EFFECT OF PROBIOTIC SUPPLEMENTATION ON GROWTH RATE, RUMEN METABOLISM, AND NUTRIENT DIGESTIBILITY IN HOLSTEIN HEIFER CALVES[1]

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Summary

Sixteen Holstein heifer calves were used in an 112-day trial to study the effects of probiotic supplementation on growth performance and rumen metabolism. Calves were divided into four groups of four calves each, with two groups receiving the probiotic supplement and two groups serving as controls. Calves were limited to 1.6 kg dry matter of a corn-barley based grain mix per day. Long-stem bromegrass hay was fed as forage the first 56 days and bromegrass silage the last 56 days of the trial. Probiotic (28 g/d/calf) was fed along with the grain mix twice daily. Data were analyzed for the entire trial and also for the separate hay and silage feeding periods. Total weight gain and average daily gain were not affected ($p > .05$) by probiotic supplementation. Dry matter intake was lower ($p < .05$) and feed efficiency (kg feed/kg weight gain) was improved slightly during the hay feeding period for the probiotic-supplemented calves. Wither height gain was greater ($p < .05$) during the hay period and lower ($p < .05$) during the silage period for probiotic-supplemented calves. Heart girth gain was improved ($p < .07$) by probiotic supplementation, particularly during the hay feeding period ($p < .05$). Total rumen volatile fatty acid (VFA) concentration was higher ($p < .05$) with the probiotic-supplemented calves. Molar proportions of individual VFA were not affected ($p > .05$). Rumen ammonia-N and plasma urea-N concentration were lower ($p < .05$) for probiotic-supplemented calves during the hay feeding period. Total tract nutrient digestibility was not affected ($p > .05$). Some improvements in animal performance and changes in rumen and blood metabolites were observed when calves were supplemented with probiotic. Effects due to probiotic supplementation were most pronounced during the hay feeding period.

(**Key Words**: Calves, Digestion, Growth, Probiotic, Rumen Fermentation)

Introduction

Administration of antibiotics in sub-therapeutic doses in an attempt to enhance animal productivity has become common practice in many animal agriculture enterprises. However, the use of antibiotics as growth promotors has a number of disadvantages. Using broad spectrum antibiotics may inhibit the growth of beneficial bacterial species in the gut, possibly increasing the potential for colonization of the gut by pathogenic species, notably coliform bacteria (Parker, 1990). There is growing concern that the use of antibiotics as growth promotors may result in the development of resistant populations of pathogenic bacteria which could make subsequent use of antibiotics for therapy ineffective. In addition, consumer concern over the possibility of antibiotic residues in animal products warrants consideration.

One possible alternative to antibiotics is the use of probiotics. Probiotics can be defined as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" (Fuller, 1989). In addition, probiotics introduce beneficial microorganisms into the gut which may alter the environment and inhibit the growth of pathogenic bacteria. At present, two main types of products based on either yeast (*Saccharomyces cerevisiae*) or fungal (*Aspergillus oryzae*) cultures, alone or in combination with other microorganisms, are available for use in animal diets (Frumholtz et al., 1989). Several functions of probiotics have

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been proposed, including: 1) the protection of young animals against enteropathic disorders such as diarrhea by inhibiting the colonization of the gut by coliform bacteria; 2) an increase in feed conversion efficiency and live weight gain in young growing animals (Fuller, 1990; Sissons, 1989). The efficacy of probiotics in enteric disease prevention is based on sound scientific evidence (Fuller, 1990; Sissons, 1989). The most consistent response to enteric disease prevention is that achieved when probiotics are fed to neonatal and young pigs, with the overall response similar to that achieved with low-dosage antibiotic administration (Collington, et al., 1988; Parker, 1990). The normal gut microflora may protect the animal against certain enteropathic disease (Fuller, 1989). Antibiotic therapy and stress can adversely affect the balance of normal populations of gut microflora, resulting in enteric disorders, lowered feed intake, and reduced production performance. The aim of the probiotic approach is to correct or prevent the deficiencies in the gut microflora (Fuller, 1990; Sissons, 1989).

