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Prevotella bryantii 25A Used as a Probiotic in Early-Lactation Dairy Cows: Effect on Ruminal Fermentation Characteristics, Milk Production, and Milk Composition[1]

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ABSTRACT

Ingestion of high levels of rapidly fermented carbohydrates after parturition often leads to the production of excessive quantities of organic acids that may exceed the buffering capacity of the rumen and cause pH to drop. Ruminal acidosis results in animal discomfort, anorexia, depression, decreased digestibility, and decreased milk production. In the present study, we examined the effects of daily addition of cells of a newly isolated strain of *Prevotella bryantii* (25A) to the rumen of 12 ruminally cannulated cows in early lactation. This strain was selected based on earlier in vitro studies that indicated its ability to grow rapidly, compete for starch, and produce organic acids other than lactate. After calving, all cows received increasing amounts of an energy-dense diet containing barley grain, corn silage, and grass silage in a 40:60 forage-to-concentrate ratio. Animals were blocked according to milk production from their previous lactation. Treatments (control and *P. bryantii*) were distributed among cows within the same block. Cows were fed once a day. Six cows were given a daily dose of *P. bryantii* (2×10^{11} cells/dose), administered directly with a syringe through the rumen cannula, from 3 wk prepartum up to 7 wk postpartum. Rumen fluid was sampled before feeding and at 2 and 3 h postfeeding on wk 1, 2, 3, 4, 6, and 7 postpartum. Feed intake and milk yield were recorded daily and milk composition was recorded 2 d/wk, up to wk 7 of lactation. Feed intake was similar between control and treated cows. *Prevotella bryantii* did not change milk production, but milk fat tended to be greater in treated cows compared with control cows (3.9 vs. 3.5%). Rumen pH was similar between the 2 groups and differed across sampling times, being higher before feeding (6.3) as opposed to 2 h (5.9) and 3 h (5.7) postfeeding. Rumen lactate concentration was similar before feeding between control and treated cows; however, 2 to 3 h after feeding, lactate concentration was lower in cows receiving *P. bryantii* compared with control cows (0.7 vs. 1.4 m*M*). This difference was maintained throughout the experimental period. Concentration of NH_3-N was greater in treated cows than in control cows (174 vs. 142 mg/L). Acetate (65.5 vs. 57.8 m*M*), butyrate (12.7 vs. 10.5 m*M*), and branched-chain C4 fatty acid (0.90 vs. 0.75 m*M*) concentrations were greater in postfeeding samples of treated cows compared with control cows. Supplementing early-lactating cows with *P. bryantii* 25A increased ruminal fermentation products and milk fat concentration. Because signs of subacute ruminal acidosis were not observed in either treated or control cows, no conclusions can be made about possible protection against acidosis by *P. bryantii*.

Key words: *Prevotella bryantii* 25A, transition dairy cow, probiotic, rumen modification

INTRODUCTION

The period after parturition, when a change in diet occurs to meet the energy required for milk production, is most critical for the health of dairy cows. Large quantities of rapidly fermentable carbohydrates are often fed to meet the increased demand for energy, and this may lead to production of organic acids (VFA and lactate) in amounts that exceed the buffering capacity of the rumen. Problems may also arise from the lack of an adequate adaptation period, during which the epithelium papillae develop and provide increased absorption of VFA (Krause and Oetzel, 2006).

If 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 subacute ruminal acidosis (**SARA**), causing animal discomfort and decreased production performance (Duffield et al., 2004).

Several dietary strategies for this critical period were reported in the review by Krause and Oetzel (2006),

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including feeding unprocessed grains that are less fermentable; providing TMR instead of separate ingredients; feeding smaller meals more frequently and at regular intervals; and introducing grains progressively so that the rumen microbial population can adapt. It is also recommended that ration formulations provide a minimum content of total and physically effective fiber. However, this is not always sufficient, because cows are able to sort out long feed particles (Bach, 2007).

