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K.A. Beauchemin, W.Z. Yang, Diego Morgavi, G.R. Ghorbani, W. Kautz, et al.. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. *Journal of Animal Science*, 2003, 81, pp.1628-1640. 10.2527/2003.8161628x . hal-02675787

HAL Id: hal-02675787

<https://hal.inrae.fr/hal-02675787>

Submitted on 31 May 2020

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Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle^{1,2}

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ABSTRACT: Two studies were conducted to determine whether a bacterial direct-fed microbial (DFM) alone or with yeast could minimize the risk of acidosis and improve feed utilization in feedlot cattle receiving high-concentrate diets. Eight ruminally cannulated steers, previously adapted to a high-concentrate diet, were used in crossover designs to study the effects of DFM on feed intake, ruminal pH, ruminal fermentation, blood characteristics, site and extent of digestion, and microbial protein synthesis. Steers were provided ad libitum access to a diet containing steam-rolled barley, barley silage, and a protein-mineral supplement (87, 8, and 5% on a DM basis, respectively). In Exp. 1, treatments were control vs. the lactic-acid producing bacterium *Enterococcus faecium* EF212 (EF; 6×10^9 cfu/d). In Exp. 2, treatments were control vs EF (6×10^9 cfu/d) and yeast (*Saccharomyces cerevisiae*; 6×10^9 cfu/d). Supplementing feedlot cattle diets with EF in Exp. 1 increased ($P < 0.05$) propionate and ($P < 0.05$) decreased butyrate concentrations, decreased the nadir of ruminal pH ($P < 0.05$), enhanced the flow of feed N

($P < 0.10$) to the duodenum but reduced that of microbial N ($P < 0.10$), reduced ($P < 0.10$) intestinal digestion of NDF, and increased ($P < 0.10$) fecal coliform numbers. Other than the increase in propionate concentrations that signify an increase in energy precursors for growth, the other metabolic changes were generally considered to be undesirable. In Exp. 2, providing EF together with yeast abolished most of these undesirable effects. Combining EF with yeast increased the DM digestion of corn grain incubated in sacco, but there were no effects on altering the site or extent of nutrient digestion. The diets used in this study were highly fermentable, and the incidence of subclinical ruminal acidosis, defined as steers with ruminal pH below 5.5 for prolonged periods of time, was high. Supplementing the diet with EF, with or without yeast, had limited effects on reducing ruminal acidosis. It seems that cattle adapted to high-grain diets are able to maintain relatively high feed intake and high fiber digestion despite low ruminal pH. The *Enterococcus faecium* bacterium and yeast used in this study were of limited value for feedlot cattle already adapted to high-grain diets.

Key Words: Acidosis, *Enterococcus*, Microbial Flora, Probiotics, Yeasts

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J. Anim. Sci. 2003. 81:1628–1640

Introduction

Direct-fed microbials (DFM), or probiotics, are live, naturally occurring bacterial supplements (Yoon and

Stern, 1995). The original concept of feeding bacterial DFM to livestock was based primarily on potentially beneficial postruminal effects, including improved establishment of beneficial gut microflora (Fuller, 1999). However, certain bacterial DFM may also improve ruminal function. Ghorbani et al. (2002) reported that steers receiving both lactate-utilizing *Propionibacterium* and lactate-producing *Enterococcus* had higher ruminal concentrations of acetate, and some of the blood variables measured indicated a reduced risk of metabolic acidosis. Although the mode of action of DFM in the rumen is not completely understood, the presence of lactate-producing bacteria is thought to help the ruminal microflora adapt to the presence of lactic acid (Ghorbani et al., 2002), whereas the presence of lactate-utilizing bacteria is thought to prevent accumulation of lactate (Nisbet and Martin, 1994; Kung and Hession,

¹Lethbridge Research Centre contribution number 387(02090).

²The authors thank B. Farr, A. Furtado, R. Wuerfel, K. Andrews, and D. Vedres for their assistance in conducting the experiment and performing laboratory analyses and the staff of the Lethbridge Research Centre metabolism unit for care of the cattle. The financial contributions of Chr. Hansen, Inc. and the Agriculture and Agri-Food Canada Matching Investment Initiative are gratefully acknowledged.

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Received September 30, 2002.

Accepted February 4, 2003.

1995). Nocek et al. (2000) reported decreased risk of acidosis for dairy cows receiving a combination of yeast and lactate-producing bacteria, *Lactobacillus* and *Enterococcus*.

There is a growing body of evidence that supplementing diets with yeast (*Saccharomyces cerevisiae*) increases milk production of dairy cows and weight gain of growing cattle (Yoon and Stern, 1995). Production responses attributed to yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen (Martin and Nisbet, 1992; Newbold et al., 1996), which may be beneficial for feedlot cattle fed high-grain diets.

The objective of this study was to evaluate whether providing *Enterococcus faecium*, a lactate-producing bacterium, alone or in combination with yeast, to feedlot cattle fed a high-grain diet could decrease the risk of acidosis and improve feed digestion.

Materials and Methods

Experimental Design and Animals

Two experiments were conducted: the first used *Enterococcus faecium* alone, while the second used *E. faecium* and yeast. Each experiment was conducted as a crossover design with two squares, four steers within each square, two periods and two diets. The squares (groups) within each experiment were run concurrently, and experiments were run consecutively.

Eight cannulated steers were used in both experiments. Steers in the first group were fitted with ruminal cannulas, whereas the steers in the second group were fitted with ruminal and duodenal cannulas. The ruminal cannulas measured 10 cm in diameter and were constructed of soft plastic (Bar Diamond, Parma, ID). The duodenal cannulas were simple, T-shaped cannulas that were placed proximal to the common bile and pancreatic duct, approximately 10 cm distal to the pylorus. Surgeries for both groups of animals were completed about 6 mo before starting the experiment. Prior to the start of the adaptation phase of the experiment, Group 1 steers had not been exposed to grain diets, whereas Group 2 had been used previously in a 4-mo study in which a high-grain barley-based diet was fed. Body weight of steers in Groups 1 and 2, respectively, was 484 ± 58 kg and 530 ± 33 kg at the start of Exp. 1 and 514 ± 68 kg and 562 ± 25 kg at the start of Exp. 2.

The length of each period was 3 wk, with a 10-d adaptation and an 11-d measurement period. Steers were housed in individual stalls bedded with rubber mats and cared for according to the guidelines of the Canadian Council on Animal Care (1993).

In order to minimize carryover effects from period-to-period, on the last day of Period 1, the rumen of each steer was emptied manually, and the ruminal contents were placed into the rumen of the next steer within the square that was to receive that treatment. Thus, each

Table 1. Ingredient and chemical composition of the total mixed diet (DM basis)

Item	%
Ingredient ^a	
Barley silage ^{bc}	7.94
Barley, steam-rolled ^d	87.13
Barley, ground	0.97
Canola meal	1.52
Calcium carbonate	1.85
Trace mineral/vitamin mix ^e	0.05
Salt	0.54
Chemical composition	
ME allowed gain, kg/d ^f	2.01
NE _g , Mcal/kg ^f	1.17
NE _m , Mcal/kg ^f	1.80
OM, %	94.2
CP, %	12.5
MP allowed gain, kg/d ^f	1.53
NDF, %	22.0
Effective NDF, %	8.09
ADF, %	10.2

^aAll ingredients pelleted, excluding steam-rolled barley and silage.

