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Site of infusion of a commercially available direct-fed microbial on performance and digestibility in lactating Holstein cows

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ABSTRACT

Objective: The objective of this research was to evaluate site of infusion of a commercially available direct-fed microbial (DFM) containing 10^9 cfu/g of *Lactobacillus acidophilus* and 10^9 cfu/g *Propionibacterium freudenreichii* on DMI, rumen kinetics, ruminal VFA, digestibility, milk production, milk components, and blood metabolites in lactating dairy cows.

Materials and Methods: Four Holstein cows equipped with ruminal cannulas were used in a Latin square design experiment with 4 periods. Each 37-d period consisted of 14 d of no treatment to prevent crossover contamination, 14 d of adaptation to treatments, 8 d of sampling, and 1 d for ruminal evacuations. Within each period, cows were assigned to 1 of 4 treatments: (1) cows were fed a TMR formulated to meet or exceed nutrient requirements plus 5 g of lactose twice daily without the addition of DFM (control); (2) cows were fed the TMR with a daily dose of DFM top dressed on the feed twice daily (TD); (3) cows were fed the TMR with ruminal infusion of the DFM administered twice daily (RuI); or (4) cows were fed the TMR plus abomasal infusion of the DFM twice daily (AbI). During the sampling period within each period, DMI and milk production were measured daily with set days for blood and rumen fluid collection. Data were analyzed using the MIXED procedure of SAS with animal within period as a random effect. Dry matter intake was not different among treatments.

Results and Discussion: No differences were detected in rumen kinetics, pH, individual VFA or VFA ratios, ammonia, or digestibility. There were no differences in kilograms of milk production ($P > 0.87$); SCC ($P > 0.54$); or percentage of butter fat ($P > 0.21$), milk protein ($P > 0.83$), lactose ($P > 0.91$), SNF ($P > 0.88$), and MUN ($P > 0.49$). No difference existed in most of the milk fatty acids except for 8:0, which had a greater concentration ($P > 0.01$) in AbI versus control, TD, and RuI.

Implications and Applications: Route of administering DFM overall had no effects on DMI, rumen kinetics, ruminal VFA, digestibility, milk production, or milk components in the present experiment.

Key words: direct-fed microbial, dairy cow, milk production, digestibility, rumen kinetics

INTRODUCTION

Many factors can influence a cow's milk production including environmental stressors, housing environment, management practices, diet, genetics, and overall health. Bacteria or yeast cultures or both in the diet, commonly referred to as direct-fed microbials (**DFM**), can help minimize or prevent obstacles that may negatively affect milk production. Fuller (1997) defined probiotics, or DFM, as "preparations consisting of live microorganisms or microbial stimulants which affect the endogenous microflora of the recipient." Additionally, microorganisms that can be used as DFM are considered normal microflora of the gut specific to host species. Such microflora are nonpathogenic and may include viruses, bacteriophages, fungi, yeast, and bacteria (Fuller, 1997). Research has been conducted to determine the effects of feeding DFM to dairy calves (Cruywagen et al., 1996), cows (Stein et al., 2006), and feedlot cattle (Ghorbani et al., 2002). Production systems may use DFM to prevent disease (prevention of subacute rumen acidosis, described by Beauchemin et al., 2003) or improve food safety (decrease the occurrence of *Escherichia coli* O157-H7 shedding, described in Krehbiel et al., 2003). The use of DFM to enhance production goals has shown promising results in milk yield (McGilliard and Stallings, 1998), in transition cows (Nocek et al., 2003), and health (Oetzel et al., 2007); however, a study into milk yield, and rumen health and digestibility disagree with these findings (Raeth-Knight et al., 2007). Previous research could not be found which determined the effects

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Table 1. Treatment assignments and dosages

Treatment	Dosage
Control	5 g of lactose top dressed on TMR
Top dress	DFM mix[1] top dressed on TMR
Rumen infusion	DFM mix[1] reconstituted in 60 mL of sterile water followed with 60 mL of sterile water
Abomasal infusion	DFM mix[1] reconstituted in 60 mL of sterile water followed with 120 mL of sterile water

[1]Direct-fed microbial (DFM) mix fed once daily: 10^9 cfu/g *Lactobacillus acidophilus* and 10^9 cfu/g *Propionibacterium freudenreichii* with 5 g of lactose.

of site delivery of DFM on apparent total-tract digestibility or milk production.