Commercial information about probiotics often includes the claim that feeding the probiotic preparations will result in increased feed conversion efficiency and live weight gain (Sissons, 1989). At present, there is insufficient research data as to whether probiotics enhance feedstuff digestion and promote increases in animal growth and feed conversion efficiency, particularly in ruminants. Of the reports published, response to probiotics has been variable. Fuller (1990) further suggested that even for the same species of animal, the probiotic effect may vary with age and diet. It may be easier to influence the gut flora of the young animal, while it is still in a state of flux. Based on the existence of some positive results it appears that under certain conditions probiotics can achieve what is claimed for them (Fuller, 1989; Sissons, 1989). It is unclear whether the responses, if any, stem directly from improved digestive performance or indirectly due to the suppression of gut pathogens which might otherwise have adverse effects on gut metabolism and animal performance. Most of the positive growth responses achieved with feeding probiotics have involved non-ruminants, particularly young growing pigs (Pollman, 1980) and chickens (Dilworth and Day, 1978). With ruminants, Adams et al. (1981) reported an increase in live weight gain and food conversion efficiency when steers were fed yeast culture (*Saccharomyces cerevisiae*) as a probiotic. Several researchers (Fallon and Harte, 1987; Hughes, 1987) reported an improvement in weight gain and feed consumption when yeast culture was added to young calf diets. In contrast, Wagner et al. (1990) reported no response in growth performance when young dairy calves were fed a yeast culture supplement.

The objectives of this experiment were to study the effects of probiotic supplementation on growth performance, rumen metabolism, and nutrient digestibility in weaned dairy heifers.

Materials and Methods

Probiotic

The probiotic used in this trial (Fastrack™, Conklin Co., Inc., Shakopee, MN) contained a source of live (viable) naturally occurring microorganisms along with materials designed to promote optimum growth of beneficial bacteria in the intestinal tract. Total microbial activity was generated at 40 billion colony-forming units per .454 kg. Ingredients in the probiotic pack included yeast culture (*Saccharomyces cerevisiae*), dried *Streptococcus faecium* fermentation product, dried *Lactobacillus acidophilus* fermentation product, dried *Aspergillus oryzae* fermentation product, dried *Bacillus subtilis* fermentation product, rice hulls, and calcium carbonate. This probiotic was fed at a recommended rate of 28 g per calf per day.

Animals and Housing

Sixteen Holstein heifer calves were divided into four groups of four calves each with two groups receiving the probiotic supplement and two groups serving as controls. Calves were started on trial at four months of age and remained on trial through seven months of age. All calves were housed indoors in a mechanically ventilated dairy barn. This barn received no supplemental heat during the winter months. Prior to being on trial, calves were placed into individual outdoor calf hutches at two days of age and remained in these hutches until weaning at six weeks old. The indoor pens provided one free-stall resting area for each calf. Under these indoor

housing conditions, environmental stress to the calves was minimized as much as possible.

Feeding Trial

Grain mix (table 1) feeding was limited to 1.6 kg (dry matter basis) per calf per day. Forage was fed free-choice throughout the trial. During the first 56 days long-stem bromegrass hay was used as the forage while bromegrass silage was fed the last 56 days of the trial. Chemical composition of the dietary ingredients is given in table 2. Grain mix was fed twice daily at 08:00 and 16:00 h. Forage was available to the calves throughout the day with the amount of forage offered recorded daily. Feed weigh-backs were measured daily at 08:00 h in order to calculate dry matter (DM) intake for each pen. Individual calf DM intake was determined daily and calculated as the total feed consumption for each pen divided by the number of calves per pen. Average daily gain (ADG) and feed-to-gain ratios were calculated for each individual calf. Probiotic was fed twice daily by mixing it into the grain mix at each feeding. One-half of the daily allotment of probiotic was fed at each grain feeding time.

TABLE 1. INGREDIENT CONTENT OF PELLETED GRAIN MIX

Ingredient	% of dry matter
Barley	26.6
Corn	26.6
Alfalfa meal	8.3
Animal fat	3.2
Beet pulp	8.3
Soybean meal	22.8
Limestone	1.9
Dicalcium phosphate	1.0
Trace mineralized salt[1]	.8
Magnesium oxide	.2
Vitamin premix[2]	.3

[1] Composition: 98% NaCl, .35% Zn, .28% Mn, .175% Fe, .035% Cu, .007% I, .007% Co.

[2] Composition: 4.4 million IU vitamin A/kg, 3.5 million IU vitamin D/kg, 8800 IU vitamin E/kg, 1.6 % Mg, 500 mg/kg Co, 12000 mg/kg Cu, 600 mg/kg I, 50000 mg/kg Fe, 47500 mg/kg Mn, 60 mg/kg Se, 48000 mg/kg Zn.