^bEffective NDF was 66%, measured as the sum of the proportion of sample retained on the top (4.2%) and bottom (61.9%) sieves of the Penn State particle separator.

^cComposition was 37.1% DM, 12.3% CP, 45.8% NDF, 28.3% ADF, and 4.8% lignin based on four samples composited by period.

^dProcessing index measured as the volume weight of the barley after processing (DM basis), expressed as a percentage of its volume weight (DM basis) before processing was 79%.

^eSupplied per kilogram of DM of diet: 15 mg of Cu, 63 mg of Zn, 27 mg of Mn, 0.65 mg of I, 0.2 mg of Co, 0.3 mg of Se, 4,200 IU of vitamin A, 415 IU of vitamin D, and 13 IU of vitamin E.

^fEstimated from NRC (1996). Animal body weight used in the model was 450 kg.

steer started the period with ruminal contents corresponding to the same treatment it was fed. At the end of Exp. 1, the ruminal contents from control steers were transferred to control steers in Exp. 2, and ruminal contents from treatment animals were transferred to treatment animals in Exp. 2.

Treatments and Diet

In Exp. 1, steers received either the control treatment or *E. faecium* EF212 (**EF**), and in Exp. 2, steers received either the control treatment or *E. faecium* EF212 with yeast (**EF + Yeast**). The treatments were supplied by Chr. Hansen, Inc. (Milwaukee, WI). The viability of the preparation was checked by Chr. Hansen, Inc., prior to starting the experiment. The bacteria and yeast were blended with calcium carbonate to supply 6×10^8 cfu of bacteria per gram (6×10^9 cfu/d) and 6×10^8 cfu of yeast per gram (6×10^9 cfu/d) when top-dressed onto the diet once daily at the time of feeding ($10 \text{ g} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$). Steers fed the control diets received only carrier ($10 \text{ g} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$).

The experimental diet contained 87% steam-rolled barley, 8% whole crop barley silage, and 5% supplement (DM basis), as shown in Table 1. The diet was formulated using NRC (1996) to meet or exceed the CP, effective fiber, mineral, and vitamin requirements of cattle

weighing 450 kg and gaining 1.5 kg/d. The diet was prepared daily using a feed mixer. Feed was offered once daily at 0900 for ad libitum intake (at least 10% orts). Feed offered and refused were recorded daily.

Intakes and Body Weight

Samples of barley silage, concentrate (comprised of rolled barley grain and pelleted supplement), and diet were collected five times weekly and composited. Dry matter was determined on a portion of each weekly composite feed sample, and the DM contents were used to adjust the silage to concentrate ratio of the diet when necessary. Weekly samples of barley silage, concentrate, and diet were then composited by period and retained for chemical analysis. Samples of orts were collected daily and composited by animal for each period. Samples were dried, and DMI for each steer was calculated based on the feed DM offered and orts DM refused.

The steers were weighed at on the first day of Period 1 and at the end of each period before feeding but before evacuating the rumen.

Ruminal pH

Ruminal pH was measured continuously for 3 d of each period on d 14 to 17 using indwelling electrodes. An electrode (model PHCN-37; Omega Engineering, Stamford, CT) was inserted into the rumen of each steer through the cannula. A weight was attached to the electrode to ensure that it remained in the ventral sac. In addition, a protective shield with large openings that allowed ruminal fluid to percolate freely was placed around the electrode to prevent it from coming in contact with the ruminal epithelium. The electrodes were removed from the rumen approximately 1 h prior to feeding each day and calibrated with pH 4.0 and pH 7.0 standards. Thus, continuous pH measurements were made for about 23 h/d. The pH was measured every 5 s, and an average of these readings was recorded every 15 min using a data logger. Ruminal pH data were summarized daily for each steer in each period as daily mean pH, maximum and minimum pH, proportion of the measurement period in which pH was below 5.8 or below 5.5, and area below pH 5.8 or pH 5.5. The proportion of time during which pH was below the particular threshold value was calculated using the actual duration that pH was measured for that animal. The threshold values of 5.5 and 5.8 were chosen because these values have been used previously to indicate subclinical ruminal acidosis (Brown et al. 2000; Ghorbani et al. 2002). The area was calculated by adding the absolute value of negative deviations in pH from pH 5.8 or 5.5 for each 15-min interval. The number was then expressed as pH units $\times$ h. The duration that pH remained below the threshold indicates the duration of subclinical acidosis, while the area between the curve and the pH threshold indicates the severity of subclinical acidosis.

Ruminal Fermentation

At 0, 6, and 12 h after feeding on d 18 and d 21, ruminal contents (250 mL per site) were obtained from four sites within the rumen (reticulum, dorsal and ventral sac, and the mat), composited, and strained through a polyester monofilament fabric (Pecap 7-355/47, mesh opening-355 μ m, Tetko Inc., Scarborough, ON, Canada). Five milliliters of filtrate was preserved by adding 1 mL of 25% (wt/vol) HPO_3 to determine VFA and lactate, and 5 mL of filtrate was preserved by adding 1 mL of 1% (wt/vol) H_2SO_4 to determine NH_3 . The samples were subsequently stored frozen at -20°C until analyses.

Microbiological Enumerations

At 0, 6, and 12 h after feeding on d 15 and d 18, ruminal contents (250 mL per site) were obtained from four sites within the rumen (reticulum, dorsal and ventral sac, and the mat), blended anaerobically under oxygen-free CO_2 , and strained through a polyester monofilament fabric (Pecap 7-355/47, mesh opening 355- μ m, Tetko Inc. Five milliliters of filtrate was preserved using 1 mL of methyl green formalin-saline solution for protozoa enumeration (Ogimoto and Imai, 1981). These protozoal samples were stored at room temperature and protected from light until counting. Protozoa were counted with the aid of a hemocytometer chamber. Duplicate preparations of each sample were counted, and if either value differed from the average by more than 10%, the counts were repeated. Blended, strained ruminal contents were serially diluted in 0.1% (wt/vol) anaerobic buffered peptone. Diluted samples were inoculated (0.2 mL/tube) in triplicate into Hungate tubes for enumeration of total (10^{-6} to 10^{-9} dilution), amylolytic (10^{-4} to 10^{-7} dilution), and lactate-utilizing (10^{-3} to 10^{-6} dilution) bacteria by the roll-tube technique. For enumeration of the total viable bacterial population, medium 10 of Caldwell and Bryant (1966) was used with glucose (0.1% wt/vol), maltose (0.1% wt/vol), cellobiose (0.1% wt/vol), DL-lactic acid solution (0.085% wt/vol), and starch (0.1% wt/vol) added as carbohydrate sources. For enumeration of amylolytic and lactate-utilizing bacteria, a selective carbohydrate agar was used based on medium 10 of Caldwell and Bryant (1966) containing 0.1% (wt/vol) soluble starch or 50 mM lactic acid as the main energy source for amylolytic and lactate-utilizing bacteria, respectively.