The direct mode of action for bacterial DFM in ruminants has not been fully elucidated, but has been suggested to include changes in ruminal fermentation, changes in the microbial population in the rumen or lower gut, improvement of DM digestibility, increase in nutrient flow to the intestines, and changes in the immune system (Yoon and Stern, 1995; Krehbiel et al., 2003; Raeth-Knight et al., 2007). The purpose of this study was to determine the effects of bacterial-based DFM on DMI, ruminal nutrient digestibility, ruminal kinetics, milk production, milk components, and milk fatty acids.

MATERIALS AND METHODS

Experimental Design and Sample Collection

Four multiparous (mean lactations = 2.25) Holstein cows (DIM = 73.25 ± 20.11) fitted with type 9C rumen cannulas (Bar Diamond) were used in a 4 × 4 Latin square design with 37-d periods, to determine the effect of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* on milk production, digestibility, and metabolism. The experiment was conducted in accordance with the policies and procedures of an approved Oklahoma State University Animal Care and Use Protocol, number AG0713. The rumen content donor, a dry Holstein cow, was fed the same TMR as the cows on the treatments to serve as a rumen material filler after rumen evacuations. Cannulation surgeries were conducted at the Oklahoma State University veterinary hospital 3 mo before the start of the experiment to allow adequate healing time. To evaluate effects of *L. acidophilus* and *P. freudenreichii* on production parameters, 4 treatments were tested at an inclusion rate suggested by the DFM manufacturer: (1) top dressing of 5 g of lactose and no DFM (negative control); (2) top dressing of 5 g of lactose, 10^9 cfu/g *L. acidophilus*, and 10^9 cfu/g *P. freudenreichii* (**TD**; positive control); (3) ruminally infused DFM containing 5 g of lactose, 10^9 cfu/g *L. acidophilus*, and 10^9 cfu/g *P. freudenreichii* (**RuI**); and (4) abomasal infusion of DFM containing 5 g of lactose, 10^9 cfu/g *L. acidophilus*, and 10^9 cfu/g *P. freudenreichii* (**AbI**; Table 1). Treatments were arranged according to a Latin square design (Table 2). Cows were housed in separate pens and fed a TMR balanced for mid-lactation Holstein cows twice daily (0630 and 1830 h; Table 3) with treatments (lactose with or without DFM) fed once daily in the morning. Cows were milked twice daily (0530 and 1730 h) in a double 6 herringbone milking parlor. There was a 14-d adjustment/wash out period (d 1 to 14), followed by a 14-d infusion/treatment period (d 15 to 27), and measurements were collected for 7 d (d 28 to 35). Complete rumen evacuations were conducted on d 37 to prepare cows for the subsequent period. To minimize cross-treatment contamination between periods, rumen contents from the control and donor were split between all 5 cows by weight. The DFM came in 5-g prepackaged foil pouches from Nutrition Physiology Corporation. Each pouch consisted of 5 g of lactose and 1×10^9 cfu/g *L. acidophilus* and 1×10^9 cfu/g *P. freudenreichii*. The pouches were stored in a −20°C freezer for 1 wk before use and then transferred to a −10°C freezer until being fed.

Prepackaged DFM was poured into a dry 60-mL syringe, and sterile water was drawn from a prefilled beaker. The syringe was then lightly shaken until the lactose was dissolved into solution. After administrating DFM via infusion lines, sterile water was flushed through the syringe and infusion line 2 times for RuI (120 mL) and 3 times for AbI (180 mL) to clean them.

For rumen infusion, 0.60 m of Tygon Fuel and Lubricant Tubing (Saint-Gobain; length = 4.8 mm, 0.48-cm inside diameter × 0.79-cm outside diameter) was inserted through the cannula into the ventral rumen. A quick-hose clamp (Andwin Scientific) was used to open and close the tube. The infusions were administered via a 60-mL syringe twice daily using sterile water and rinsed with 120 mL of sterile water, followed by 60 mL of air to push remaining fluid through the tubing. Abomasal infusions were conducted by using the technique described by Gressley et al. (2006). A similar apparatus was used with 2.74 m of tubing and the addition of a rubber flange held in place by hose clamps that were cut and ground down to create smooth surfaces that held the infusion tubing postruminally. Four 1.27-cm holes were drilled into the outer corners of the flange to aid the passage of digesta and was placed through the omasal-abomasal ridge. The infusions