Measurements and Sample Collection

Body weight, wither height, heart girth, and paunch girth were recorded at the beginning and end of the experimental period and biweekly during the experiment. Rumen fluid and jugular blood samples were collected monthly during the experiment. Rumen fluid was collected by applying vacuum to an esophageal tube fitted with a stainless steel suction strainer. Measurements of pH were taken immediately after collection of the rumen fluid. Fifty ml of rumen fluid was placed into plastic vials containing 2 ml 50% sulfuric acid in order to retard further microbial activity. Acidified samples were then frozen until analyzed for ammonia N by steam distillation (Bremner and Keeney, 1965), lactate by colorimetric analysis (Sigma Chemical Co., St. Louis, MO, procedure No. 726-UV/826-UV) and volatile fatty acid (VFA) content by gas chromatography. Rumen fluid samples were prepared for VFA

TABLE 2. CHEMICAL COMPOSITION OF DIETARY INGREDIENTS

Item	Grain mix	Bromegrass hay	Bromegrass silage
Dry matter (%)	88.0	81.4	29.2
		% of dry matter	
Organic matter	92.2	95.2	92.4
Crude protein	21.5	13.2	11.9
Neutral detergent fiber	16.2	60.9	62.2
Acid detergent fiber	7.6	31.1	34.6
Ether extract	4.90	1.29	3.79
Ca	1.18	.32	.46
P	.55	.29	.23
Mg	.31	.15	.20
K	1.26	1.21	1.22

analysis using the method of Erwin et al. (1961). Volatile fatty acids were analyzed on a Perkin-Elmer model 8320 capillary gas chromatograph using a Nukol fused silica 30 m × .25 mm ID capillary column (Supelco, Inc., Bellefonte, PA). Operating conditions were as follows: column temperature, 182°C; injector and detector temperatures, 232°C and 292°C, respectively; linear velocity, 20 cm/sec, helium; detector, FID; sample size 1 microliter; split ratio 100:1.

Jugular vein blood samples were drawn into heparinized vacutainer tubes at the time of rumen sampling. Samples were centrifuged at 1000 × g for 15 min at 10°C to separate plasma. The separated plasma was removed from the vacutainer tube using a serum filter isolator (Isc-filter, Becton-Dickinson Co., Oxnard, CA). Plasma was frozen until analyzed for urea-N (Sigma Chemical Co., St. Louis, MO, procedure No. 640) and glucose (Sigma Chemical Co., St. Louis, MO, procedure No. 510).

Individual calf fecal samples were collected three times per day for three consecutive days (total of nine fecal sampling times) at the end of each month. The nine individual fecal samples were composited into one sample for subsequent chemical analyses. These samples were used to estimate apparent total tract nutrient digestibility. Acid detergent lignin (ADL) was used as the digestibility marker. Grain mix and forage samples were collected weekly for chemical analyses. Feed and fecal samples were oven dried at 55°C for 48 h and ground through a 1 mm Wiley mill screen for subsequent analysis. A 1 g sample of the ground feeds and fecal samples was dried at 105°C for 24 h to determine absolute DM percentage. Organic matter (OM) was determined after samples were ashed in a muffle furnace at 550°C for 12 h. Crude protein (CP), ether extract, Ca, P, and Mg were determined according to AOAC (1984) procedures. Neutral detergent fiber, (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially by the method of Van Soest and Robertson (1980).

Statistical Analysis

Data were analyzed using the general linear models procedure of SAS (1985). All data, with the exception of the feed intake data, were analyzed using a model that contained treatment, rep, and treatment × rep as independent variables. The model used for the feed intake data included only treatment as an independent variable since the measurement was determined on a group basis. Data were analyzed first on an entire 112 day trial basis (both hay and silage feeding periods data analyzed together), and also on a separate forage feeding period basis (separate analysis for both the hay and silage feeding periods).

Results and Discussion

Data are presented for the entire 112-day trial as well as for the 56-day hay and silage feeding periods. Results are presented as the treatment mean ± SEM. For purposes of clarification, a P-value of equal to or less than .05 was considered to be statistically significant.

Growth Performance and Feed Intake

Animal growth and feed intake data are presented in tables 3 and 4. Initial and final body weights were similar ($p > .05$) between treatments. Total weight gain was not affected ($p > .05$) by probiotic supplementation. In agreement with the present study, Wagner et al. (1990) reported no growth response when yeast culture was used as a probiotic in either corn- or wheat-based high concentrate diets for young dairy calves. In contrast, Hughes (1987) reported increased gain and feed efficiency when yeast culture was added into the concentrate portion of weaned-calf diets. Fallon and Harte (1987) added yeast culture to high-energy calf starter diets and reported increased feed intake and significant improvements in weight gain with barley-soya based diets, but not with corn gluten-barley based diets. This may be related to the fact that soya is generally more readily and extensively fermented or degraded in the rumen than is corn gluten. The addition of yeast may have had a positive effect on the ruminal fermentation of the barley-soya diet possibly by increasing the ruminal buffering and pH, resulting in increased feed intake and animal performance due to a more stable rumen environment.