Fecal samples were obtained from the rectum on d 16 at 0 h for enumeration of total coliform bacteria. Fecal samples were diluted (10^{-2} to 10^{-5}) in 0.1% (wt/vol) anaerobic buffered peptone and inoculated in triplicate into Petri dishes, mixed with molten MacConkey agar media (Becton Dickinson Microbiology Systems, Sparks, MD) and allowed to solidify. Colonies were enumerated after 24 h incubation at 39°C .

In Sacco Digestion

To evaluate the potential effects of the treatments on rate and extent of feed digestion, samples of rolled

corn, rolled barley, and alfalfa hay were incubated in sacco for 0, 2 (grain only), 4, 6, 12, 24, 48, and 96 (hay only) h starting on d 14. Samples were incubated in duplicate (triplicate for the final time points) each period in two of the four steers in Group 1.

The rolled grains were used as is, but the hay was dried at 55°C for 48 h and ground through a 4.5-mm screen. Five grams of DM were then weighed into small bags (10 × 20 cm) made of monofilament Pecap polyester (pore size-51 ± 2 µm; B. & S. H. Thompson, Ville Mont-Royal, QC, Canada) and heat-sealed. Individual bags were placed in mesh retaining sacs that allowed ruminal fluid to percolate among the bags. The bags were then soaked in warm (39°C) water for 10 min and placed in the rumen (except the 0-h bags). Zero-hour bags were prepared in quadruplicate and incubated for 30 min in artificial ruminal fluid (Goering and Van Soest, 1970) without the micromineral solution. All bags were removed at the end of the incubation period, machine washed (without the spin cycle) for 10 min in cold water, and machine rinsed for 3 min. Bags were then manually washed under cold tap water and checked for broken seams. Bags were dried at 55 to 60°C for 48 h and weighed to determine DM disappearance.

Kinetics of DM disappearance in sacco were estimated using the non linear regression procedure of SAS (SAS Inst., Inc., Cary, NC). For each steer, period, and feed (rolled barley grain, rolled corn grain, and ground alfalfa hay), the model proposed by Ørskov and McDonald (1979) and modified by McDonald (1981) to incorporate a lag was fitted to the percentage of DM disappearance as follows:

$$y = a + b(1 - e^{-c(t-L)}) \text{ for } t > L$$

where a = soluble fraction (%), b = slowly degradable fraction (%), c = fractional rate constant at which b is degraded (%/h), L = lag time (h), and t = time of incubation (h).

Model parameters were then used to estimate DM disappearance at 12, 24, and 48 h of incubation for the three feeds.

In Vivo Digestion and Microbial N Synthesis

Duodenal flow and apparent digestibilities of nutrients in the total tract were determined using YbCl₃ (Rhône-Poulenc, Inc., Shelton, CT). Ammonia ¹⁵N ([¹⁵NH₄]₂SO₄, 10.6 atom % ¹⁵N; Isotec, Miamisburg, OH) was used as a ruminal microbial marker. During the last 11 d of the period, marker solutions were continuously infused into the rumen of steers in Group 2 via the ruminal cannula using an automatic pump. Daily amounts infused were 1.5 g of Yb and 180 mg of ¹⁵N dissolved in 550 mL of water for each animal. Ruminal (from the reticulum, dorsal, and ventral sac; 800 mL total), duodenal (300 mL), and fecal samples (100 g) were collected four times daily, every 6 h moving ahead 1.5 h each day, for the last 4 d of the period. This

schedule provided 16 representative samples of ruminal, duodenal, and fecal contents taken at 1.5-h intervals.

Ruminal samples were immediately squeezed through two layers of cheesecloth. Ruminal particles in the retentate were blended (400 g of ruminal particles plus 400 mL of 0.9% NaCl) in a Waring blender (Waring Products Division, New Hartford, CT) for 1 min and then squeezed through four layers of cheesecloth. The filtrates obtained by squeezing the ruminal samples and the filtrate obtained from squeezing the blended retentate were combined and centrifuged (800 × g for 10 min at 4°C) to remove protozoa and remaining fine feed particles, and the supernatant was centrifuged (27,000 × g for 30 min at 4°C) to obtain a mixed ruminal bacteria pellet. Bacterial pellets, accumulated by steer each period, were freeze-dried and ground using a mortar and pestle. Subsamples of the ground bacterial pellets were further ground to a fine powder using a ball mill (Mixer Mill MM2000; Retsch, Haan, Germany) for determination of N content and ¹⁵N enrichment.

Duodenal samples were pooled by steer within each period and mixed using a blender (model MX-9100, Toshiba, Tokyo, Japan), freeze-dried, and retained for chemical analysis. Fecal samples were collected from the rectum of each steer. The samples were dried at 55°C, ground through a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and retained for chemical analyses to determine digestibility.

Blood Chemistry

On d 17 and 21 of each period, blood samples were taken 5 h after feeding from the jugular vein of each steer, alternating sides at each collection. The blood was collected in 10-mL vacuum tubes containing Na-heparin (Vacutainer, No. 6480, green stopper, Becton Dickinson, Franklin Lakes, NJ), and blood pH and CO₂ were analyzed within 2 h by the Lethbridge Regional Hospital (Lethbridge, AB, Canada). The pH was analyzed on a blood gas analyzer (model IL1715, Instrumintation Laboratory Co., Lexington, MA), and CO₂ was determined using a chemistry analyzer (Dimension RXL, Dade Behring Inc., Newark, DE). Packed cell volume was determined by collecting a blood sample in a 7-mL vacuum tube containing K₃EDTA solution (Vacutainer, No. 6450, purple stopper, Becton Dickinson). The blood was transferred to a microhematocrit capillary tube, the end was sealed, and the tube was centrifuged for 6 min with a hematocrit centrifuge and read with a microcapillary reader (model MH, International Equipment Co. Boston, MA). A third sample was collected in a 10-mL vacuum tube containing Na-heparin and silica gel (Vacutainer, No. 366672, green/gray stopper, Becton Dickinson) and centrifuged at 3,000 × g (model IEC Centra-4B, International Equipment Co., Needham Heights, MA) for 20 min. A subsample (1 mL) of the plasma was centrifuged at 16,000 × g for 2 min (Spectrafuge 16M, National Labnet Co., Woodbridge,

NJ) to remove fibrinogen, and the supernatant was used to analyze glucose (slide No. 8130536), lactate dehydrogenase (slide No. 8351082), amylase (slide No. 8208191), Ca^{2+} (slide No. 8048191), and Mg^{2+} (slide No. 1080266) using a dry chemistry analyzer (VetTest analyzer, model 8008, IDEXX Lab, Westbrook, ME).

