

Modification of Digestive System Microbiome of Lactating Dairy Cows by Feeding Bovamine[®]: Effect on Ruminal Fermentation

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Summary and Implications

We evaluated the effect of Bovamine (*Lactobacillus acidophilus* strain NP51 and *Probiobacterium freudenreichii* strain NP24) feeding on the digestive system microbiome of dairy cattle during late lactation (average DIM = 202). To unveil the underlying mechanisms, we examined Bovamine effects on ruminal total volatile fatty acid (VFA) concentration and the *Firmicutes* : *Bacteroides*. Dairy cows were randomized to treatment groups that were either fed the Bovamine or the lactose carrier top-dressed on the total mixed ration (TMR) for six weeks. Feeding Bovamine favorably modified the digestive system microbiome as determined by the bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). Fecal *Firmicutes* : *Bacteroides* was decreased, suggesting decreased shedding of potential pathogens. Conversely, ruminal *Firmicutes* : *Bacteroides* was increased indicating increased energy harvest. As a result, concentrations of the total VFA were increased significantly in the rumen. Also, natural killer cells (NK) frequency was increased under stimulation with mitogen concanavalin (ConA) in vitro, suggesting improved cattle immunity. Results advocate that feeding Bovamine to lactating dairy cows favorably alters their digestive system microbiome, improves the dietary energy availability, and health of cattle with significant implications to transition and early lactation dairy cows. A future study will be conducted to determine possible improvement in feed efficiency of mid-lactation dairy cows.

Introduction

Bovamine is a probiotic formulation that contains probiotic *Lactobacillus acidophilus* strain NP51, a lactic acid producer, and probiotic *Probiobacterium freudenreichii*, a lactic acid utilizer. These Bovamine metabolic properties allow the feeding concentrates to high producing dairy cows to increase milk production. It has been established that *Lactobacillus acidophilus* strain NP51

favorably modifies the immune responses of dairy cows and of a mouse model of Johne's disease. Thus, the use of Bovamine to improve ruminal fermentation and enhance immunity of dairy cows is a research objective to improve overall productivity and health, and thus profitability of dairy cattle.

Materials and Methods

Dairy cows (24 Holsteins and 24 Jerseys; average DIM = 202 days) were assigned randomly to one of two treatment groups: Bovamine (n = 24) or control (n = 24). Each treatment group contained 12 Holstein and 12 Jersey cows housed separately. Following an adaptation period, the Bovamine group was fed the Bovamine probiotic mixture at 1×10^9 CFU of *Lactobacillus acidophilus* strain NP51 plus 2×10^9 CFU of *Probiobacterium freudenreichii* · cow⁻¹·day⁻¹ for six weeks. The Bovamine dose was blended with 1.04 g of lactose carrier that was mixed with 472 g of ground corn and top-dressed on the TMR fed *ad libitum*. Similarly, the control group was fed the lactose carrier at 1.04 g·cows⁻¹·day⁻¹. On wk 3 and wk 6 relative to Bovamine feeding, ruminal (100 mL) and fecal (15 g) samples were collected and used for examination of ruminal and fecal microbiota. Additionally, part of the ruminal samples was used to determine the concentrations of FVA.

Results and Discussion

Feeding Bovamine to late lactation dairy cows modified significantly the concentrations of ruminal VFA. By wk 3, Bovamine feeding substantially increased ($P = 0.0001$; Table 1) total VFA by 34% compared with those of the control dairy cows. Also, by the end of wk 6, Bovamine feeding significantly ($P = 0.005$) increased total VFAs by 32.5% to be greater than that of the control dairy cows (Table 1). Elucidation of the microbiomic mechanism via which Bovamine increases ruminal VFA concentration showed that Bovamine feeding tended ($P = 0.06$) to increase ruminal *Firmicutes* : *Bacteroides* (Figure 1). As a result, the energy capture was enhanced because VFA concentration increased (Figure 1). Also, Bovamine feeding increased NK cells in peripheral blood when stimulated with ConA (Table 2) implying improved cows immunity and health. These data indicate that Bovamine feeding to dairy cows advantageously modifies the ruminal microbiome to enhance cows immunity and increase the dietary energy capture with significant implications for transition and early lactation dairy cows.

Acknowledgements

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Table 1. Effects of Bovamine feeding to dairy cattle on ruminal total VFA production at time 0, wk 3, and wk 6 relative to Bovamine feeding. Data are mean \pm SEM. (SEM = 6.03-8.44).