Table 2. Treatment assignments for 4 × 4 Latin square[1]

	Cow			
Period	**1**	**2**	**3**	**4**
1	Control	Top dress	Rumen	Abomasal infusion
2	Top dress	Abomasal infusion	Control	Rumen
3	Abomasal infusion	Rumen	Top dress	Control
4	Rumen	Control	Abomasal infusion	Top dress

[1]Top dress = direct-fed microbials (DFM) applied to the top of the TMR; rumen = DFM dosed to rumen via rumen infusion apparatus; abomasal infusion = DFM dosed postruminally via infusion apparatus.

Table 3. Ingredient and nutrient composition of the diet (DM basis)

Item	Value
Ingredient	
Bermuda grass, %	10.45
Alfalfa, %	25.25
Whole cottonseed, %	5.81
Distillers dried grain with solubles, %	12.10
Corn gluten feed, %	9.97
Lactation cow grain mix	
Ground corn, %	21.56
Soybean meal, 48% CP, %	1.44
Soybean hulls, %	9.51
RUMOLAC,[1] %	1.17
Limestone, %	1.23
Sodium bicarbonate, %	0.82
Calcium diphosphate, %	0.18
Magnesium oxide, %	0.16
Salt white, %	0.18
Lactating premix, %	0.18
Components	
DM, % (as fed)	56.06
CP, %	15.15
ADF, %	21.14
NDF, %	40.14
NE_l, Mcal/kg	1.70
Crude fat, %	6.50
Ash, %	3.81
Ca, %	0.96
P, %	0.41
Mg, %	0.36
K, %	1.05
Na, %	0.30
Zn, mg/kg	86
Cu, mg/kg	15
Mn, mg/kg	58
Mo, mg/kg	0.50

[1]Robt Morgan Inc.; RUMOLAC contains fat (as fatty acids), 84%; calcium, 9.0%; NE_l, 1.34 Mcal/kg.

were mixed with sterile water and then administered via 60-mL syringe twice daily and flushed with 180 mL of sterile water, followed by 60 mL of air. Before infusion the dosing tube was physically checked to ensure proper location.

Ruminal Evacuation

To decrease the possibility of cross-treatment contamination between periods, and in consultation with a rumen microbiologist, complete rumen evacuations were conducted on d 37. Contents were collected in treatment-specific, 208.2-L Rubbermaid trashcans (Rubbermaid) and weighed. Contents of any cow receiving a DFM was discarded and replaced with a mixture of fresh feed along with the rumen contents from the control and donor cow. To ensure each cows rumen was full, the donor cow was used to supply further rumen digesta that was added to the control rumen contents and feed mix. Extra feed was also offered on evacuation day to allow the cows to consume adequate amounts for fill.

Feed Intake

To evaluate daily DMI (d 28 to 35), TMR and orts were collected daily, weighed, and immediately placed in a −10°C freezer. Composition and ingredients of the TMR are listed in Table 1. To achieve *ad libitum* intake, animals were fed the TMR twice daily with a target of 10% orts. Samples of orts (100 g) from each treatment were collected before the morning feeding from d 28 to 35. Samples of the TMR (100 g) were collected and weighed before both feedings. At the end of each period, TMR (1,600 g) and orts (800 g) samples were thawed and composited by weight. The composited sample was subsampled (100 g) and dried at 60°C for 72 h for nutrient composition analysis. Dried samples were then ground in a Wiley Mill to pass through a 2-mm screen. All samples were evaluated for CP (LECO Truspec CN, LECO Corporation), NDF and ADF (ANKOM^{200}, ANKOM Technology Corporation), DM, OM, and ash (Galyean, 1997). The percent DM was used to correct all as-fed TMR and orts sample to a 100% DM basis. Daily DMI was then calculated by subtracting the

dry weight of the orts from the dry weight of the TMR offered each day.