Chemical Analysis

All chemical analysis was performed on each sample in duplicate, and when the coefficient of variation for the replicate analysis was >5%, the analysis was repeated. Feed DM was determined by oven drying at 55°C for 48 h. Analytical DM content of the samples was determined by drying at 135°C for 3 h (AOAC, 1995), followed by hot weighing. The OM content was calculated as the difference between DM and ash contents (AOAC, 1995; Method 942.05). The NDF and ADF contents were determined by the methods described by Van Soest et al. (1991) with amylase and sodium sulfite used in the NDF procedure. Samples were reground using a ball ground (Mixer Mill MM2000; Retsch, Haan, Germany) for determination of starch and N. The concentration of CP ($\text{N} \times 6.25$) in feed was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruminals, Milan, Italy), and ^{15}N enrichment in duodenal content (i.e., to determine bacteria) was measured by flash combustion with isotope ratio mass spectrometry (VG IsoTech, Middlewich, England). Starch was determined by enzymatic digestion as described by Rode et al. (1999).

The processing index of the barley grain was calculated by measuring the volume weight of the barley after processing, expressed as a percentage of its volume weight before processing on a DM basis, to quantify the degree of processing of the barley grain (Yang et al., 2000). The effective fiber content of barley silage was measured as the sum of the proportion of the sample retained on the top and bottom sieves of the Penn State particle separator.

Ruminal VFA were quantified using colonic acid as the internal standard, and gas chromatography (model 5890, Hewlett Packard, Little Falls, DE) with a capillary column (30 m $\times$ 0.25 mm i.d., 1 μm phase thickness, bonded PEG, Supelco Nukol, Sigma-Aldrich Canada, Oakville, ON) and flame ionization detection. The oven temperature was 100°C for 1 min, which was then ramped by 20°C/min to 140°C, and then by 8°C/min to 200°C and held at this temperature for 5 min. The injector temperature was 200°C, the detector temperature was 250°C, and the carrier gas was helium. Ruminal $\text{NH}_3\text{-N}$ concentration was determined by the salicylate-nitroprusside-hypochlorite method using a flow injection analyzer (Sims et al., 1995). Concentrations of Yb in the samples were determined using atomic absorption spectrophotometry (AOAC, 1990). Lactic acid was determined by gas chromatography after derivatization with boron trifluoride-methanol as described by Supelco (1998).

Calculations and Statistical Analyses

Procedures used to calculate site and extent of digestion are explained in further detail by Titgemeyer (1997). Briefly, the flow of DM to the duodenum and the amount of DM excreted in feces were calculated by dividing Yb infused (1.5 g of Yb per animal daily) by Yb concentration (grams of Yb per kilogram of DM) in the duodenal digesta or feces, respectively. Flow of other nutrients to the duodenum or feces was calculated by multiplying DM flow by the concentration of the nutrient in duodenal or fecal DM. Ruminal microbial N flow to the duodenum for each steer was estimated by the ratio of atom percentage excess of ^{15}N flow at the duodenum to ^{15}N atom percentage excess of ruminal bacteria. Organic matter truly digested in the rumen was calculated by first subtracting the amount of OM contributed by the microbial mass from the flow of OM to the duodenum.

For each period, means for individual steers were calculated for all variables. Data for microbial counts were converted before analysis using a log transformation. Data were analyzed by experiment with the mixed model procedure of SAS (SAS Inst. Inc.) to account for effects of group (or square), steer within group, period, and treatment. Period within square was not considered in the model because both squares were conducted simultaneously, and thus the effect of period was considered to be the same for both squares. Group, steer within group, and period were considered as random effects and treatment was considered a fixed effect. The restricted maximum likelihood method was used to estimate the variance components and the Kenwardroger method was used to approximate the degrees of freedom. Data for ruminal pH were summarized by day and then analyzed using the same mixed model but with day included as a repeated measure and using compound symmetry. Similarly, data for VFA within experiment were analyzed by sampling time using a repeated measures model, although means for sampling time were obtained by combining data for both experiments. Treatment effects were declared significant at $P < 0.05$, and trends were discussed at $P < 0.10$, unless otherwise stated.

Results and Discussion

Feed Intake and Body Weight

The diet used in this study was typical of diets used commercially by the feedlot industry in western Canada. Dry matter intake averaged 7.82 kg throughout the study and was not affected by treatments (Table 2). Steers fed EF were heavier than Control steers ($P < 0.05$), but the ADG was similar. However, proving yeast with EF had no effect on BW. Because the study was conducted as a metabolism study designed as a Latin square, the growth performance may not reflect the commercial situation. In production studies, there have

Table 2. Body weight and dry matter intake of steers fed *Enterococcus faecium* with and without yeast (n = 8)

Item	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Body weight						
Mean for the period, kg	519 ^b	525 ^a	24	552	555	26
ADG, kg/d	0.62	0.89	0.18	0.67	0.87	0.14
DMI, kg/d	7.78	7.78	0.49	7.95	7.75	0.45

^{a,b}Within a row and experiment, means that do not have a common superscript differ ($P < 0.05$).

been some reports of positive effects of bacterial DFM on milk production of dairy cows (Ware et al., 1988) and weight gain of steers (Swinney-Floyd et al., 1999; Galyean et al., 2000; Rust et al., 2000); however, many of these studies have not been published in scientific peer-reviewed journals. Furthermore, many differences exist among bacterial species and strains used as DFM, making it difficult to make comparisons with the literature.

Ruminal pH

The diets used in this study were highly fermentable, and the incidence of subclinical ruminal acidosis was high. We considered subclinical ruminal acidosis to occur when ruminal pH remained below 5.5 in a steer for more than 50% of the time on one of the days of measurement. In Exp. 1, six of the eight steers in Period 1, and five of the eight steers in Period 2, experienced subclinical ruminal acidosis. In Exp. 2, five steers experienced subclinical ruminal acidosis in Period 1, and four in Period 2. The pH values recorded in Exp. 1 during the evaluation of EF were lower than those recorded in Exp. 2 during the evaluation of EF + Yeast (Table 3). This indicates that there was increased adaptation of the cattle to these highly fermentable diets over time. The ruminal pH conditions in which EF was evaluated were harsher than the conditions in which

EF + Yeast was evaluated. The mean pH values (5.68 to 5.74) reported in Exp. 2 are similar to the range of 5.69 to 5.72 reported previously by Ghorbani et al. (2002), but lower than the range of 5.79 to 6.06 reported by Beauchemin et al. (2001) for feedlot cattle fed a similar high-concentrate barley-based diet. Thus, the pH values reported in Exp. 1 were lower than previously reported for feedlot cattle fed barley-based diets possibly because four of the steers had not been exposed to high-concentrate diets before starting the adaptation phase of this study. In commercial feedlots, cattle are fed backgrounding diets that contain 25 to 40% grain (DM basis) for several months before receiving high-grain finishing diets. Thus, they are adapted slowly to grain.