In spite of these precautions, some animals are more susceptible to a pH drop, and additional measures to prevent SARA could prove beneficial. Several studies investigated the role of monensin (an antibiotic-ionophore) in preventing SARA, and although some reported elevation of rumen pH by reducing concentrations of VFA with no effect on rumen lactate (Burrin and Britton, 1986), others reported no effect on rumen pH (Mutsvangwa et al., 2002; Osborne et al., 2004). However, because of general concerns about antibiotics, recent research has evaluated the use of live microorganisms with the aim of maintaining greater ruminal pH when feeding early-lactation dairy cows (Nocek et al., 2002; Beauchemin et al., 2003).

In the present study, we used a new strain of *Prevotella bryantii* that was selected and isolated based on its ability to grow rapidly on starch media and to produce end products other than lactate (mainly succinate and propionate; Rodriguez, 2003). In a preliminary study in which *P. bryantii* 25A was introduced in the rumen of 3 goats submitted to a lactic acidosis challenge, Rodriguez (2003) observed that ruminal pH was lower in control animals after an acidosis challenge. Rumen lactate concentration 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 decline to less than 3 m*M* was also observed in treated animals after 12 h. The hypothesis of the present study was that dosing the rumen with *P. bryantii* 25A would modulate rumen fermentation and reduce transient spikes of rumen lactate, with a resulting increase in rumen pH and animal performance, during early lactation.

MATERIALS AND METHODS

Animals, Feeding, and Sampling Procedure

Twelve multiparous rumen-fistulated dairy cows were used. All cows were fed a high-forage diet consisting of grass hay with mineral and vitamin supplements, from wk −6 to −3 relative to parturition (Table 1). Three weeks before calving, cows received the diet shown in Table 1. After calving, all cows received increasing amounts of an energy-dense diet containing barley grain, corn silage, and grass silage in a 40:60 forage-to-concentrate ratio (Table 1). Animals were blocked according to milk production from their previous lactation. Treatments (control and *P. bryantii*) were distributed between cows within the same block. Milk production from the previous lactation was, on average, 9,333 and 9,349 kg for cows identified as control or receiving *P. bryantii*, respectively. All cows received an additional 1.5 kg/d of hay during the first week after parturition. They were fed once a day at 0900 h throughout the experimental period to increase the variation in postfeeding ruminal pH (Krause and Oetzel, 2006). Six cows were given *P. bryantii* once a day before feeding via the rumen cannula, from 3 wk prepartum to 7 wk postpartum. Rumen fluid was sampled before feeding, 2 and 3 h postfeeding (2- and 3-h samples were pooled before analysis) on wk 1, 2, 3, 4, 6, and 7 postpartum. Feed intake and milk yield were recorded daily up to wk 7 of lactation. Milk samples were taken on 4 consecutive milkings each week for analysis of milk composition. Rumen fluid samples were analyzed for pH (immediately after collection), VFA, NH_3-N, and lactate. All animals were cared for according to standards set by the Canadian Council on Animal Care (1993).

Preparation of the Probiotic Strain

Cells of *Prevotella bryantii*, strain 25A, that had been isolated and described by Rodriguez (2003) were grown under a CO_2 atmosphere in an anaerobic medium containing mineral salts, cysteine:HCl, yeast extract, and a mixture of VFA, with wheat starch as substrate. Cells were grown to the late-log phase in a 100-L fermenter and were harvested by centrifugation following a 15-fold concentration by using a hollow fiber system (Amicon DC 10L, Amicon, Beverly, MA). The cell paste was suspended in approximately 2.5 L of spent medium containing 10% dimethylsulfoxide and was dispensed in individual doses in plastic syringes (25 mL), which were frozen and maintained at −86°C until used. Precautions were taken to limit exposure of the harvested cells to air until frozen. Before inoculation of cows, viable cell concentrations of these preparations were determined by serial dilution and plate count on agar medium in an anaerobic chamber (Bactron 1, Sheldon Manufacturing Inc., Cornelius, OR). Counts ranged from 2 to 29 × 10^{10} cfu/dose.