Average daily gain (ADG) was nearly identical for the control and probiotic-supplemented calves, for the entire 112-day trial as well as for the

TABLE 3. BODY WEIGHT GAIN AND FEED PERFORMANCE OF HOLSTEIN HEIFER CALVES SUPPLEMENTED WITH A PROBIOTIC

Measurement[1]	Treatment	
	Control	Probiotics
No. of calves	8	8
Final weight (kg)		
Entire trial	224.7±3.8	222.2±4.9
Initial weight (kg)		
Entire trial	125.9±3.7	123.6±3.9
Weight gain (kg)		
Entire trial	98.8±2.6	98.6±3.6
Hay period	48.1±1.6	47.9±2.3
Silage period	50.7±1.9	50.7±3.0
Days on trial		
Total	112	112
Hay period	56	56
Silage period	56	56
Average daily gain (kg/d)		
Entire trial	.88±.02	.88±.03
Hay period	.86±.03	.85±.04
Silage period	.91±.03	.91±.05
Dry matter intake (kg/d/calf[2])		
Entire trial	4.75±.02	4.58±.08
Hay period	4.50±.10[a]	4.05±.05[b]
Silage period	5.00±.10	5.10±.10
Dry matter intake (% of BW[3])		
Entire trial	2.80±.02	2.70±.03
Hay period	3.00±.02[a]	2.75±.05[b]
Silage period	2.55±.05	2.60±.02
Feed efficiency (kg feed/kg gain)		
Entire trial	5.41±.14	5.24±.20
Hay period	5.28±.18	4.82±.26
Silage period	5.58±.20	5.77±.37

[1] Mean±SEM

[2] Calculated as total feed consumption of each pen divided by number of calves per pen.

[3] BW = body weight.

[a,b] Means within a row with different superscripts differ ($p < .05$).

TABLE 4. BODY MEASUREMENTS OF HOLSTEIN HEIFER CALVES SUPPLEMENTED WITH A PROBIOTIC

Measurement[1]	Treatment	
	Control	Probiotics
No. of calves	8	8
Final wither height (cm)		
Entire trial	111.7±1.0	110.9± .5
Initial wither height (cm)		
Entire trial	101.4±1.2	99.6±1.4
Wither height gain (cm)		
Entire trial	10.3± .7	11.3± .9
Hay period	2.6± .6[b]	5.4± .9[a]
Silage period	7.7± .7[a]	5.9± .6[b]
Final heart girth (cm)		
Entire trial	142.9±1.9	144.1±1.7
Initial heart girth (cm)		
Entire trial	118.8±1.7	115.8±1.4
Heart girth gain (cm)		
Entire trial	24.1±1.0[d]	28.2±1.8[c]
Hay period	12.1±1.0[b]	14.4± .8[a]
Silage period	12.0±1.5	13.8±1.2
Final paunch girth (cm)		
Entire trial	179.9±1.6	177.0±1.8
Initial paunch girth (cm)		
Entire trial	149.9±1.9	144.7±2.0
Paunch girth gain (cm)		
Entire trial	30.0±1.3	32.3±2.3
Hay period	14.9±3.2	17.5±1.6
Silage period	15.1±3.2	14.8±2.6

[1] Mean±SEM.

[a,b,c,d] Means within a row with different superscripts differ ([a,b]$p < .05$; [c,d]$p < .07$).

56-day hay and silage feeding periods. An ADG of .88 kg/day was achieved throughout the trial, indicating that calves were growing at a very acceptable rate during the trial. Wagner et al. (1990) reported no response in ADG as a result of yeast culture supplementation, while Fallon and Harte (1987) reported an increased ADG for calves fed a barley-soya diet supplemented with yeast culture. In the present experiment, ADG was slightly greater during the silage feeding period compared to the hay feeding period. The faster rate of growth during the silage feeding period is likely a reflection of the greater feed intake during this period compared to the hay feeding period. Dry matter intake (DMI) was slightly lower ($p < .14$) for probiotic-supplemented

calves during the entire 112-day trial. Dry matter intake was significantly lower ($p < .05$) for the probiotic-supplemented calves during the hay feeding period. This is in contrast to the increased DMI reported by Fallon and Harte (1987) as a result of yeast culture supplementation. The reasons for the lower DMI during the hay feeding period are unknown. Dry matter intake expressed as a percentage of body weight followed a similar trend as that of DMI expressed as kg/day.