The risk of acidosis in this study decreased as time progressed. Steers in Exp. 1 had a ruminal pH below 5.8 for about 74% of the time, and below 5.5 for about 55% of the time, whereas steers in Exp. 2 had a ruminal pH below 5.8 for about 57% of the day and below 5.5 for about 39% of the day (Table 3). Because the diet was the same in both experiments, it appears that the pH increased, and acidosis decreased, as time progressed and the animals adapted to this high-grain diet.

Treatments had no effect on decreasing acidosis, and, in fact, EF alone may have exacerbated the problem. Although there was no effect of DFM on mean ruminal pH (Exp. 1: 5.53; Exp. 2: 5.71; Table 3), or maximum

Table 3. Ruminal pH of steers fed *Enterococcus faecium* with or without yeast (n = 8)

Item	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Mean	5.56	5.50	0.10	5.68	5.74	0.10
Minimum	5.15 ^a	5.03 ^b	0.06	5.18	5.25	0.12
Maximum	6.21	6.33	0.10	6.40	6.40	0.07
Proportion of day						
pH < 5.8, %	73.5	74.6	7.5	61.4	53.2	8.9
Area < pH 5.8, pH × h	8.5	10.1	1.5	6.1	5.8	1.5
Proportion of day						
pH < 5.5, %	50.1	59.5	9.6	40.4	36.8	8.7
Area < pH 5.5, pH × h	3.1	4.4	0.9	2.5	2.6	0.9

^{a,b}Within a row and experiment, means that do not have a common superscript differ ($P < 0.05$).

Table 4. Characteristics of ruminal fermentation in steers fed *Enterococcus faecium* with or without yeast (n = 8)

Item	Exp. 1			Exp. 2			Time after feeding, h			
	Control	<i>Enterococcus</i>	SE	<i>Enterococcus</i> +			0	6	12	SE
				Control	Yeast	SE				
VFA										
Total, mM	105.9	108.4	3.1	109.0	108.9	3.6	104.7 ^b	111.0 ^a	108.5 ^{ab}	3.7
Acetate (A), mol/100 mol	52.7	48.9	2.3	51.6	52.8	1.6	51.7	51.4	51.5	1.7
Propionate (P), mol/100 mol	25.4 ^b	33.1 ^a	3.1	27.6	28.2	2.8	27.5 ^b	29.3 ^a	28.7 ^{ab}	2.1
Butyrate, mol/100 mol	16.1 ^c	13.1 ^d	1.6	15.4	13.9	2.2	15.1 ^a	14.3 ^b	14.5 ^{ab}	1.0
Isobutyrate, mol/100 mol	0.84	0.81	0.07	1.04 ^a	0.90 ^b	0.09	1.04 ^a	0.83 ^b	0.82 ^b	0.07
Valerate, mol/100 mol	2.3	1.8	0.6	2.1	1.8	0.2	2.1 ^a	1.9 ^b	1.8 ^b	0.2
Isovalerate, mol/100 mol	2.1	1.7	0.2	1.9	2.1	0.2	2.1 ^a	1.9 ^b	1.9 ^b	0.3
Caproic, mol/100 mol	0.56	0.61	0.12	0.39	0.31	0.16	0.44	0.48	0.48	0.10
A:P	2.46 ^c	1.66 ^d	0.39	2.17	2.06	0.28	2.17 ^a	2.02 ^b	2.08 ^{ab}	0.24
Lactate, mM	ND ^e	ND		ND	ND	ND	ND	ND	ND	
NH ₃ N, mg/L	6.38	5.39	1.35	11.45	10.69	1.82	12.49 ^a	6.33 ^b	6.62 ^b	1.72

^{a,b}Within a row and experiment, means that do not have a common superscript differ ($P < 0.05$).

^{c,d}Within a row and experiment, means that do not have a common superscript differ ($P < 0.10$).

^eND = not detectable.

pH, lowest pH was lower ($P < 0.05$) for steers receiving EF than for Control steers. There was no effect of treatment on the proportion of time or area below pH 5.8 or 5.5. The results of the current study do not confirm the findings of some other studies in which yeast or DFM based on lactic acid-utilizing bacteria have been used. In those studies, use of DFM decreased the risk of acidosis. Supplementing dairy diets with yeast decreased the drop in ruminal pH following meals in the study by Williams et al. (1991). Huffman et al. (1992) reported that steers receiving *Lactobacillus acidophilus* had reduced time that pH was below 6.0. Nocek et al. (2000) reported daily low pH was higher, and area under pH 5.5 was lower, for dairy cows receiving *Enterococcus* and *Lactobacillus*. It should be noted that the incidence of subclinical ruminal acidosis was more severe in the present study than in those previous studies. The lack of effect of bacterial DFM on preventing subclinical ruminal acidosis observed in this study confirms the lack of effect on ruminal pH reported previously for feedlot cattle fed high-grain diets supplemented with mixtures of lactic acid-producing and lactic acid-utilizing bacteria (Kim et al., 2000; Ghorbani et al., 2002). However, in view of the observed difference in ruminal pH conditions between Exp. 1 and Exp. 2, it is questionable whether a crossover design, even with ruminal emptying, is appropriate methodology for assessing biologically active compounds such as DFM. As there was a significant effect of day on most of the ruminal pH variables, it is clear that pH should be measured over several days, as previously reported by Ghorbani et al. (2002).

Ruminal Fermentation

Low ruminal pH was due to the accumulation of VFA rather than lactate (Table 4), which is typical for adapted steers (Hristov et al., 2001; Ghorbani et al., 2002). Ruminal concentrations of lactate were ex-

tremely low and below the level of detection (<1 mM). There was no effect of the treatments on total concentration of VFA, which averaged 108 mM. However, supplementing the diet with EF increased ($P < 0.05$) the proportion of propionate and, consequently, decreased ($P < 0.10$) the proportion of butyrate in ruminal fluid compared with the control. These results are consistent with the expectation that supplemental EF stimulate the presence of lactic acid-utilizing bacteria, which produce propionate. Similarly, Kim et al. (2000) reported increased propionate concentrations in steers fed *L. plantarum*, another lactic acid-producing bacterium. In Exp. 2, however, providing yeast with EF negated any effects on propionate proportion. It is not clear whether the lack of effect of EF in the presence of yeast was attributed directly to the yeast or to the fact that the ruminal pH was higher during Exp. 2 compared with Exp. 1.

As a result of a higher propionate concentration, the acetate:propionate ratio tended to decrease ($P < 0.10$) with EF supplementation, whereas EF + Yeast had no effect on acetate:propionate ratio (Table 4). Other than a decrease ($P < 0.05$) in isobutyrate proportion for steers fed EF + Yeast, the treatments had no effect on other individual VFA proportions or concentration of NH₃.

Time after feeding had a significant effect on concentrations of total VFA, most individual VFA proportions, and concentration of NH₃ as expected (Table 4). In general, differences were noted between prefeeding (0 h) and postfeeding (6, 12 h) sampling times, but no differences occurred between 6 and 12 h postfeeding. Prefeeding, total concentration of VFA was lower, proportions of propionate were lower, proportions of butyrate, isobutyrate, valerate, and isovalerate were higher, acetate:propionate ratio was higher, and NH₃ concentrations were higher compared with postfeeding.