DMI, Milk Yield, and Composition

Feed intake was recorded daily from parturition to wk 7 of lactation. Weighed TMR, allowing approxi-

Table 1. Composition of the rations (DM basis) served to dry and lactating cows

Item	6 wk prepartum	3 wk prepartum	Postpartum
Ingredient (%)			
Grass silage	—	—	20.0
Corn silage	—	27.0	20.0
Barley grain	—	—	41.0
Protein supplement[1]			9.0
Soybean meal		12.0	8.0
Grass hay	98.0	30.0	—
Mineral and vitamin supplement[2,3]	1.5	0.5	1.5
Limestone	0.5	0.5	0.5
Protein supplement for transition cows[4]	—	30.0	—
Formulated chemical composition (% of DM)			
DM (%)	86.8	55.7	48.0
CP	11.5	4.1	18.3
NDF	56.8	49.2	29.8
NSC	20.9	27.7	42.7
Ca	0.61	0.64	0.73
P	0.42	0.43	0.45
K	2.68	1.59	1.33
Mg	0.32	0.30	0.27
Na	0.15	0.17	0.24

[1]Protein supplement contained the following: corn distillers grain (25%), wheat distillers grain (15%), canola meal (15%), SoyPLUS (45%; Omnigrains, St-Germain de Grantham, Québec, Canada).

[2]Vitamin-mineral mix (prepartum) contained the following: major minerals (%): Ca (3.0), P (12.0), Mg (12.0), Na (2.4), K (1.4), S (2.3); minor minerals (mg/kg): Fe (4,860), Mn (6,580), Zn (7,720), Cu (1,670), F (1,200), I (205), Co (122), Se (40); vitamins (IU/kg): vitamin A (811,200), vitamin D (245,000), vitamin E (7,600).

[3]Vitamin-mineral mix (postpartum) contained the following: major minerals (%): Ca (9.5), P (5.5), Mg (5.5), Na (13.0), Cl (15.0), K (1.4), S (2.1); minor minerals (mg/kg): Fe (2,745), Mn (2,065), Zn (3,000), Cu (495), I (69), Co (33), Se (20); vitamins (IU/kg): vitamin A (501,859), vitamin D (65,000), vitamin E (2,600).

[4]The following ingredients constituted 80% of the supplement: canola meal, sugarbeet pulp, soyhulls, cornmeal, wheat, gluten feed, gluten meal, wheat distillers grain, wheat middlings. A mineral-vitamin premix constituted the remaining 20% (Coop Fédérée, Montreal, Québec, Canada).

mately 10% refusals, was distributed to each cow with a feed cart with load cells (Rovibec, model 530, Coaticook, Québec, Canada). After 24 h, feed refusals were weighed for each cow. The TMR was sampled once a week and hay was sampled on wk 1 for DM analysis by near-infrared spectroscopy (Foss NIR Systems Inc., Laurel, MD). Milk yield was also recorded daily from parturition to wk 7 of lactation by using Metatron units with 2% accuracy (Westfalia Surge, Victoriaville, Québec, Canada) in a herringbone-type milking parlor. Milk samples were taken on 4 consecutive milkings (Monday evening, Tuesday morning, Tuesday evening, and Wednesday morning) during each week from parturition to wk 7 of lactation. Milk samples were kept at 4°C, using bronopol as a preservative, and were shipped weekly to Valacta (the DHI organization responsible for milk recording in the province of Québec). The content of milk fat, protein, and urea N was determined by using a near-infrared analyzer (Foss Electric, Hillerød, Denmark) according to AOAC (1990).

