the tables. Least squares means are associated with confidence intervals instead of standard errors of the means for these 2 variables. Significance was declared at $P \leq 0.05$, and trends were accepted if $P \leq 0.10$.

RESULTS AND DISCUSSION

DMI, Milk Production, and Composition

Dry matter intake was depressed during SARA (22.0 kg/d) compared with adaptation (24.4 kg/d; $P < 0.001$) and did not recover to adaptation level during rest (22.3 kg/d; Table 4). During the 4 d of SARA, DMI decreased linearly and in a quadratic manner, the most important decrease being recorded on the second day of SARA, followed by somewhat constant DMI for the remaining SARA phase (Table 4). Depression of DMI is commonly reported in grain-induced SARA (Gozho et al., 2005, 2006; Fairfield et al., 2007). As rumen pH decreases following a meal rich in carbohydrates, the first response of the animal is a depression in feed intake (Oetzel et al., 2007). In a previous study with late-lactation cows, DMI during adaptation was only 20.7 kg/d, as opposed to 24.4 kg/d in the present study, and DMI was not decreased during SARA (Chiquette, 2009). It may well be that along with the dietary makeup, the DMI of cows before the induction of SARA could affect the severity of depression observed during SARA, similarly to the premise brought forward for milk fat depression during SARA by Gozho et al. (2007). It has been proposed that the decreased DMI observed during SARA is a consequence of decreased rumen motility (Kleen et al., 2003). The increased osmolarity of rumen content due to a reduced absorption of VFA through the epithelium would be responsible for decreased rumen motility (Owens et al., 1998).

Very few studies have reported production parameters during the recovery phase following SARA induction. Krause and Oetzel (2005) reported a 25% decrease in DMI during SARA (2 d of SARA) and recovery to baseline level during rest (2 d of measurements). The short phase of rest (2 d as opposed to 10 d in the present study) during which DMI was recorded could explain the difference between the Krause and Oetzel (2005) study and the present study. Probiotics had no effect on DMI independently of the feeding phase considered.

Similarly to DMI, milk production decreased during SARA (31.8 kg/d) compared with the adaptation phase (34.0 kg/d; $P = 0.003$) and did not recover during rest

Table 4. Effect of feeding phases and days of SARA on DMI, milk production, and composition

Item	Adaptation (A)	SARA (S)	Rest (R)	SEM (feeding phases)	Effect of feeding phases (<i>P</i> -value)		Days of SARA ¹				SEM (days of SARA)	Effect of days of SARA (<i>P</i> -value)		
					A vs. S	R vs. S	d 1	d 2	d 3	d 4		Linear	Quadratic	
DMI (kg/d)	24.4	22.0	22.3	0.51	<0.0001	0.66	24.2	18.7	19.3	20.0	1.11	<0.0001	<0.0001	
Milk production (kg/d)	34.0	31.8	30.7	0.55	<0.0001	0.12	33.0	32.2	31.3	30.6	1.26	0.003	0.97	
Milk efficiency ²	1.38	1.45	1.37	0.02	0.005	0.01	1.41	1.78	1.67	1.58	0.09	0.13	<0.001	
Milk fat (%)	3.15	3.18	3.05	0.13	0.98	0.68	ND ³	ND	ND	ND	ND	ND	ND	
Fat yield (kg/d)	1.04	0.98	0.92	0.04	0.34	0.19	ND	ND	ND	ND	ND	ND	ND	
Milk protein (%)	3.30	3.24	3.31	0.03	0.04	0.01	ND	ND	ND	ND	ND	ND	ND	
Protein yield (kg/d)	1.09	1.01	1.00	0.03	0.11	0.88	ND	ND	ND	ND	ND	ND	ND	
Milk urea (%; mg/100 mL)	16.3	13.6	13.7	0.77	0.02	0.99	ND	ND	ND	ND	ND	ND	ND	

¹Days of challenge with SARA.

²Milk efficiency = kilograms of milk/kilograms of DMI.

³ND = not determined.

(30.7 kg/d). During the 4 d of SARA, milk production decreased linearly (Table 4).

The depression in milk production recorded from the present study (2.2 kg/d), during induced SARA, was less than what was reported by Stone (1999; 3 kg/cow per day), Krause and Oetzel (2005; 3.5 kg/d), and Khafipour et al. (2009a; 3.3 kg/d). These latest values of milk depression compare better with that recorded during the rest phase of the present study (3.3 kg/d).