Microorganisms

Protozoal numbers were high (Table 5), confirming previous studies with feedlot cattle fed high-grain diets

Table 5. Bacterial and protozoal numbers in the ruminal fluid and feces of steers fed *Enterococcus faecium* with or without yeast (n = 8)^a

Item	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Ruminal fluid						
Lactate-utilizing bacteria ($\times 10^7$), cfu/mL	4.30	6.80	1.55	2.60	2.21	0.57
Amylolytic bacteria ($\times 10^8$), cfu/mL	3.91	2.49	2.08	3.66	4.78	1.40
Total bacteria ($\times 10^{10}$), cfu/mL	3.77	6.20	1.35	2.51	2.60	0.32
Protozoa ($\times 10^6$), cells/mL	1.24	0.77	0.32	1.38	1.39	0.22
Feces						
Coliforms ($\times 10^6$), cfu/g	3.8 ^c	16.9 ^b	7.4	5.3	8.7	5.9

^aData were log transformed prior to statistical analysis.^{b,c}Within a row and experiment, means that do not have a common superscript differ ($P < 0.10$).

(Hristov et al., 2001; Ghorbani et al., 2002). Most of the protozoal populations were identified as *Entodinium*. Supplementing the diet with EF tended to decrease ($P < 0.15$) protozoal numbers, which is considered undesirable in terms of preventing acidosis because ruminal protozoa, especially *Entodinium*, play a critical role in utilizing lactic acid in the rumen (Newbold et al., 1987). The decrease in protozoal numbers with EF supplementation contrasts with the results of Van Koeveering et al. (1994) who reported that including lactobacillus cultures in the diet prolonged retention of protozoa. The decrease in protozoal numbers with supplementation of EF was also opposite to the increase in protozoal numbers observed in our previous study (Ghorbani et al., 2002) for steers receiving *Propionibacterium*, which converts lactate and glucose to acetate and propionate. In the present study, the effect of EF on lowering protozoal numbers was not apparent when diets were also supplemented with yeast.

Treatments had no effects on lactate-utilizing bacteria, amylolytic bacteria, or total bacterial numbers (Table 5). This result was somewhat surprising because both EF and yeast were expected to increase the presence of lactate-utilizing bacteria.

The number of fecal coliform bacteria tended to ($P < 0.10$) increase with EF supplementation indicating a postruminal effect. This effect was negated when yeast was provided. The reasons for this undesirable effect of EF on fecal coliforms are not clear and need to be evaluated in further experiments. It is possible that increased numbers of fecal coliform bacteria in cattle fed EF was the result of increased colon acidification, which may have occurred as a result of lower minimum ruminal pH. Diez-Gonzalez et al. (1998) reported that decreased colon pH, which corresponded to lower ruminal pH as a result of feeding higher grain diets, resulted in an increase in fecal coliform numbers.

In Sacco Digestion

In sacco digestion of feedstuffs was affected by treatments (Table 6). In general, providing EF alone negatively affected the in sacco digestion of corn grain, bar-

ley grain, and alfalfa hay. In contrast, providing EF with yeast generally improved the digestion of these substrates.

For corn, EF alone decreased 12-h ($P < 0.05$), 24-h ($P < 0.05$), and 48-h ($P < 0.10$) disappearance, but EF + Yeast increased ($P < 0.10$) 24-h and 48-h disappearance (Table 6). Furthermore, EF decreased ($P < 0.05$) the size of the degradable fraction, whereas EF + Yeast increased ($P < 0.05$) it as well as tended to decrease ($P < 0.10$) the rate of digestion. For barley, EF alone decreased ($P < 0.05$) the size of the degradable fraction and the 48-h ($P < 0.05$) disappearance. The negative effect of EF on barley digestion was negated when EF was provided with yeast. For alfalfa hay, EF alone decreased ($P < 0.05$) 24-h disappearance, but this effect was not evident when EF was provided together with yeast. However, EF + Yeast decreased ($P < 0.05$) the digestion rate and tended ($P < 0.10$) to increase the size of the potentially digestible fraction.

In Vivo Digestion and Microbial N Synthesis

Supplementation with EF, alone or with yeast, had no effect on total tract digestibility of DM, but tended ($P < 0.10$) to decrease OM digestibility (Table 7). Mean total-tract digestibility of DM and OM during the study was 73.5% and 76%, respectively, which is similar to previously reported values for barley-based feedlot diets (Beauchemin et al., 2001). Fifty-four percent of the DM and OM ingested was digested in the rumen with no effect due to treatments. These values are in agreement with the range of 47 to 63% for OM ruminal digestion presented previously for barley-based diets (Beauchemin et al., 2001). A slightly higher ruminal OM digestibility (62 to 66%) was reported by Zinn (1993) compared to the present study, probably due to the use of readily digestible ingredients such as molasses (8% of dietary DM).

Starch was almost completely digested (98%) in the total tract due to extensive digestion in the rumen (Table 8). About 83% of the starch ingested was digested in the rumen, which would account for the high total VFA concentrations and low ruminal pH values ob-

Table 6. In sacco digestion kinetics of corn grain, barley grain, and alfalfa hay in steers fed *Enterococcus faecium* with or without yeast (n = 2)

Feedstuff	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Corn						
Soluble fraction (A), %	15.8	15.9	0.7	15.2	15.3	0.1
Degradable fraction (B), %	51.5 ^c	36.9 ^b	12.8	38.7 ^c	52.7 ^b	1.4
Degradation rate (c), %	0.054	0.072	0.032	0.060 ^d	0.048 ^e	0.002
Lag, h	0.3	0	0.2	0.7	0.9	0.6
12-h disappearance ^a	35.9 ^b	34.0 ^c	1.2	34.1	37.0	0.6
24-h disappearance ^a	46.9 ^b	42.1 ^c	1.2	44.2 ^e	50.5 ^d	0.5
48-h disappearance ^a	57.5 ^d	48.6 ^e	6.5	51.5 ^e	62.4 ^d	1.1
Barley						
Soluble fraction (A), %	14.5	13.4	1.1	14.1	13.0	0.9
Degradable fraction (B), %	58.6 ^b	45.6 ^c	6.8	50.2	53.7	6.5
Degradation rate (c), %	0.066	0.105	0.018	0.110	0.083	0.028
Lag, h	0	0	0	2.0	0	0.6
12-h disappearance ^a	46.0	45.5	3.1	45.2	46.8	2.2
24-h disappearance ^a	60.5	54.7	4.3	57.5	59.3	2.5
48-h disappearance ^a	70.4 ^b	58.5 ^c	6.2	63.2	65.6	5.3
Alfalfa hay						
Soluble fraction (A), %	32.1	32.5	0.3	32.1	32.4	0.2
Degradable fraction (B), %	36.3	31.0	2.8	31.6 ^d	34.7 ^e	2.6
Degradation rate (c), %	0.045	0.045	0.002	0.054 ^b	0.048 ^c	0.001
Lag, h	1.1	0.1	0.4	2.5	0.9	0.7
12-h disappearance ^a	46.2	45.2	0.9	44.9	46.6	0.6
24-h disappearance ^a	55.5 ^b	52.7 ^c	1.7	53.9	55.6	1.4
48-h disappearance ^a	64.0	59.8	2.4	61.0	63.5	2.2