Rumen Sampling and pH Measurement

Rumen content was sampled on wk 1, 2, 3, 4, 6, and 7 of lactation. On each of those weeks, samples were taken before feeding and at 2 and 3 h postfeeding. Handfuls of rumen content (solid and liquid) were sampled through the rumen cannula from the ventral, posterior, and anterior sacs of the rumen. The content was squeezed to remove liquid from the solid (approximately 750 mL), and some solid particles were added to a 1-L container. The content was mixed and subsampled for bacterial DNA quantification (unpublished data). The remaining content was strained through 4 layers of cheesecloth to remove large particles. Rumen pH was measured within 15 min of collection by using an Oakton 1000 pH meter (Fisher Scientific, Nepean, Ontario, Canada). Samples for NH_3-N and lactate were kept at −20°C. Samples for VFA were acidified [5 mL of filtered rumen fluid and 1 mL of H_2SO_4 (0.5 *M*)] before freezing at −20°C. Postfeeding samples were pooled by cow and by day before analysis.

VFA and Lactate Determination

Upon thawing, VFA samples were centrifuged (29,000 × *g*, 20 min, 4°C). Approximately 1 mL of resin (Dowex 50 WX8-100, Sigma-Aldrich, St. Louis, MO) was added and incubated for 10 min, and samples were filtered through a 0.22-μm syringe filter. Subsamples (0.5 μL)

Table 2. Effect of *Prevotella bryantii* 25A and sampling time on concentrations of VFA (m*M*), lactate (m*M*), NH_3-N (mg/L), and pH before feeding and at 2 and 3 h after feeding

	Prefeeding				Postfeeding			
Item	Control	*P. bryantii*	SEM	*P*-value	Control	*P. bryantii*	SEM	*P*-value
Total VFA	79.4	82.7	2.32	0.33	98.0	108.7	3.91	0.07
Acetate (A)	49.3	52.6	1.34	0.10	57.8	65.5	1.56	0.004
Propionate (P)	20.8	19.0	1.15	0.27	26.3	26.3	1.38	0.99
Butyrate	6.59	8.24	0.50	0.03	10.5	12.7	0.53	0.01
Valerate	0.90	0.94	0.07	0.71	1.42	1.51	0.18	0.72
Br-C4[1]	0.73	0.80	0.04	0.18	0.75	0.90	0.04	0.02
Br-C5[2]	1.03	1.13	0.08	0.36	1.26	1.67	0.15	0.08
A:P	2.51	2.93	0.14	0.05	2.31	2.60	0.12	0.10
Lactate[3]	0.21	0.21	—	0.56	1.40	0.70	—	0.05
NH_3-N	132	156	11.7	0.17	152	195	13.9	0.03
pH	6.33	6.24	0.05	0.17	5.84	5.79	0.04	0.39

[1]Branched-chain C4 acids.

[2]Branched-chain C5 acids.

[3]Because there was a lack of normality in the distribution of lactate values, they were analyzed with a generalized linear mixed model by using the gamma distribution and the logarithmic link function of the GLIMMIX procedure, which best fit the lactate data distribution. Standard errors cannot be generated with this model; therefore, minimum and maximum values associated with each mean are presented for control prefeeding (0.13, 0.45), *P. bryantii* prefeeding (0.14, 1.43), control postfeeding (0.21, 7.35), and *P. bryantii* postfeeding (0.18, 3.24).

were analyzed by using a Hewlett-Packard model 6890 gas chromatograph (Agilent Technology Canada Inc., Mississauga, Ontario, Canada). The column used was a Stabilwax-DA (30 m × 0.53 mm × 0.50 μm; Chromatographic Specialties, Brockville, Ontario, Canada). Temperatures of the injector and detector were 250 and 300°C, respectively. Filtered rumen fluid (5 mL) was added in duplicate vials and analyzed for lactate by using the colorimetric assay of Taylor (1996).

NH3-N

Filtered rumen content was centrifuged (17,750 × *g*, for 5 min at 4°C) and the supernatant was analyzed by using the phenol-hypochlorite reaction (Weatherburn, 1967) with volumes adapted for microplates.

Statistical Analysis

Variables were analyzed as a randomized complete block design, with blocks as a random factor and *P. bryantii* versus the control as a fixed factor. Repeated measurements in time (week) were added to the model when appropriate, and the analysis was performed with the MIXED procedure of SAS (release 9.1, 2002, SAS Institute Inc., Cary, NC).