Feed efficiency, expressed as kg feed/kg body weight gain, was not significantly ($p > .05$) affected by treatment. However, a consistent numerical difference ($p < .19$) was noted for feed efficiency during the hay feeding period, with the probiotic-supplemented calves being more efficient in converting feed to gain. The effect was reversed slightly during the silage feeding period. Adams et al. (1981) reported an increase in feed conversion efficiency when steers were fed diets supplemented with yeast culture as a probiotic. Hughes (1988) also reported an improvement in feed efficiency when calves were supplemented with yeast culture. In agreement with the present study, Wagner et al. (1990) found no significant differences in feed efficiency when dairy calves were supplemented with yeast culture.

Body measurements are given in table 4. Initial measurements for wither height, heart girth, and paunch girth were similar ($p > .05$) between groups of calves. Wither height gain over the entire trial was slightly higher for the probiotic supplemented calves, although the difference was not significant ($p > .05$). During the hay feeding period, wither height gain was greater ($p < .05$) for the probiotic supplemented calves. This effect was reversed during the silage feeding period ($p < .05$), although the actual difference between the two treatments was less during the silage feeding period than during the hay feeding period. Heart girth gain tended to be greater ($p < .07$) for the probiotic supplemented calves. The difference was significant ($p < .05$) during the hay feeding period, while a numerical advntage was found for the probiotic group during the silage feeding period. Paunch girth gain was not significantly different ($p > .05$) between the two treatment groups, although a consistent numerical advantage was noted for the probiotic supplemented calves for the entire 112-day trial and during the hay feeding period. Overall, it appeared that probiotic supplemented calves showed a small but consistent advantage in body measurement gain, especially during the hay feeding period. The advantage was not as pronounced during the silage feeding period. The reasons for this are unclear. However, it may be related to the fact that silage already contains fermentation products and bacterial cultures as a result of the normal ensiling process, while dried hay does not contain any significant amounts of fermentation products. Another possible factor may be related to the age of the calves during each of the forage feeding periods. Calves were on average two months younger during the hay feeding period. It has been proposed that probiotics are most beneficial in younger animals, particularly when young animals are stressed (Fuller, 1989; Sissons, 1989). In the present study, it is possible that stress due to weaning of the calves carried over into the time of the hay feeding period. This stress was most likely not present during the latter silage feeding period. Therefore, it is likely that probiotic supplementation would be most beneficial during the earlier hay feeding period. In further support of this concept, Pollman et al. (1980) obtained better results with starter than with growing-finishing pigs when a probiotic was fed. Fuller (1990) also suggested that even for the same species of animal the probiotic effect may vary with age and diet. It may be easier to influence the gut flora of younger animals while it is still in a state of flux.

Rumen and Blood Metabolites

Rumen pH and VFA are presented in table 5. Rumen pH was not affected ($p > .05$) by probiotic supplementation. Values averaged 6.87 across both treatments and forage sources. This relatively high pH value is indicative of a high forage diet. Williams et al. (1989) reported an increase in rumen pH when steers were given yeast culture (*Saccharomyces cerevisiae*). They attributed the increase in pH to a reduction in rumen lactate. The changes in pH and lactate were detected in steers fed ground barley and hay but not in steers fed only hay. Several other researchers (Wagner et al., 1990; Wiedmeier et al., 1987) reported no change in pH when either yeast culture or fungal culture (*Aspergillus oryzae*) was fed to dairy calves and non-lactating cows. Frumholtz et al. (1989) reported that the

TABLE 5. RUMEN pH AND VOLATILE FATTY ACIDS IN HOLSTEIN HEIFER CALVES SUPPLEMENTED WITH A PROBIOTIC

Measurement[1]	Treatment	
	Control	Probiotics
Rumen pH		
Entire trial	6.87± .03	6.87± .02
Hay period	6.74± .04	6.79± .03
Silage period	7.01± .04	6.96± .04
Total VFA (mM)		
Entire trial	86.1 ±1.0[b]	92.2 ±1.9[a]
Hay period	91.5 ±3.0	99.1 ±3.2
Silage period	80.7 ±2.4	85.2 ±2.7
Individual VFA (mol/100 mol):		
Acetate		
Entire trial	63.4 ± .4	63.3 ± .2
Hay period	64.2 ± .3	64.3 ± .2
Silage period	62.5 ± .5	62.3 ± .5
Propionate		
Entire trial	20.8 ± .3	20.8 ± .3
Hay period	19.9 ± .5	19.7 ± .3
Silage period	21.8 ± .3	21.9 ± .6
Butyrate		
Entire trial	11.0 ± .3	11.1 ± .2
Hay period	11.4 ± .3	11.5 ± .2
Silage period	10.6 ± .3	10.7 ± .2
Valerate		
Entire trial	1.36± .04	1.30± .04
Hay period	1.41± .06[c]	1.29± .04[d]
Silage period	1.33± .04	1.31± .05
iso-Butyrate		
Entire trial	1.26± .04	1.25± .03
Hay period	1.20± .07	1.16± .04
Silage period	1.32± .03	1.35± .05
iso-Valerate		
Entire trial	1.70± .11	1.82± .10
Hay period	1.48± .14	1.63± .15
Silage period	1.92± .09	2.02± .08