Similarly to DMI, milk production was not affected by probiotics at any of the feeding phases. This could be due to the lactation stage of the animals. Studies where probiotics improved milk production were with early-lactation cows (Nocek and Kautz, 2006) but this could not be tested here because the Latin square design of the present study extended over 4 mo.

Milk efficiency increased during SARA (1.45 kg of milk/kg of DMI), compared with that during adaptation (1.38 kg of milk/kg of DMI; $P = 0.005$) or rest (1.37 kg of milk/kg of DMI; $P = 0.01$). Similar observations can be made from data reported by Krause and Oetzel (2005). The increased milk efficiency during SARA followed a quadratic curve (i.e., reaching a peak on d 2 and decreasing thereafter). Probiotics did not affect milk efficiency in any feeding phases.

Milk protein concentration slightly decreased during SARA (3.24%) compared with that during adaptation (3.30%; $P = 0.04$) and rest (3.31%; $P = 0.01$). This decrease in protein concentration during SARA is slightly less (2%) than the 3% reported by Stone (1999). Milk protein yield tended to decrease during SARA and remained low during rest (Table 4). A similar pattern in milk protein yield between pre-SARA, SARA, and recovery was reported by Krause et al. (2009).

Milk urea concentration also decreased during SARA (13.6 mg/100 mL) compared with adaptation (16.3 mg/100 mL; $P = 0.02$) and did not recover to adaptation level during rest (13.7 mg/100 mL). Enemark (2008) reported that normal urea concentration should be between 3 to 5 mM, but <3 mM during SARA. In the present study, milk urea concentrations during SARA and adaptation corresponded to 3 and 2 mM, respectively. Milk was only sampled on 2 d during SARA so that linear and quadratic effects could not be determined.

Milk fat percentage and milk fat yield were not affected by SARA. Although milk fat depression is often associated with SARA, it is not always the case. Alzahal et al. (2010) reported that the effect of ruminal pH on milk fat is inconsistent in the literature. Allen (1997) summarized the association between ruminal pH and milk fat concentration from 23 studies and concluded that ruminal pH explained 39% of the variation in milk fat percentage. More recently, Enemark et al. (2004)

reported that correlation coefficients between ruminal pH and milk fat synthesis in cows over 30 DIM were only 0.305. An explanation brought forward by Krause and Oetzel (2005) is that a short duration of SARA (4 d) is not sufficiently long to allow the metabolic change to occur. Krause et al. (2009) reported that decreased milk fat percentage probably occurs during repeated bouts of SARA. Probiotics had no effect on the concentration of milk constituents independently of the feeding phases considered.

Rumen pH

Characteristics of pH measured during adaptation weeks and SARA weeks showed that SARA conditions were successfully induced with the grain challenge model used. All pH characteristics were indicative of greater acidic conditions in the rumen during SARA (Table 5). Time with rumen pH below 5.6 corresponded to 5, 13, and 4 h during adaptation, SARA, and rest, respectively, which represented ruminal conditions slightly more acidic than the ones reported by Krause et al. (2009), where rumen pH was below 5.6 for 2 h pre-SARA, 11 h during SARA, and 3 h during recovery. These conditions can be considered as severe SARA when compared with most of the SARA challenges reported in the literature (Gozho et al., 2007; Khafipour et al., 2009a,c). For most of the rumen pH characteristics of the present study, a linear increase toward more acidic conditions occurred with days of SARA. An increase was observed in the percentage of time during which rumen pH was below 5.2 from d 1 (18%) to 4 (40%), corresponding to 4 to 9.6 h/d from d 1 to 4, respectively. During the rest phase that followed SARA, rumen pH recovered to adaptation values. This suggests that the rest phase was sufficiently long to allow the recovery of normal fermentation.