The model for VFA and NH_3-N was that of double repeated measures, with hours and week as the 2 repeated measures. Because there was a lack of normality in their distribution, lactate values were analyzed with a generalized linear mixed model by using the gamma distribution and the logarithmic link function of the GLIMMIX procedure, which best fit the lactate data distribution. Standard errors cannot be generated with this model; therefore, minimum and maximum values associated with each mean are presented in Table 2 for lactate.

RESULTS AND DISCUSSION

VFA

Total VFA concentration was greater ($P = 0.0001$) in postfeeding than in prefeeding samples (103.4 and 81.1 m*M* for post- and prefeeding, respectively; Table 2). Cows receiving *P. bryantii* tended to have a greater concentration of total VFA postfeeding ($P = 0.07$). There was no effect of weeks after parturition on total VFA. Similarly, there was more of each individual VFA postfeeding than prefeeding (Table 2). Before feeding, acetate concentration tended to be greater in treated cows compared with control cows ($P = 0.10$), but it was greater in treated cows postfeeding ($P = 0.004$) throughout the experimental weeks. Butyrate concentration was greater with the *P. bryantii* 25A treatment compared with the control treatment in both the prefeeding ($P = 0.03$) and postfeeding ($P = 0.01$) samples. Propionate concentration was similar between the treated and control groups at both sampling times (19.9 and 26.3 m*M*, for pre- and postfeeding concentrations, respectively). *Prevotella bryantii* 25A was shown to produce succinate as a result of starch fermentation in vitro, and given that succinate is rapidly metabolized to propionate in the rumen (Blackburn and Hungate, 1963), we would have expected a greater propionate concentration associated with treated cows. Rumen succinate concentration was not measured in the present study.

The concentrations of branched-chain C4 and branched-chain C5 acids were similar in control and treated cows before feeding. Postfeeding, however, the branched-chain C4 acid concentration was greater in treated animals compared with the control group ($P = 0.02$) and the branched-chain C5 concentration tended to be greater in treated than in control cows ($P = 0.08$). This increased concentration of branched-chain VFA in the presence of *P. bryantii* suggests an increased rate of proteolysis and AA metabolism in animals receiving *P. bryantii* 25A. The increased concentrations of acetate and butyrate would suggest an increased rate of fiber fermentation. Moreover, *P. bryantii* 25A could itself have cellulolytic activity, because Rodriguez (2003) reported that *P. bryantii* 25A could ferment xylan in vitro. More recently, Sawanon and Kobayashi (2006) reported synergistic fibrolysis in the rumen by cellulolytic *Ruminococcus* and noncellulolytic *Selenomonas ruminantium*. Synergistic fibrolysis by *P. bryantii* and cellulolytic bacteria cannot be ruled out, given the fermentation characteristics observed in the present study. The possible increase in cellulolytic activity when dosing the rumen with *P. bryantii* 25A will need further investigation.

Before feeding, the acetate-to-propionate ratio was greater in treated animals compared with control animals ($P = 0.05$) and it tended to be greater in postfeeding samples ($P = 0.10$). Important fluctuations in the ratio were recorded in control animals during the first 2 wk after parturition, although the ratio was more stable in treated cows. These results differ from those obtained by Rodriguez (2003) with goats (3 animals per treatment) subjected to a lactic acidosis challenge and inoculated with freshly grown, nonpreserved *P. bryantii* cultures. In their study, goats receiving *P. bryantii* had greater concentrations of ruminal acetate, butyrate, propionate, and valerate compared with control goats. They observed no difference in ruminal isoacid concentrations. Several key differences between these studies (animal species, challenging the animal for acidosis, freshly grown as opposed to preserved *P. bryantii* cultures) may explain the contrasting results.