Time	Total VFA, mM		Statistics (P value)
	Control	Bovamine-fed	
Time 0	69.7 \pm 4.9	64.8 \pm 5.4	0.50
Wk 3	81.7 \pm 4.5	109.1 \pm 4.4	0.0001
Wk 6	56.0 \pm 4.6	74.2 \pm 4.4	0.005

Bovamine, (*Lactobacillus acidophilus* strain NP51 and *Probiobacterium freudenreichii* strain NP24).

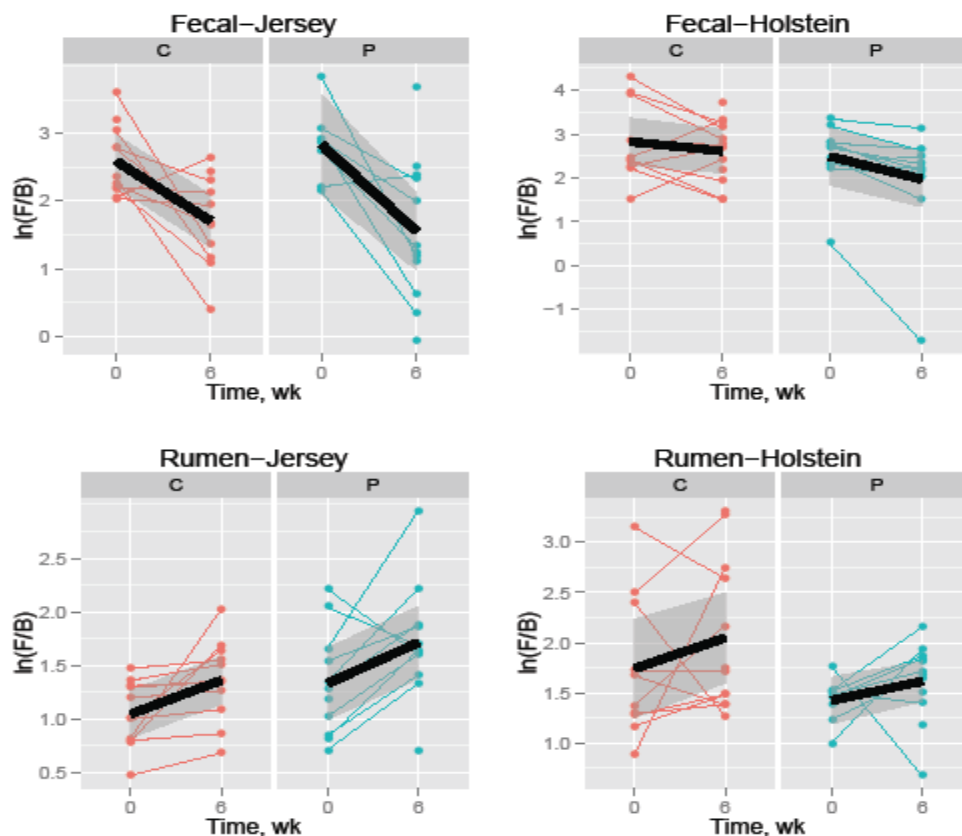


Figure 1. Effects of Bovamine feeding to dairy cattle on the ratio of the relative abundance of *Firmicutes* to *Bacteroides* (F/B) in rumen. Points represent individual observations, and subjects are linked with a line. Average changes are denoted by the black lines. (C) stands for control and (P) stands for probiotic Bovamine.

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Table 2. Effects of Bovamine feeding to dairy cattle on CD335⁺ NK cell repertoire in PMNs on time 0, wk 3, and wk 6 relative to Bovamine feeding. Data are mean \pm SEM. (SEM = 0.11-0.13).

Time	CD335 ⁺ NK cells repertoire frequency			Statistics (P value)
	Stimulation	Control	Bovamine-fed	
Time 0	NS	1.7 \pm 0.08	1.7 \pm 0.10	0.99
	ConA	2.5 \pm 0.08	2.6 \pm 0.1	0.33
Wk 3	NS	1.7 \pm 0.07	1.6 \pm 0.08	0.79
	ConA	2.5 \pm 0.07	3.5 \pm 0.08	0.0001
Wk 6	NS	1.9 \pm 0.08	1.8 \pm 0.08	0.73
	ConA	2.6 \pm 0.08	3.9 \pm 0.08	0.0001

Stimulation: stimulation with concanavalin A (**ConA**) or no stimulation (**NS**); NK, cell, natural killer cells. **Bovamine**, (*Lactobacillus acidophilus* strain NP51 and *Probiobacterium freudenreichii* strain NP24)