^aEstimated using the equation: DM disappearance = A + B * (1 - e^{-c(t-lag)}).^{b,c}Within a row and experiment, means that do not have a common superscript differ (*P* < 0.05).^{d,e}Within a row and experiment, means that do not have a common superscript differ (*P* < 0.10).**Table 7.** Intake, duodenal flow, and site and extent of digestion of DM and OM in steers fed *Enterococcus faecium* with and without yeast^a

Item	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
DM						
Intake (digestion phase)	8.32	8.24	0.18	8.37	8.46	0.24
Duodenal flow, kg/d	5.55	5.43	0.36	5.64	5.88	0.34
Digestibility, % of intake						
Ruminal	57.5	55.5	4.4	53.0	51.7	2.2
Intestine	41.5	39.0	4.5	40.6	42.7	3.8
Total tract	74.7	73.2	2.2	73.2	73.2	1.6
OM						
Intake, kg/d	7.94	7.84	0.18	7.75	7.81	0.24
Duodenal flow, kg/d						
Total	4.47	4.44	0.32	4.62	4.80	0.16
Microbial	1.10	0.95	0.13	0.97	1.04	0.08
Digestibility, % of intake						
Ruminal ^b	57.5	55.5	4.4	53.0	51.7	2.2
Intestine						
Total OM ^c	34.8	33.2	4.1	35.2	37.2	2.8
Feed OM ^d	21.0	21.1	4.1	22.7	24.0	3.0
Total tract	78.5 ^e	76.6 ^f	2.4	75.6	75.7	1.7

^aMeasured in four duodenally cannulated steers.^bCorrected for microbial OM.^cIncludes feed and microbial components.^dCalculated as the difference between total tract and ruminal digestion.^{e,f}Within a row and experiment, means that do not have a common superscript differ (*P* < 0.10).

Table 8. Intake, duodenal flow, and site and extent of digestion of starch and fiber in steers fed *Enterococcus faecium* with and without yeast^a

Item	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Starch						
Intake, kg/d	3.88	3.84	0.12	3.75	3.79	0.26
Duodenal flow, kg/d	0.58	0.68	0.21	0.68	0.65	0.10
Digestibility, % of intake						
Ruminal	84.9	82.2	5.9	81.9	82.9	27
Intestine	13.6	16.3	5.1	15.8	14.9	3.0
Total tract	98.5	98.5	0.8	97.7	97.8	0.6
NDF						
Intake, kg/d	2.55	2.55	0.26	2.49	2.49	0.11
Duodenal flow, kg/d	1.33	1.17	0.15	1.30	1.40	0.09
Digestibility, % of intake						
Ruminal	47.4	53.9	7.0	47.8	43.6	2.5
Intestine	10.1 ^d	0.1 ^e	6.3	5.4	7.5	3.9
Total tract	57.5	54.0	8.7	53.1	51.1	4.0
ADF						
Intake, kg/d	0.86	0.87	1.02	0.91 ^e	0.93 ^d	0.05
Duodenal flow, kg/d	0.64	0.60	4.9	0.65 ^e	0.71 ^b	0.07
Digestibility, % of intake						
Ruminal	25.9	30.9	5.2	29.6 ^b	23.5 ^e	4.7
Intestine	5.3	0.1	6.7	5.5	9.2	6.4
Total tract	31.2	30.9	3.9	35.1	32.7	5.3

^aMeasured in four duodenally cannulated steers.^{b,c}Within a row and experiment, means that do not have a common superscript differ ($P < 0.05$).^{d,e}Within a row and experiment, means that do not have a common superscript differ ($P < 0.10$).

served in this study. Treatments had no effect on site or extent of starch digestion.

Total tract digestibility of NDF and ADF averaged 54 and 32%, respectively during the study, with no effect of treatment (Table 8). About 48% of the NDF ingested and 27% of the ADF ingested was digested in the rumen. Thus, despite the low ruminal pH of the steers in this experiment, ruminal and total tract digestion of fiber remained high. Feeding EF + Yeast decreased ($P < 0.05$) ruminal ADF digestibility, and, consequently, duodenal flow of ADF increased ($P < 0.05$), although EF + Yeast had no effects on NDF digestion. The reason for decreased ruminal digestion of ADF as a result of feeding EF + Yeast is uncertain. Supplementing diets with EF alone tended ($P < 0.10$) to lower intestinal digestion of NDF, but this was not the case for steers fed EF + Yeast. The reason for decreased intestinal digestion of fiber for steers receiving EF is not clear but may be related to the lower nadir ruminal pH in Exp. 1.

Intake of N and flow of nonammonia N from the rumen were similar for all treatments (Table 9). However, the composition of the nonammonia N flow differed between steers fed control and those fed EF. Feeding EF tended ($P < 0.10$) to decrease the flow of microbial N from the ruminal but ($P < 0.10$) increase the flow of feed N. Decreased flow of microbial N from the rumen resulted from the numerical decrease in efficiency of microbial N synthesis, measured as grams of microbial N per kilogram of OM digested in the ruminal, because

OM intake and ruminal digestion were similar for both diets. Decreased microbial N synthesis was opposite to expectations, based on the fact that the EF treatment numerically decreased protozoal numbers by 38%. Protozoa contribute to recycling of N in the rumen and decreasing protozoal numbers is expected to increase efficiency of protein utilization (Jouany, 1996; Koenig et al., 2000). Increased flow of feed N from the rumen was the result of decreased ($P < 0.10$) ruminal digestion of feed N. This decrease in ruminal digestion of feed N caused by EF supplementation confirms the results for DM observed in sacco (Table 6). These results indicate that the EF supplement shifted the ruminal environment in a manner similar to that observed with ionophore supplementation: EF increased propionate concentrations, decreased proteolytic activity, and reduced protozoal numbers (Bergen and Bates, 1984).

Providing EF + Yeast negated all effects of EF on N metabolism (Table 9). However, supplementation with yeast failed to increase the bacterial population in the rumen, alter ruminal digestibility, or change the flow of microbial N from the rumen, unlike in some other studies (Newbold et al., 1996; Yoon and Stern, 1996). Yoon and Stern (1996) reported for dairy cows that yeast culture increased ruminal OM and CP digestion and decreased OM and N flow to the duodenum.