Lactate

Lactate concentration in rumen fluid before feeding did not differ between the 2 groups of cows and was 0.21 m*M* (Table 2). Cows receiving *P. bryantii* 25A had lower postfeeding ruminal lactate concentrations ($P = 0.05$) throughout the experimental period, averaging 0.7 m*M* compared with 1.4 m*M* in the control cows, with concentrations ranging from 0.2 to 7.4 m*M* in the control group and 0.2 to 3.2 m*M* in the treated group. These results are in accordance with what was previously observed in vitro and with goats receiving *P. bryantii* 25A (Rodriguez, 2003), although the difference between control and treated animals was less pronounced in the present study. Both studies reflect the ability of the strain to ferment starch and to compete with lactic acid-producing bacteria for this substrate. Lactate concentrations observed in the present study were within what is considered normal (<5 m*M*; Oetzel et al., 1999), with the exception of 3 postfeeding samples at 5.5, 5.8, and 7.4 m*M*. Krause and Oetzel (2005) reported a lactate concentration peak of 16 m*M* during SARA.

Ruminal pH

As expected, rumen pH was lower in the 2- and 3-h postfeeding samples compared with the prefeeding samples in control and treated cows ($P = 0.0001$), but there was no effect of treatment on rumen pH (Table 2). It is possible that the increased concentration of VFA observed in treated animals relative to the control (11 m*M*) was partly cancelled out by the decreased lactate concentration in those same animals (0.7 m*M*). Considering that lactic acid is approximately 10 times stronger than VFA (pKa 3.9 vs. 4.9; Nagaraja and Titgemeyer, 2007), the decrease in lactic acid would correspond to a 7 m*M* decrease in VFA, which still results in a net positive increase in VFA (4 m*M*) in treated animals. This could explain the lower ruminal pH that was systematically observed in treated animals compared with the control, although this was not significant (Figure 1). In the present study, pH remained above 5.5, which is greater than what is generally considered as the SARA zone ($5.2 < pH < 5.6$; Owens et al., 1998; Keunen et al., 2002; Nagaraja and Titgemeyer, 2007). Average pH was 6.0 and 5.9 for control and treated cows, respectively. Cows did not show any signs of SARA, such as intermittent feed intake or decreased milk production. Usually SARA is more likely to occur in high-producing dairy cows (Osborne et al., 2004). Krause and Oetzel (2006) reported that primiparous cows are more at risk for SARA than are multiparous cows. Cows in the present study were multiparous and produced an average of 35 kg of milk daily.

Undoubtedly, continuous pH measurement would have allowed a better evaluation of the duration and severity of pH drop after feeding. Continuous pH measurements are reported in the literature, although usually with a smaller number of animals (4 to 9; Keunen et al., 2002; Mutsvangwa et al., 2002; Nocek et al., 2002).

Ruminal NH_3-N

Rumen NH_3-N concentration increased with weeks postpartum in the control and treated animals ($P =$