Probiotics had no effect on rumen pH characteristics during the adaptation or the phase of rest when compared with CON. During SARA, EFSC reduced the percentage of time during which pH was <6.0 (71%) compared with CON (85%; $P = 0.03$) and increased the maximum pH recorded ($P = 0.05$). *Enterococcus faecium* and *Saccharomyces cerevisiae* tended to increase mean pH recorded over 24 h (5.65) compared with CON (5.45; $P = 0.10$; Table 6). Although not significant, a decrease was observed in the proportion of time with pH below 5.6 when the animals received EFSC (46%) compared with CON (62%). In a previous study with late-lactation cows (4) in a Latin square design, including a phase with SARA challenge (Chiquette, 2009), EFSC also tended to increase rumen pH during SARA (5.84) when compared with the control (5.41; $P = 0.06$) and increased the nadir pH (5.02) compared with the

Table 5. Effect of feeding phases and days of SARA on rumen pH characteristics

pH	Adaptation (A)		SARA (S)	Rest (R)	SEM (feeding phases)	Effect of feeding phases (P-value)		Days of SARA ¹				SEM (days of SARA)	Effect of days of SARA (P-value)	
	A vs. S	R vs. S				d 1	d 2	d 3	d 4	Linear	Quadratic			
Mean	5.95	5.56	6.07	0.05	<0.001	5.62	5.54	5.62	5.43	0.06	0.06	0.06	0.23	
Min ²	5.21	4.78	5.40	0.06	<0.001	4.93	4.72	4.78	4.64	0.07	0.07	0.07	0.50	
Max ³	6.62	6.45	6.63	0.04	<0.001	6.51	6.39	6.46	6.41	0.06	0.06	0.06	0.46	
Pct <6.0 ⁴	51.3	77.4	40.0	4.48	<0.001	80.0	81.1	69.6	82.5	3.48	3.48	3.48	0.08	
Pct ≥5.6, <6.0	30.1	23.4	24.4	3.14	0.82	29.0	25.5	20.9	16.4	3.70	3.70	3.70	0.88	
Pct <5.6	21.2	54.0	15.6	3.54	<0.001	51.1	55.1	48.7	65.9	4.88	4.88	4.88	0.11	
Pct ≥5.2, <5.6	17.1	25.9	15.0	2.66	0.004	32.9	25.9	20.7	25.5	3.21	3.21	3.21	0.06	
Pct <5.2	4.10	28.1	0.65	1.81	<0.001	18.0	29.7	28.0	40.2	0.001	0.001	0.001	0.93	

¹Days of challenge with SARA.²Minimum pH recorded.³Maximum pH recorded.⁴Pct = percentage of time during which pH values recorded were below 6.0.

control (4.43; $P = 0.05$). As in the present study, the proportion of time during which pH was below 5.6 was numerically but not statistically lower with EFSC (28%) compared with the control (66%). This probiotic mixture was used by Nocek et al. (2002), who suggested that at the level of 10^5 cells/mL of rumen fluid, EFSC increased mean rumen pH.

Prevotella bryantii 25 A had no effect on any of the rumen pH characteristics recorded during SARA. In a previous study (Chiquette et al., 2008), *P. bryantii* 25 A decreased ruminal lactate concentrations in dairy cows during 7 wk postpartum. Because signs of SARA were not observed in either treated or control cows in that study, no conclusions could be made about possible protection against acidosis by *P. bryantii*. It appears from the present results that *P. bryantii* 25A cannot prevent or mitigate SARA symptoms from challenged dairy cows.

Rumen VFA, Lactate, and Ammonia

Total VFA concentration was greater during SARA (106 mM) than during rest (77 mM; Table 7). Except for butyrate, the proportion of each individual VFA was also affected by the feeding phase. Proportions of propionate and valerate were greater during SARA compared with rest, whereas proportions of acetate and *iso*-acids were less during SARA than during rest. The relative change in the proportion of each VFA is consistent with the greater proportion of rapidly fermentable carbohydrate during SARA than during rest and is similar to the change in the proportion of VFA during a SARA challenge, reported by Blanch et al. (2009). In the present study, it is interesting to observe the dynamic of the fermentation pattern during the 4 d of SARA with a rapid modification within 24 h of SARA induction and stabilization thereafter. Valerate proportions, on the other hand, increased linearly from d 1 to 4 of SARA.