Milk Production and Composition

Milk weights were recorded at each milking (d 28 to 35) by using Heart of America DHIA milk meters and sampling equipment. All meters were calibrated by Heart of America DHIA to DHIA standards before the start of the experiment. Meters were tested monthly during the herd's routine DHIA testing program. Average milk production was calculated daily. Milk samples were taken at the p.m. milking on d 32 and at the a.m. milking on d 33 of each period. Two samples were collected: one in a DHIA milk sample tube with Micro-Tabs (milk preservative from DHIA) added to be used for milk composition analysis, and one in a 50-mL polypropylene conical tube for milk fatty acid analysis. Two composite samples consisting of a.m. and p.m. milkings from each treatment cow were composited by the milk yield from each a.m. and p.m. milking. After compositing milk samples, the sample tubes were shipped to Heart of America DHIA to evaluate butter fat, lactose, MUN, protein, solids-not-fat, and SCC. All remaining samples were frozen (−20°C) for fatty acid analysis via gas chromatography using a procedure based on that of Bligh and Dyer (1959) for total lipid extraction. The fatty acid and total conjugated linoleic acid derivatization procedure was based on that of Nuernberg et al. (2002). Gas chromatography analysis was performed on a Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a Hewlett-Packard 7673A Auto-Sampler and a J&W BD23 column (30 m × 25 mm × 0.25 μm film thickness; Agilent). The gas chromatograph was set at an inlet temperature of 250°C, split 1:25; a detector temperature of 300°C; and a flow of 1.0 mL/min at a temperature of 170°C, and the carrier gas was helium. The oven program was set for a temperature of 120°C held for 2 min, and the temperature was then increased by ramp 1 (12°C/min to 190°C) and ramp 2 (2.0°C/min to 224°C). Fatty acids analyzes consisted of total SFA: all fatty acids without any double bond (8:0 to 24:0); total UFA: all fatty acids with double bond(s) (12:1 to 22:6n-3); total MUFA: all fatty acids with a single double bond (12:1 to 22:1); total PUFA: all fatty acids with 2 or more double bonds (18:2 *trans*-10,*cis*-12 to 22:6n-3); total n-6 PUFA: 18:2n-6, 20:3n-6, and 20:4n-6; total n-3 PUFA: 18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3; and conjugated linoleic acid: 18:2 *trans*-10,*cis*-12, and 18:2 *cis*-9,*trans*-11.

Digestibility

Chromic oxide was used as an indigestible marker to measure fecal output. The marker was dosed (10 g) in the rumen via the rumen cannula in preweighed 14.79-mL Torpac gelatin capsules twice daily, at 0700 and 1900 h, for 7 d (d 27 to 35) before collection. On d 32 to 35, fecal grab samples were collected at 0700 and 1900 h. Samples were stored in rectal palpation gloves in a −10°C freezer. At the end of each period, the fecal samples were thawed and then composited by treatment at 100 g each on a wet-weight basis. The samples were dried at 60°C for 72 h and then ground with a Wiley Mill (Thomas Scientific) to pass through a 2-mm screen. Samples were then prepared using the procedure described by Williams et al. (1962) and analyzed by ICP (inductively coupled plasma-atomic emission spectroscopy) for chromium at the Oklahoma State University Soil, Water and Forage Analytical Laboratory. The chromic oxide was used as an estimator of fecal output to calculate the digestibility of CP (LECO Truspec CN, LECO Corporation), NDF and ADF (ANKOM^{200}, ANKOM Technology Corporation), DM, OM, and ash (Galyean, 1997).

Ruminal Fluid Analysis

Rumen fluid was sampled starting on d 34 at 3-h intervals for 24 h starting at 0700 h and ending at 0700 h the following day (8 collection times) for analysis of VFA, ammonia, and pH. Rumen fluid was also collected on d 35 at 3-h intervals for 24 h starting at 0700 h (8 collection times) for analysis of cobalt EDTA dilution. A total of 4 collection devices were used and assigned per treatment. The ruminal collection devices were made up of PVC pipe capped on the sampling end, which was 1.27 cm in diameter and 60.96 cm in length, with 0.32-cm holes drilled randomly for the first 7.62 cm of pipe, starting at the sampling end. An Erlenmeyer flask, assigned per treatment, was affixed to the ruminal collection device for sample collection. A second Erlenmeyer flask was used as a vacuum trap and attached to the collection Erlenmeyer flask by 0.50 m of Tygon Fuel and Lubricant Tubing (Saint-Gobain; 4.8 mm in length, 0.48-cm inside diameter × 0.79-cm outside diameter). The vacuum trap was then connected to a portable vacuum pump for ruminal fluid sampling. Rumen fluid was collected in 3 different locations of the ventral sac of the rumen via collection apparatus inserted through the cannula opening. Rumen fluid was thoroughly mixed after collection. After mixing, rumen pH was evaluated with a VWR SympHony SP70P pH meter. Recorded ruminal pH was converted to H ion for statistical analysis. Meta-phosphoric acid was added to two 50-mL polypropylene conical tubes to which the mixed rumen fluid was added, making a 4:1 ratio of rumen fluid to meta-phosphoric acid. The samples were inverted 6 times and immediately stored in a −10°C freezer until frozen solid. All samples were then transferred to, and stored in, a −20°C freezer until analysis.