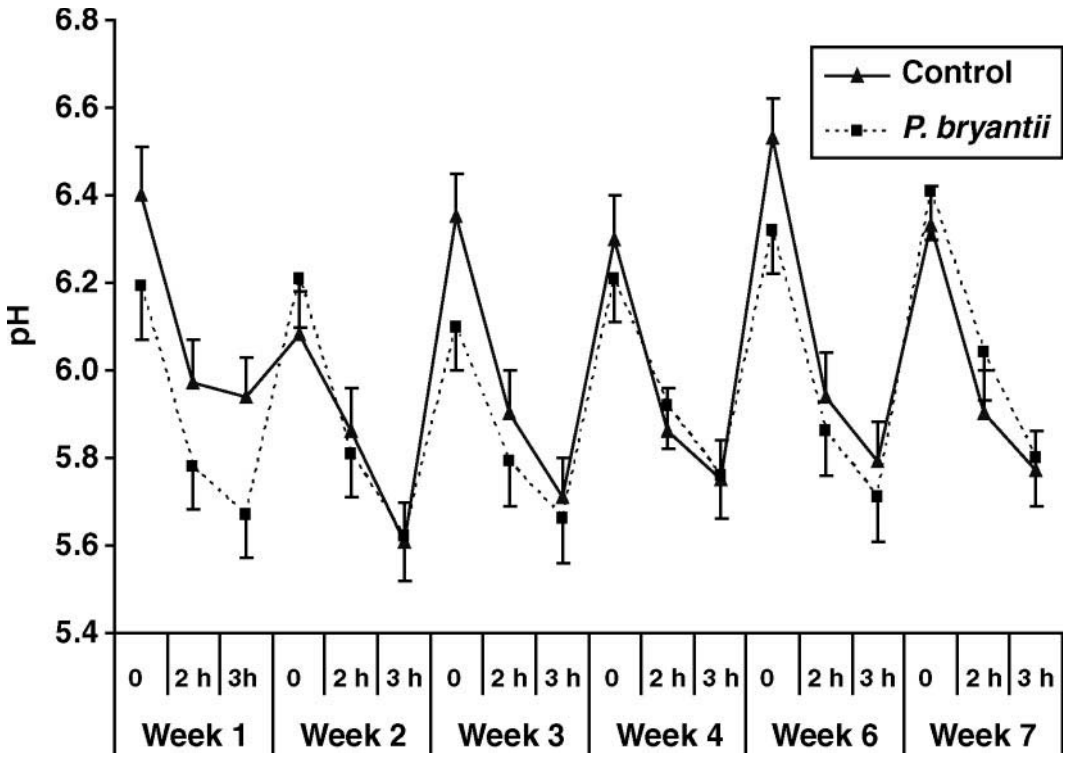

Figure 1. Effect of *Prevotella bryantii* 25A on ruminal pH at 0, 2, and 3 h after feeding for 7 wk after parturition (bars represent SD).

0.0003) as the level of feed intake increased. Concentration of NH_3-N was greater in postfeeding samples (173.5 mg/L) compared with prefeeding samples (141.8 mg/L; $P = 0.001$) throughout the experimental period. Treated cows had a greater rumen NH_3-N concentration than that recorded in the control cows postfeeding (195.4 and 152.2 mg/L for treated and control cows, respectively ($P = 0.03$; Table 2). We do not have a definitive explanation for this, although it could be indicative of a greater proteolytic and deamination activity by *P. bryantii*, which is generally thought to make a significant contribution to the degradation of starch, proteins, and peptides in the rumen (Stewart et al., 1997). It is also in accordance with the greater concentrations of iso-acids observed in treated cows. These iso-acids are produced by bacteria that deaminate and decarboxylate branched-chain AA (Allison et al., 1962).

These NH_3-N concentrations are within the limits of what is generally reported in the literature (Einarson et al., 2005; Benchaar et al., 2006; Raeth-Knight et al., 2007) but are beyond the concentration of 50 mg/L generally recognized as the minimum concentration for microbial protein synthesis (Satter and Roffler 1974). For other authors, the optimum NH_3-N concentration would be in the range of 85 to 162 mg/L (Ørskov et al., 1972; Kang-Meznarich and Broderick 1980; Pisulewski et al., 1981).

DMI, Milk Production, and Composition

Dry matter intake increased linearly from wk 1 to 5 of parturition ($P = 0.0001$) and was, on average, 14.6 kg/d on wk 1 and 23.5 kg/d on wk 5 after parturition, after which it stabilized, resulting in a quadratic effect ($P = 0.0001$; Table 3). Dry matter intake was not different between animals in the *P. bryantii* and control groups (21.2 kg of DM/d; $P = 0.49$).

Milk production (35.1 kg/d) and FCM (33.4 kg/d) were not different between treatments. These results for DMI and milk production should be interpreted with caution because of the relatively small number of animals used for production data. Milk production increased linearly ($P = 0.0001$) from wk 1 (26.5 kg/d) to wk 5 (37.9 kg/d) of lactation. Quadratic and cubic effects were observed with weeks of milk production (Table 3). A similar evolution in DMI and milk production during lactation was reported by Pollott (2004).