No effect of probiotics was observed on total VFA concentration or on the proportion of each individual VFA during SARA or rest. This is consistent with results from a previous study (Chiquette, 2009) where EFSC was given to late-lactating cows submitted to a SARA challenge. Very little data exists in the literature on the effect of this particular combination of probiotics over the proportion of ruminal VFA. In a study with steers fed 87% rolled barley, Beauchemin et al. (2003) used a similar combination of EFSC but with only 1 strain of *E. faecium* as opposed to 2 strains in the present study and reported no effect of the probiotic on the proportion of individual VFA or total VFA. Similarly to a previous study with early-lactating dairy cows (Chiquette et al., 2008), *P. bryantii* 25A had no

Table 6. Effects of probiotics on rumen pH characteristics during SARA

pH	Probiotic ¹			SEM	P-value	
	CON	ES	25A		ES vs. CON	25A vs. CON
Mean	5.45	5.65	5.55	0.06	0.10	0.50
Min ²	4.74	4.83	4.73	0.10	0.74	1.00
Max ³	6.33	6.59	6.41	0.07	0.05	0.62
Pct <6.0 ⁴	84.7	71.1	79.1	3.24	0.03	0.39
Pct ≥5.6, <6.0	22.2	24.5	22.1	3.79	0.87	1.00
Pct <5.6	62.3	46.3	57.0	5.32	0.11	0.70
Pct ≥5.2, <5.6	27.8	24.1	26.9	2.78	0.56	0.96
Pct <5.2	34.9	22.0	30.1	5.70	0.24	0.78

¹CON = control; ES = mixture of *Enterococcus faecium* and *Saccharomyces cerevisiae* at 2 g/cow per day; 25A = *Prevotella bryantii* 25A (25 mL/head per day, containing 2×10^{11} cells/dose).

²Minimum pH recorded.

³Maximum pH recorded.

⁴Pct = percentage of time during which pH values recorded were below 6.0.

effect on propionate proportions. This is particularly intriguing because *P. bryantii* 25A was selected for its ability to produce succinate in vitro as the main end product of carbohydrate fermentation. Succinate is known to be readily metabolized to propionate in the rumen environment (Blackburn and Hungate, 1963) and, therefore, is rarely present in appreciable amounts. It was not quantified in the present study.

Lactate concentrations increased during SARA (1.31 mM) compared with rest (0.41 mM; Table 7) but were kept far below detrimental levels characteristics of acute lactic acidosis (40 mM; Owens et al. 1998). The evolution of lactate concentration from d 1 to 4 of SARA followed a quadratic curve, peaking on d 2 of SARA and decreasing thereafter to the level of d 1. The highest lactate concentration was recorded 2 h after the morning meal during SARA (3.9 mM) compared with prefeeding (0.3 mM; $P < 0.001$; data not shown). During rest, the highest lactate concentrations were recorded 1 h after the morning meal (1.3 mM) and decreased thereafter to values not different from prefeeding concentration (0.2 mM; data not shown). A similar trend and lactate levels were observed by Blanch et al. (2009) during and after a SARA challenge.

Although lactate concentrations were numerically lower with EFSC during SARA, *P. bryantii* 25A and EFSC had no effect on lactate concentrations during SARA or rest. No interaction existed between probiotic and sampling times or days of SARA. The previous decrease in lactate concentrations when early-lactating cows received *P. bryantii* from wk 1 to 6 postpartum (Chiquette et al., 2008) was not reproduced under short-term SARA challenge. Khafipour et al. (2009c), who studied rumen microbiome composition using 2 nutritional models of SARA, identified *Prevotella* spp. as being potential probiotics to use in the prevention of SARA, based on the high abundance of *Prevotella* spp.

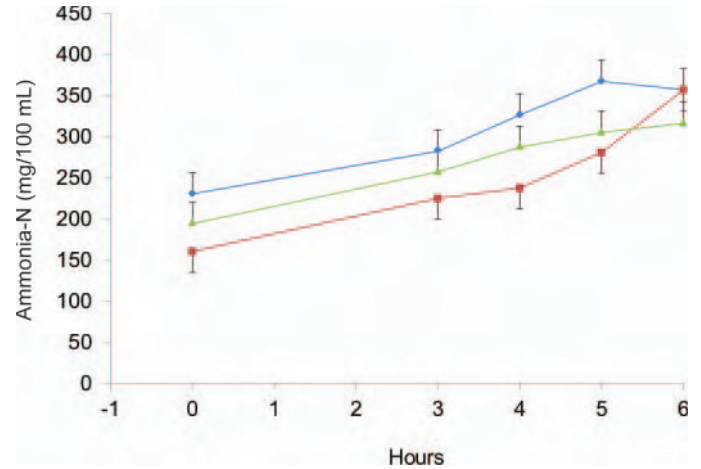
in alfalfa pellet-induced SARA. In light of the present study, the particular strain of *P. bryantii* 25A, which has been shown to decrease lactate concentrations in the rumen of early-lactating cows, did not prove effective in decreasing lactate concentrations or controlling rumen pH when added to the rumen of cows experiencing an induced SARA challenge.