[1] Mean±SEM.

[a,b,c,d] Means within a row with different superscripts differ ([a,b] $p < .01$; [c,d] $p < .10$).

addition of a fungal fermentation extract to *in vitro* cultures eliminated the transient fall in pH that normally occurred following the addition of subtrate to the culture vessels. In contrast, Harrison et al. (1988) and Martin et al. (1989) reported a decrease in pH when yeast culture was supplemented to lactating cows and *in vitro* ruminal fermentations, respectively. It is conceivable that rumen pH could decline as a result of probiotic (yeast or fungal cultures) supplementation particularly if ruminal fermentation of high starch feedstuffs is increased. A lowered pH value may be reflected in changes in total VFA concentration or proportion of individual VFA. Molar percentage of propionate tends to increase as the pH drops to 6.0 or below (Davis, 1979).

Total VFA concentration was increased ($p < .01$) by probiotic supplementation for the entire 112-day trial. A consistent numerical increase in total VFA concentration was noted for both the hay ($p < .13$) and silage ($p < .24$) feeding periods as a result of probiotic supplementation. It appears that ruminal fermentation was stimulated by probiotic supplementation. Molar proportions of individual VFA were not affected ($p > .05$) by probiotic supplementation, with the exception that valerate tended to decrease ($p < .10$) in probiotic supplemented calves during the hay feeding period. In agreement with this study, Martin et al. (1989) and Frumholtz et al. (1989) reported increased total VFA concentration with *in vitro* ruminal cultures following yeast culture and fungal culture supplementation. Martin et al. (1989) further reported an increase in propionate production while Frumholtz et al. (1989) reported a shift from propionate towards butyrate, valerate, and branched-chained VFA. The increase in valerate reported by Frumholtz et al. (1989) is in contrast to the lower valerate during the hay feeding period in the present study. Other researchers (Harrison et al., 1988; Wagner et al., 1990; Wiedmeier et al., 1987) reported no change in total VFA concentration following yeast culture or fungal culture supplementation. With dairy calves, Wagner et al. (1990) found no differences in individual VFA proportions as a result of yeast culture supplementation. With non-lactating cows, Wiedmeier et al. (1987) also reported no changes in individual VFA proportions following yeast culture or fungal culture supplementation. These results are in agreement with the present study. With lactating dairy cows fed a 60% concentrate diet, Harrison et al. (1988) reported lower molar

proportions of acetate and isovalerate and higher proportions of propionate and valerate in cows supplemented with yeast culture. Dietary differences such as grain level, type of grain, and forage type may have a direct influence on the individual VFA profile following probiotic (yeast or fungal culture) supplementation. Individual VFA may be altered more readily when diets contain high amounts of readily available carbohydrates. This would help to explain the lack of response in individual VFA proportions in the present study, since grain feeding was limited while forage was offered free-choice throughout the trial.

Rumen ammonia-N concentration (table 6) was lower ($p < .05$) for the probiotic supplemented calves during the hay feeding period. For the entire 112-day trial, ammonia-N was numerically lower ($p < .19$) with probiotic treatment. Lower values may indicate less protein breakdown in the rumen, especially during the hay feeding period. Lower ammonia-N concentration may also indicate more utilization of the ammonia-N for ruminal microbial protein synthesis and growth. Frumholtz et al. (1989) reported that total viable bacterial numbers nearly doubled in an *in vitro* rumen simulation system when a fungal fermentation extract (*Aspergillus oryzae*) was added to the culture flasks. The cellulolytic population increased threefold as a result of fungal culture supplementation. However, ammonia concentration was increased by over 30% in fermentation vessels receiving the fungal culture. Arambel et al. (1987) suggested that *Aspergillus oryzae* fermentation extract may increase rumen proteolysis. In agreement with results obtained during the hay feeding period in the current experiment, Harrison et al. (1988) reported a lower rumen ammonia-N concentration when yeast culture was supplemented to lactating cows. They suggested that the greater concentrations of total anaerobic bacteria and cellulolytic bacteria as a result of yeast culture supplementation may explain why ruminal ammonia-N concentrations were lower in cows fed yeast culture. Ammonia-N is the preferred nitrogen source for many rumen bacterial species, particularly the cellulolytic bacteria (Allison, 1980). Lower ammonia-N may be a reflection of increased uptake of ammonia into microbial protein. Wiedmeier et al. (1987) reported no change in rumen ammonia-N concentration when yeast culture or fungal fermentation extract was fed to non-lactating cows.