Cobalt EDTA was prepared as described by Udén et al. (1980), before the start of each sampling day. Analysis of cobalt EDTA dilution was conducted on d 35 at 3-h intervals for 24 h starting at 0700 h. The 0-h samples were collected, followed by dosing 300 mL of cobalt EDTA through the rumen cannula (Galyean, 1997). After dosing into the rumen, the cobalt EDTA was thoroughly mixed into rumen contents. Rumen fluid was collected in 3 differ-

ent locations of the ventral sac of the rumen via collection apparatus inserted through the cannula opening. Rumen fluid was thoroughly mixed after collection. Sampled rumen fluid was immediately stored in a −10°C freezer until frozen solid. All samples were then transferred to, and stored in, a −20°C freezer until analysis. Rumen fluid were slowly thawed in a −2°C refrigerator. Samples were then prepared as described by Galyean (1997) and analyzed for cobalt EDTA by ICP (inductively coupled plasma-atomic emission spectroscopy) for cobalt EDTA at the Oklahoma State Soil, Water and Forage Analytical Laboratory. Results were used to calculate rumen kinetics such as fluid dilution rate, fluid flow rate, ruminal fluid volume, and turnover time (Galyean, 1997).

Rumen fluid with a 4:1 ratio of rumen fluid to metaphosphoric acid was slowly thawed in a −2°C refrigerator. Rumen fluid was prepared as described by Erwin et al. (1961), and Goetsch and Galyean (1983), and analyzed by gas chromatography (Hewlett-Packard 5890 Series II Gas Chromatograph; Hewlett-Packard) equipped with a Hewlett-Packard 7673A Auto-Sampler with a Phenomenex ZB-FFAP column (30 m × 0.53 mm × 1 μm) for VFA. Inlet temperature was 250°C, and the flame ionization detector was set at 280°C. Oven parameters were set with the initial temperature at 80°C (held 0.2 min), which was then increased 15°C/min to 145°C (held 0.5 min) and then increased 45°C/min to 235°C, with a final hold of 2.0 min. The carrier gas was helium, set at a flow rate of 8 mL/min.

Rumen ammonia was analyzed according to Broderick and Kang (1980) and adopted to 96-well microplates (Beckman Coulter). The modified procedure was as follows (all centrifuge temperatures were at 4°C): (1) centrifuge rumen fluid at 20,000 × *g* for 10 min in 12-mL centrifuge tubes; (2) pipette 2 mL of supernatant into 2-mL micro-centrifuge tubes and place centrifuge tubes in a tabletop micro-centrifuge (Fisher Scientific [Model 235C]) for 15 min at 24,000 × *g*; (3) add 3 μL of centrifuged rumen fluid, distilled water for blank, and working standards to individual wells; (4) add 150 μL of phenol reagent, put plate cover on and mix on plate shaker (VWR Micro Plate Shaker model 980130) at 300 rpm for 30 s, and cover entire shaker containing plates with foil; (5) add 120 μL of hypochlorite reagent and put plate cover on and mix on plate shaker at 300 rpm for 30 s under foil; (6) place covered micro plate on prewarmed 95°C plate warmer [VWR (model 980130)] for 5 min; and (7) allow plates to cool to room temperature. Absorbance was measured with a plate reader (Multiskan Spectrum; Thermo Scientific) according to the procedure of Broderick and Kang (1980). Intra- and inter-assay CV for ammonia were below 5%.