Given that EFSC affected rumen pH, one would expect an effect of EFSC on total VFA or on lactate concentration. The difference in sampling frequencies between these variables might explain the absence of such effect. Indeed, pH was recorded continuously over a 24-h period as opposed to finite VFA and lactate samplings concentrated in the hours (1 to 4) following feeding, where most of the fermentative activity occurs. It could also be that under SARA conditions, where lactate concentration is kept under control by the activity of lactate-utilizing bacteria, the addition of *E. faecium*, which is believed to boost the population of lactate-utilizing bacteria, does not give additional advantage.

Ammonia-N was lower during SARA (133 mg/100 mL) than during rest (186 mg/100 mL; $P < 0.001$; Table 7). A drastic and linear decrease in NH₃-N concentration occurred on the first 3 d of SARA ($P < 0.001$; Table 7), possibly caused by the detrimental effect of low pH on the activity of proteolytic bacteria. Khafipour et al. (2009b) reported a similar decrease in NH₃-N concentration in the rumen (−20%) as rumen pH decreased. Generally probiotics had no effect on NH₃-N concentrations during SARA; however, on the first day of SARA, *P. bryantii* 25A increased NH₃-N (313 mg/L) compared with CON (232 mg/L) at all sampling times ($P = 0.04$; Figure 1). A similar increase in NH₃-N was observed with *P. bryantii* 25A in a previous study (Chiquette et al., 2008) and is consistent with the known proteolytic activity of this bacterium.

Table 7. Effect of feeding phases and days of SARA on molar proportions (mol/100 mol) of individual VFA and concentration (mM) of total VFA, lactate, and NH₃-N

VFA	SARA (S)		Rest (R)		SEM (feeding phases)		Effect of feeding phases (P-value)		Days of SARA ¹				SEM (days of SARA)		Effect of days of SARA (P-value)					
									d 1		d 2		d 3		d 4		Linear		Quadratic	
	SARA (S)	Rest (R)	SEM (feeding phases)	R vs. S	P-value	d 1	d 2	d 3	d 4	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	SEM (days of SARA)	
C ²	54.8	65.7	0.94	0.001	0.001	59.5	54.5	52.0	50.9	0.97	<0.0001	0.011								
C ³	33.1	18.4	1.10	<0.0001	<0.0001	25.8	34.3	36.6	37.0	1.05	<0.0001	<0.0001								
C ² /C ³	1.78	3.63	0.13	<0.0001	<0.0001	2.40	1.69	1.51	1.42	0.12	<0.0001	<0.0001								
C ⁴	8.65	12.0	0.64	0.99	0.99	11.2	8.17	7.91	8.08	0.58	<0.0009	<0.0001								
C ⁵	1.96	1.21	0.25	<0.0001	<0.0001	1.20	1.56	2.24	2.83	0.23	<0.0001	0.37								
iso C ⁴	0.54	0.83	0.03	0.05	0.05	0.74	0.47	0.41	0.42	0.03	<0.0001	<0.0001								
iso C ⁵	0.93	1.82	0.09	<0.0001	<0.0001	1.38	0.89	0.67	0.60	0.09	<0.0001	0.002								
Total VFA	106	77.3	2.85	<0.0001	<0.0001	103	108	103	106	3.05	0.80	0.66								
Lactate ⁶	1.31	0.41		<0.0001	<0.0001	0.79	2.56	1.70	0.84		0.76	<0.0001								
NH ₃ -N	133	186	12.7	0.0004	0.0004	273	146	68.3	46.1	13.0	<0.0001	<0.0001								

¹Days of challenge with SARA.²C² = acetate.³C³ = propionate.⁴C⁴ = butyrate.⁵C⁵ = valerate.⁶Lactate = data were log transformed. Least squares means presented are back-transformed to millimolar concentration and associated with CI (in brackets) instead of SEM.**Figure 1.** Ruminal ammonia-N concentrations on d 1 of SARA challenge in dairy cows receiving *Prevotella bryantii* 25A (25 mL/head per day, containing 2×10^{11} cells; ●); 2 g/head per day of a probiotic combination (Probios TC; Chr. Hansen, Milwaukee, WI), 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 no probiotics (control; ■). *Prevotella bryantii* versus control: $P = 0.04$. Error bars represent SE. Color version available in the online PDF.