There was a tendency for greater milk fat content in cows receiving *P. bryantii* (3.87%) as compared with control cows (3.54%; $P = 0.06$) throughout the lacta-

Table 3. Evolution of DMI, milk production, and milk composition for 7 wk postpartum

Item	Weeks postpartum							SEM	Linear effect ($P \leq$)	Quadratic effect ($P \leq$)	Cubic effect ($P \leq$)
	1	2	3	4	5	6	7				
DMI (kg/d)	14.6	18.3	21.2	22.4	23.5	23.3	23.5	0.88	0.01	0.01	0.15
Milk production (kg/d)	26.5	32.7	35.8	37.2	37.9	37.9	37.8	1.64	0.01	0.01	0.01
4% FCM	26.7	31.6	34.5	35.1	34.9	34.6	36.1	1.84	0.01	0.01	0.01
Milk fat											
%	4.06	3.76	3.77	3.67	3.50	3.48	3.71	0.14	0.05	0.08	0.52
kg/d	1.06	1.23	1.34	1.34	1.30	1.29	1.39	0.09	0.01	0.07	0.02
Milk protein											
%	3.91	3.37	3.19	3.04	3.07	3.09	3.08	0.06	0.01	0.01	0.01
kg/d	1.03	1.09	1.13	1.12	1.15	1.15	1.15	0.07	0.02	0.07	0.45
MUN (mg/100 mL)	10.8	11.0	13.2	13.5	13.8	13.6	12.5	1.86	0.04	0.02	0.42

tion weeks. This would be in accordance with greater acetate and butyrate concentrations in the rumen of treated cows, because those VFA are precursors for milk fat synthesis. Milk fat yield, milk protein content, milk protein yield, and MUN concentrations were not affected by treatment and averaged 1.3 kg/d, 3.25%, 1.1 kg/d, and 12.6 mg/100 mL, respectively. There was no interaction between treatment and lactation weeks for milk components. Milk fat percentage showed a much slower decrease than milk protein percentage throughout the lactation weeks, with the minimum milk fat percentage observed at wk 6, as compared with wk 4 for the minimum milk protein percentage (Table 3). Very similar observations in the evolution of milk fat and protein content throughout lactation were reported by Martin and Sauvant (2007). Conversely, milk fat yield showed a faster increase in early lactation compared with milk protein yield, with a peak at wk 3 (1.34 kg/d) compared with wk 5 for the peak of protein yield (1.15 kg/d; Table 3). Pollott (2004) reported similar behavior in time for milk fat and protein yields in early lactation, with the exception that their model predicted that fat yield would peak in wk 5 (1.20 kg/d) and protein yield would peak in wk 8 (1.05 kg/d).

Similar to DMI and milk production, MUN concentration increased up to wk 5 of lactation and decreased slowly thereafter. A concomitant peak in MUN and milk production was observed by Rajala-Schultz and Saville (2003). They associated the lower concentration of MUN at the beginning of lactation with the difficulty of high-producing cows to ingest a sufficient amount of feed to meet their requirements for energy, resulting in suboptimal functioning of the ruminal flora. Wattiaux and Karg (2004) reported an average of 12.5 mg/100 mL of MUN with their high-protein diet (17.5%) as compared with 11.6 mg/100 mL with their low-protein diet (16.4%). In the present study, the average MUN was 12.6 mg/100 mL and the postpartum diet was formulated to provide 18.3% CP.

CONCLUSIONS

Dosing the rumen with *P. bryantii* 25A increased the concentration of fermentation products in the rumen, indicating that feed digestion might be increased. Milk fat tended to increase in animals receiving *P. bryantii* 25A, which is in accordance with increased acetate and butyrate concentrations in the rumen of treated cows. The increased iso-acid concentration in treated cows is indicative of increased proteolytic and deamination activity in the presence of *P. bryantii* 25A. *Prevotella bryantii* also decreased lactate concentration after feeding compared with the control treatment. Both of these effects (reduction in lactate concentration and increase

in NH_3-N concentration) would contribute toward prevention of rumen acidosis in early-lactating cows.

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