TABLE 6. RUMEN AMMONIA AND LACTATE, AND PLASMA UREA-N AND GLUCOSE IN HOLSTEIN HEIFER CALVES SUPPLEMENTED WITH A PROBIOTIC

Measurement[1]	Treatment	
	Control	Probiotics
Rumen ammonia-N (mg/100 ml)		
Entire trial	18.6 ±1.0	17.1 ± .5
Hay period	22.4 ±1.4[a]	19.2 ± .9[b]
Silage period	14.8 ±1.0	15.0 ±1.4
Rumen lactate (mmol/l)		
Entire trial	.68± .04	.64± .02
Hay period	.69± .04	.61± .03
Silage period	.67± .05	.67± .05
Plasma urea-N (mg/100 ml)		
Entire trial	19.8 ± .6	18.5 ± .6
Hay period	20.3 ± .7[a]	18.0 ± .7[b]
Silage period	19.4 ± .6	19.0 ± .5
Plasma glucose (mg/100 ml)		
Entire trial	76.6 ±2.6[a]	69.4 ±2.1[b]
Hay period	74.3 ±3.2	68.4 ±4.0
Silage period	78.9 ±4.1	70.4 ±2.5

[1] Mean±SEM.

[a,b] Means within a row with different superscripts differ ($p < .05$).

Rumen lactate concentration was not significantly affected ($p > .05$) by probiotic supplementation, although a consistent numerical difference ($p < .17$) was noted during the hay feeding period with lactate being lower in calves receiving probiotic. Lactate concentrations were relatively low throughout the trial as might be expected when forage is offered free-choice. Lactate did not appear to be significantly influenced by forage type. With *in vitro* fermentations, Frumholtz et al. (1989) reported no significant effects on lactate concentration as a result of fungal fermentation extract supplementation. Williams et al. (1989) reported a decrease in lactate concentration in steers fed diets of hay and ground barley and supplemented with yeast culture. The expected peak in lactate concentration following barley feeding was also prevented by yeast culture supplementation. A lowered lactate concentration may result in a higher and more stabilized rumen pH. Although lactate concentration was slightly lower

during the hay feeding period in the present study, rumen pH was not affected by probiotic supplementation.

Probiotic supplementation resulted in lower ($p < .05$) plasma urea-N during the hay feeding period. A slightly lower ($p < .13$) plasma urea-N was observed for the entire 112-day trial when probiotic was fed. These changes in plasma urea-N are a reflection of the changes in rumen ammonia N levels as a result of probiotic supplementation. Plasma glucose was lower ($p < .05$) during the entire 112-day trial as a result of probiotic supplementation. Plasma glucose during the hay ($p < .25$) and silage ($p < .11$) feeding periods was also numerically lower when calves received probiotic. Reasons for these differences are unclear. Changes in rumen fermentation may account for part of these differences although specific glucose precursors such as rumen propionate were not affected by probiotic supplementation.

Nutrient Digestibility

Total tract DM, OM, CP, and fiber digestibilities are given in table 7. Values are apparent digestibilities since no adjustment has been made for microbial DM, OM, or CP contributions to the fecal DM. There were no statistical differences ($p > .05$) with regard to total tract digestibility, although ADF digestibility was numerically higher ($p < .14$) with probiotic supplementation during the hay feeding period. In general agreement with this study, several researchers (Araimbel et al., 1987; Chademana and Offer, 1990; Harrison et al., 1988; Martin et al., 1989) reported no effect on *in vitro* DM or fiber digestibility or *in vivo* total tract nutrient digestibility when yeast culture or fungal culture was fed as a probiotic. With several different forage-to-concentrate ratios, Chademana and Offer (1990) found no differences in *in vivo* organic matter or NDF digestibility as a result of yeast culture supplementation in sheep. Although fiber digestibility was not affected, Harrison et al. (1988) reported an increase in the rumen concentration of cellulolytic bacteria in cows fed yeast culture. With *in vitro* fermentation cultures, Frumholtz et al. (1989) also reported an increase in the cellulolytic population as a result of fungal culture supplementation. However, Jung and Varel (1987) noted that increases in the number of cellulolytic bacteria did not correspond to increases in digestion of cell wall, cellulose, or hemicellulose. Gomez-Alarcon et al. (1990) reported an increase in rumen and total tract digestibility of fiber when fungal culture (*Aspergillus oryzae*) was fed to mature Holstein cows. They also reported an increased *in vitro* DM disappearance from alfalfa, milo, and wheat straw as a result of fungal culture supplementation. Tapia et al. (1989) also reported