Bacterial Populations

Populations of *R. flavefaciens* were similar between the 2 feeding phases (average population = 2.6×10^8 cells/mL), whereas *F. succinogenes* and *R. albus* populations were lower ($P < 0.001$) during SARA compared with rest (Table 8). Cellulolytic bacteria are known to be sensitive to low rumen pH; however, it appears from the results of the present study that *R. flavefaciens* would be more resistant to low rumen pH than other cellulolytics. Results from a previous study (Chiquette, 2009) also showed greater populations of *R. flavefaciens* (3.3×10^8 cells/mL) compared with *R. albus* (5.1×10^6 cells/mL) or *F. succinogenes* (5×10^5 cells/mL) during a SARA challenge. On the other hand, *M. elsdenii* increased 10-fold during SARA (1.49×10^7 cells/mL) compared with rest (0.12×10^7 cells/mL; $P < 0.001$). Blanch et al. (2009) also observed an important increase in *M. elsdenii* population during an acidosis challenge (104 ng/mL) compared with prechallenge (0.087 ng/mL), although in their study, the difference was not significant. As long as *M. elsdenii* can metabolize the lactate produced during the fermentation of grain, no accumulation of lactate occurs. The increase in *M. elsdenii* during SARA seems to have prevented lactate accumulation, as shown by low levels of ruminal lactate during SARA.

Sampling time (pre-feed vs. 3 h post-feed) had no effect during rest, for all bacterial species under study. However, during SARA, all bacterial populations in-

Table 8. Effect of feeding phases and sampling time on rumen bacterial populations (cells/mL)¹

Bacteria	Effect of feeding phases (P-value)			SARA			Rest		
	SARA (S)	Rest (R)	R vs. S	Prefeed	3 h postfeed	Effect of sampling time (P-value)	Prefeed	3 h postfeed	Effect of sampling time (P-value)
<i>Fibrobacter succinogenes</i> ($\times 10^5$)	0.95 [0.52, 1.71]	4.46 [3.26, 6.09]	0.0001	0.52 [0.27, 0.98]	1.00 [0.60, 1.67]	0.0045	3.65 [2.62, 5.11]	4.48 [3.30, 6.09]	0.32
<i>Ruminococcus albus</i> ($\times 10^6$)	2.00 [1.44, 2.78]	4.78 [3.84, 5.94]	0.0001	1.02 [0.53, 1.96]	2.94 [2.00, 4.33]	0.0001	4.65 [3.87, 5.58]	4.31 [3.57, 5.20]	0.54
<i>Ruminococcus flavefaciens</i> ($\times 10^8$)	2.52 [2.04, 3.11]	2.73 [2.23, 3.34]	0.58	1.76 [1.22, 2.54]	3.16 [2.38, 4.18]	0.0022	2.61 [2.06, 3.29]	2.56 [2.02, 3.23]	0.90
<i>Megasphaera elsdenii</i> ($\times 10^7$)	1.49 [0.63, 3.51]	0.12 [0.05, 2.76]	0.0001	0.79 [0.001, 901]	2.73 [0.003, 2,680]	0.02	0.13 [0.05, 0.35]	0.12 [0.04, 0.33]	0.61

¹Data were log transformed. Least squares means presented are back-transformed to cells per milliliter and associated with CI (in brackets) instead of SEM.

creased significantly 3 h post-feed compared with pre-feed (Table 8).

Enterococcus faecium and *Saccharomyces cerevisiae* increased the population of *R. flavefaciens* during SARA (3.64×10^8 cells/mL) compared with *P. bryantii* (2.28×10^8 cells/mL; $P = 0.05$) and CON (1.92×10^8 cells/mL; $P = 0.01$; data not shown). Addition of probiotics had no effect on the other cellulolytic bacterial populations during SARA and rest phases.

CONCLUSIONS

The role of EFSC in regulating rumen pH was confirmed, with a possible effect at maintaining *R. flavefaciens* populations during SARA. In light of the present study, the particular strain of *P. bryantii* 25A, which has been shown to decrease lactate concentration in the rumen of early-lactating cows, did not prove effective when added to the rumen of cows experiencing an induced SARA challenge.

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