

Use of *Prevotella bryantii* 25A as a Probiotic to Reduce the Risk of Ruminal Acidosis in Dairy Cows

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Summary and Implications

This study was conducted to determine if adding relatively large quantities of a rapid growing, starch fermenting rumen bacterium, *Prevotella bryantii*, strain 25A, to the rumen of dairy cows would modulate rumen fermentation to reduce the risk of sub-acute ruminal acidosis (SARA). Twelve rumen fistulated dairy cows were fed an energy dense barley diet during a 3 week pre-partum period and then for 7 weeks post partum. Cells of *P. bryantii* that had been grown in a 100 liter fermenter were stored as frozen cells and then added daily to the rumen of six of the cows. Cows receiving the inoculum had lower post-feeding ruminal lactic acid, higher ruminal acetate and butyrate, and higher production of milk fat. However, since neither experimental nor control cows developed signs of SARA under the feeding conditions of this experiment, conclusions about protection against SARA by *P. bryantii* can not be reached.

Introduction

Various probiotics have been proposed as possible ways to limit lactic acidosis. A common theme has been to inoculate the rumen with bacteria that metabolize or utilize lactic acid. The current study is based on a different approach. Our approach would limit lactic acid production by inoculating the rumen with bacteria that are able to compete with the lactic acid producers for their main energy source, starch. Our candidate organism is strain 25A of *P. bryantii*, a rapidly growing starch fermenter that was isolated from the rumen of a cow adapted to concentrate. Rumen inoculations with this strain markedly reduced production of lactic acid in short-term experiments in goats and in cows (F. Rodriguez, Ph.D. thesis, Iowa State University, 2003). The experiments described here were designed to test this strain as a probiotic under practical dairy production conditions.

Materials and Methods

Beginning at 3 weeks before calving and continuing for 7 weeks after that, 12 rumen-fistulated dairy cows were fed increasing amounts of an energy rich diet (Table 1). During the first week following parturition all cows received 1.5 kg/day of hay. All cows were fed once a day and prior to

the feeding the 6 test cows were given *P. bryantii* cells through the rumen canula. Rumen fluid from the 6 test cows and from 6 control cows was sampled prior to feeding and at 2h and 3h post feeding during each week post-partum. Measurements were made of feed intake, milk yield, and milk composition.

Cells of *P. bryantii* (overnight growth, in a 100 liter fermenter at the ISU Fermentation Facility) were harvested using a hollow fiber system and by centrifugation. Cells were suspended in spent culture medium with added dimethyl sulfoxide (10% DMSO) and were dispensed in individual cow doses in 25 ml plastic syringes which were stored frozen until use. Each daily cow dose was approximately the cells from 1 liter of fermenter culture and culture counts of the frozen preparation were $2 - 29 \times 10^{10}$ viable cells per cow dose.

Results and Discussion

Cows receiving *P. bryantii* (Fig. 1) had lower post-feeding ruminal lactate concentrations ($P < 0.05$) than control cows throughout the experimental period (6.3 mg vs 12.6 mg/100 ml). Transient spikes in lactate concentrations were observed in both the control and treated groups with concentrations reaching 66.2 mg/100 ml in the control group and 29.2 mg/100 ml in the treated group. Although differences between lactate concentrations in treated vs control groups agreed with our proposed hypothesis, since 66.2 mg/100 ml, is really less than 8 mM, and much lower than total VFA concentrations (which exceeded 100 mM), the lactic acid found here did not contribute much to total ruminal acidity.

Cows receiving *P. bryantii* tended to have a greater concentrations of total VFA post feeding than did control cows ($P = 0.07$). Although propionate concentrations were not affected by treatment, concentrations of both acetate and butyrate were higher in rumen fluid from the treated cows. This increase in precursors for milk fat synthesis may account for the increased fat content ($P < 0.06$) in milk from treated cows (Fig 2.).

In this study mean ruminal pH remained above 5.5 for both treated and control groups and no effect of inoculation with *P. bryantii* on ruminal pH was detected. As neither treated nor control cows showed clinical signs of SARA, no conclusions were reached concerning protection by use of *P. bryantii* as a probiotic.