Blood Chemistry

Enterococcus faecium, with or without yeast, had no effect on any of the blood variables measured (Table

Table 9. Intake and metabolism of N in the digestive tract of steers fed *Enterococcus faecium* with and without yeast^a

Item	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Intake, g/d	188.2	185.5	8.8	191.1	192.6	2.3
Flow to duodenum						
Total						
g/d	184.4	182.9	8.3	196.3	198.4	8.1
% of intake	98.1	99.2	7.9	102.9	103.1	4.8
NAN ^b						
g/d	178.8	176.3	8.0	190.0	192.7	7.6
% of intake	95.2	95.7	8.0	99.5	100.0	4.5
Feed + endogenous						
g/d	73.2 ^e	86.4 ^d	5.2	91.1	87.1	5.6
% of intake	38.9 ^e	46.7 ^d	2.4	47.8	45.2	3.4
Microbial						
g/d	105.6 ^d	89.9 ^e	1.5	98.8	105.6	7.8
% of intake	56.3 ^d	49.0 ^e	7.8	51.8	54.9	4.2
g/kg of RFOM ^c	23.3	20.6	1.8	24.2	26.1	2.0
Fecal, g/d	61.2	61.8	4.1	68.2	66.5	3.7
Digestibility						
Ruminal (truly), %	61.1 ^d	53.3 ^e	2.4	52.2	54.8	3.4
Postruminal						
% of intake	65.6	65.8	8.6	67.1	68.5	4.1
% of flow to duodenum	65.7	64.4	4.0	64.1	65.4	2.1
Total tract						
% of intake	67.5	66.6	1.4	64.3	65.5	2.0

^aMeasured in four duodenally cannulated steers.^bNon ammonia N.^cOrganic matter truly digested in the rumen.^{d,e}Within a row and experiment, means that do not have a common superscript ($P < 0.10$).

10). The lack of effect of DFM treatments on blood pH was expected, because the acid-base balance in blood is highly regulated, and blood is saturated with bicarbonate (Owens et al., 1998). Thus, blood pH rarely fluctuates.

In a previous study in which *E. faecium* was provided together with *Propionibacterium* to feedlot steers, a trend towards decreased concentration of blood CO₂ was observed (Ghorbani et al., 2002). Lower concentration of CO₂ is indicative of reduced risk of metabolic acidosis (Brown et al., 2000). During metabolic acidosis

the concentration of blood CO₂ increases due to the reduction of the blood bicarbonate ion (Owens et al., 1998). Effects of bacterial DFM on blood CO₂ observed previously were not confirmed by the present study in which EF alone was evaluated.

In addition, previously reported (Ghorbani et al., 2002) beneficial effects of feeding a combination of *E. faecium* with *Propionibacterium* on lowering blood lactate dehydrogenase concentrations of steers were not confirmed in this study for EF or EF + Yeast. Decreased blood lactate dehydrogenase concentration reflects the

Table 10. Blood variables for steers fed *Enterococcus faecium* with or without yeast (n = 8)

Variable	Exp. 1			Exp. 2		
	Control	<i>Enterococcus</i>	SE	Control	<i>Enterococcus</i> + Yeast	SE
Blood pH	7.38	7.38	0.02	7.39	7.39	0.01
CO ₂ , mEq/L	25.56	26.19	1.02	25.88	26.69	0.61
PCV, % ^a	32.86	33.34	1.67	31.58	31.9	1.64
Glucose, mg/L	1.02	1.03	0.02	0.97	0.99	0.03
LDH, units/L ^b	8,109	8,393	535	8,108	8,021	568
Amylase, units/L	40.25	37.94	2.14	45.06	44.50	3.34
Ca ²⁺ , mg/L	92.32	91.84	0.96	91.23	90.11	1.01
Mg ²⁺ , mg/L	22.99	23.34	0.36	23.82	23.57	0.33

^aPacked cell volume.^bLactate dehydrogenase.

reduced need to metabolize lactate from tissue metabolism and absorption from the digestive tract (Owens et al., 1998). Thus, decreased lactate dehydrogenase concentration reflects a reduced risk of acidosis. Lack of effect of DFM in this study on blood lactate dehydrogenase is consistent with the very low concentrations of lactate in ruminal fluid (Table 4), although tissue lactate levels were not measured.

Lack of treatment effects on packed cell volume is consistent with the lack of effects on packed cell volume observed previously using *E. faecium* with *Propionibacterium* (Ghorbani et al., 2002). The blood cations Na, K, and Mg usually decrease during acute acidosis, while blood glucose and amylase activity increase (Owens et al., 1998; Brown et al. 2000). Although K was not measured in this study, the lack of effect of treatments on Ca, Mg, and amylase further confirms that the DFM studied had little effect on mitigating the effects of subclinical ruminal acidosis.

In summary, supplementing feedlot cattle diets with the lactic-acid producing bacterium *E. faecium* caused small shifts in the microbial ecosystem that led to increased propionate and decreased butyrate concentrations, decreased nadir ruminal pH, a change in the composition of nonammonia N flowing to the duodenum, reduced intestinal digestion of NDF, and increased numbers of fecal coliforms. The increase in propionate concentrations indicates an increase in energy precursors that would likely benefit growing cattle. However, the other metabolic changes are generally considered to be undesirable. Providing EF together with yeast eliminated the undesirable effects due to feeding EF, but there was little advantage in feeding the combined DFM compared with the Control.

The changes associated with supplementing diets with both bacteria and yeast were marginal with no effects on ruminal fermentation or ruminal microorganisms. Although the DM digestion of corn grain incubated in sacco increased, increased digestion of the barley-based diet was not confirmed by measurements of site or extent of nutrient digestion within the steers. It is possible that the EF + Yeast treatment would have been more effective had a corn-based diet been fed to the steers. The potentially beneficial effects of yeast may be diet and strain dependent. Newbold et al. (1995) reported differences in the metabolic effect of various strains of yeast, even when they were grown in an identical manner. This study supports the prior evidence that the beneficial effects of yeast are greater in forage-based diets than in high-concentrate diets (Sullivan and Martin, 1999).

Treatments had no effect on decreasing acidosis, and in fact providing *E. faecium* alone may have exacerbated acidosis as indicated by a lower nadir pH. Despite the low ruminal pH and high incidence of subclinical ruminal acidosis caused by feeding highly fermentable grain-based diets, ruminal and total tract digestion of OM and fiber remained high. For feedlot cattle accustomed to consuming high-grain diets, there was limited

value in providing bacterial DFM based on *E. faecium* bacterium, even when provided along with yeast.

Implications

Although bacterial direct-fed microbials might have the potential to improve feed utilization of feedlot cattle, the choice of bacterial species is crucial to formulating an effective product. The lactic acid-producing bacterium *Enterococcus faecium* used in this study was shown to be metabolically active and caused small shifts in the microbial ecosystem of feedlot cattle fed high-grain diets. However, these changes in digestive function were generally viewed to be of minor importance or even undesirable. Furthermore, supplementing the diet of feedlot cattle with *Enterococcus faecium*, with or without yeast, had limited effects on reducing ruminal acidosis or improving feed utilization. Thus, based on our results, a direct-fed microbial based on *Enterococcus faecium*, with or without yeast, is of limited value for feedlot cattle adapted to high-grain diets. However, the possibility that supplemental *Enterococcus faecium* bacteria may benefit nonadapted cattle, such as newly received cattle, cannot be excluded.

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