TABLE 7. APPARENT TOTAL TRACT NUTRIENT DIGESTIBILITY IN HOLSTEIN HEIFER CALVES SUPPLEMENTED WITH A PROBIOTIC

Measurement[1]	Treatment	
	Control	Probiotics
	digestibility, % of intake	
Dry matter		
Entire trial	70.6± .8	70.9± .5
Hay period	76.9± .5	77.2± .7
Silage period	64.3±1.4	64.6± .5
Organic matter		
Entire trial	72.6± .8	72.9± .5
Hay period	78.5± .5	78.7± .6
Silage period	66.7±1.3	67.1± .5
Crude protein		
Entire trial	71.4±1.2	71.0± .8
Hay period	77.4± .7	76.5±1.2
Silage period	65.4±1.8	65.5± .6
Neutral detergent fiber		
Entire trial	61.6±1.1	62.5± .6
Hay period	68.6± .8	69.4± .6
Silage period	54.8±1.5	55.6± .6
Acid detergent fiber		
Entire trial	60.0± .9	61.0± .6
Hay period	66.1± .7	67.5± .7
Silage period	53.9±1.1	54.5± .6
Hemicellulose		
Entire trial	63.3±1.1	64.2± .5
Hay period	71.1± .9	71.3± .6
Silage period	55.6±1.7	57.0± .7
Cellulose		
Entire trial	70.2± .7	71.1± .6
Hay period	74.9± .6	75.9± .6
Silage period	65.6±1.1	66.4± .7

[1] Mean±SEM.

an increased *in vitro* DM disappearance of corn stover, ryegrass, and alfalfa hay when fungal cultures were added to the culture tubes. With yeast and fungal cultures, Wiedmeier et al. (1987) reported an increase in total tract digestibility of DM, CP, and hemicellulose in Holstein cows. Percentage ruminal cellulolytic organisms was also increased as a result of fungal and yeast culture supplementation. They suggested that yeast culture provided stimulatory growth factors for cellulolytic bacteria, while the fungal culture was directly involved in cellulolysis. It appears that fiber digestibility is most often affected in comparison to other DM components as a result of yeast, fungal, or probiotic supplementation. Although not significantly, ADF digestibility did appear to be increased slightly in the present experiment when calves were supplemented with probiotic, particularly during the hay feeding period.

Overall, some benefits in animal performance and changes in rumen fermentation were found with probiotic supplementation. Most of the animal performance benefits were observed during the earlier hay feeding period. Reasons for this may be related to age of the calves, weaning stress, and differences in forage source. Discussion of these possibilities was presented earlier in this paper. Beneficial responses to probiotic supplementation were found for body measurement gains and feed efficiency, primarily during the hay feeding period. Average daily gain was largely unaffected by probiotic supplementation. Fuller (1989) stated that the growth stimulatory effect in itself is bound to be variable, as it will operate only when the animals are stressed by the presence of a growth depressing microflora. For the most part, animals used in this experiment did not appear to be under stress. Animals were housed indoors away from drafts and feed (forage) and water were provided on a free-choice basis. There were no incidents of diarrhea or other observable illnesses during the trial. However, early stress due to weaning cannot be discounted, although animals were allowed a two week adaptation period between weaning and the start of the trial. If present, weaning stress would have appeared during the hay feeding period, the time in which the probiotic appeared to be most beneficial. In addition, grain constituted a larger percentage of the total diet DM during the hay feeding period since animals were smaller during this period and consumed less forage and total feed DM. Grain mix was limited to 1.6 kg DM per day throughout the trial regardless of animal size; therefore, the proportion of forage in the diet increased with time. It was suggested previously in this paper that yeast and fungal cultures fed as probiotics may have a grater influence on ruminal fermentation and possible animal performance when animals are receiving a higher amount of grain in their diet. This concept could help to explain why the probiotic used in this study appeared to have its greatest influence during the hay feeding period, when the grain constituted a larger percentage of the total dietary dry matter.

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