Table 1. Composition of the total mixed ration (DM basis).

Ingredients	%
Grass silage	20
Corn silage	20
Barley grain	41
Top supplement ¹	9
Soybean meal	8
Mineral mix ²	1.5
Limestone	0.5

¹ Protein supplement contained: Corn distillers grain (25%), wheat distillers grain (15%) canola meal (15%), SoyPLUS (45%).

² Vitamin-mineral mix 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) and vitamins (UI/kg): Vitamin A (501,859), Vitamin D (65,000), Vitamin E (2,600).

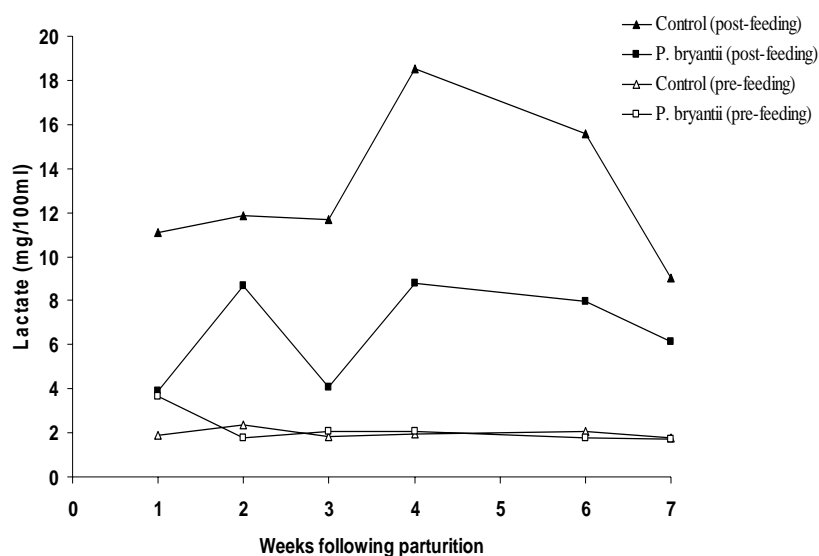


Figure 1. Effect of *P. bryantii* 25A and sampling time (prior to feeding or 2 + 3 h post-feeding) on ruminal lactate concentration (mM), during 7 weeks following parturition.

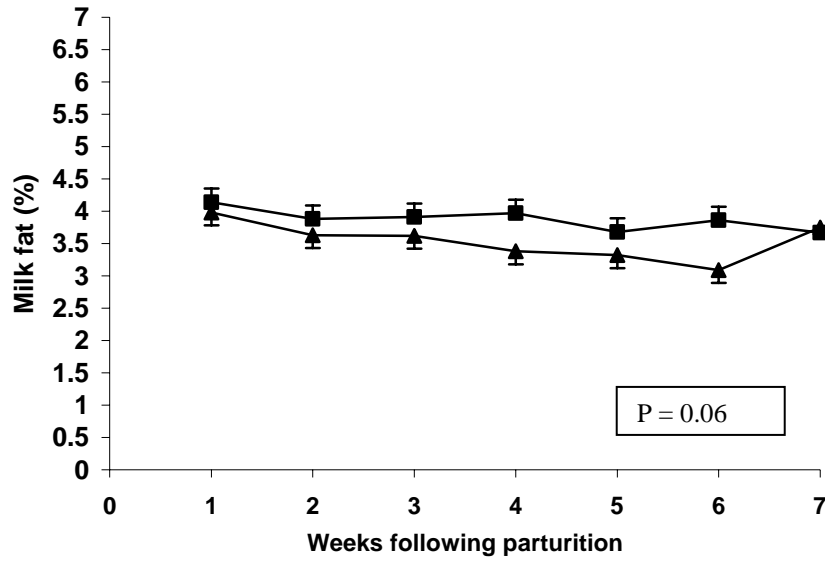


Figure 2. Effect of *P. bryantii* 25A on milk fat (%) during 7 weeks following parturition. (Bars represent standard deviations).