Blood Samples and Analysis

On d 34, pre- and post-prandial (0530 and 0730 h) blood samples were collected via coccygeal venipuncture into serum separating, sodium fluoride and sodium heparin tubes. Serum samples were allowed to sit overnight in a 5°C refrigerator. All blood samples were centrifuged at 4°C at 3,000 × *g* for 20 min to separate plasma or serum, which was pipetted into 2-mL micro-centrifuge tubes. Samples were frozen at −20°C until analyses were conducted to evaluate total protein, BHB, BUN, glucose, IGF-1, insulin, lactate, and nonesterified fatty acids (**NEFA**). All blood samples were processed in the Oklahoma State University Animal Science Ruminant Nutrition Laboratory.

Commercially available kits were used for the colorimetric determination of BUN (Urea Nitrogen Reagent, Teco Diagnostic), total protein [Total Protein (Biuret) Reagent Set, Pointe Scientific], NEFA (HR Series NEFA-HR [2], Wako Pure Chemical Industries Ltd.), BHB (β-hydroxybutyrate Reagent Set, Pointe Scientific), lactate [Lactate (Liquid) Reagent Set, Pointe Scientific], glucose [Liquid Glucose (Hexokinase) Reagent Set, Pointe Scientific], insulin (Insulin ELISA, DSL-10-1600, Diagnostic Systems Laboratories), and IGF 1 (Non-Extraction IGF-1 ELISA, DSL-10–2800, Diagnostic Systems Laboratories) concentrations. Microplates (96-well; Beckman Coulter) were used for all analyses. Absorbance was measured according to manufacturer recommendations for each metabolite using a plate reader (Multiskan Spectrum; Thermo Scientific). Intra- and inter-assay CV for analysis of each metabolite were below 5%.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS with animal within period as a random effect using LSM and orthogonal contrasts (C1: control vs. TD, RuI, AbI; C2: TD vs. RuI, AbI; C3: RuI vs. AbI) to separate significant treatment differences. Values were considered significant at $P < 0.05$; at $P < 0.10$ it was considered a tendency toward significance. Treatment × time interactions were tested for DMI, ruminal H ion (pH), ruminal NH_3, VFA, and milk production. If there was no significant treatment × time interaction, the data are presented by treatment. Data are presented as LSM.

RESULTS AND DISCUSSION

Feed Intake

Site of infusion or top dressing *L. acidophilus* and *P. freudenreichii* had no effect on DMI comparing TD, RuI, and AbI to the control (Table 4); however, top dressing the DFM tended to result in lower DMI (25.6 kg) compared with infusing it in the rumen or abomasum (27.2 kg; $P = 0.09$). No treatment × time interaction was observed. Kilograms of fecal output did not differ ($P = 0.70$) between treatments. Previous research demonstrated similar results with no difference in DMI when DFM containing *Enterococcus faecium*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* (Nocek et al., 2002) or *S. cerevisiae* (Biomate yeast plus) and 2 strains of *Enterococcus* spp.

Table 4. Effects of type of administration of direct-fed microbials on DMI, fecal output, and apparent total-tract digestibility of nutrients in lactating dairy cows[1]

						P-value[2]			
Item	Control	TD	RuI	AbI	SEM	TRT	C_1	C_2	C_3
DMI, kg	27.28	25.56	27.42	26.92	0.76	0.29	0.46	0.09	0.64
Fecal output,[3] kg	8.35	7.89	9.54	8.78	1.01	0.70	0.75	0.32	0.60
Total-tract digestibility									
DM, %	66.81	70.62	66.96	66.01	3.26	0.76	0.78	0.32	0.84
OM, %	68.98	72.05	68.51	67.94	2.82	0.74	0.87	0.29	0.89
CP, %	67.89	71.03	67.26	66.03	3.33	0.75	0.95	0.30	0.80
NDF, %	54.73	62.74	56.38	55.87	5.29	0.71	0.56	0.32	0.95
ADF, %	54.69	62.16	56.17	55.90	4.91	0.71	0.56	0.32	0.97

[1]Data presented are LSM; treatment, n = 4.

[2]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

[3]Fecal output (kg): [Cr_2O_3 (dosed g/d)/Cr_2O_3 concentration in feces (g/g of DM)]/1,000.

(Nocek et al., 2003) was fed to postpartum dairy cows. In addition, a field study conducted by Oetzel et al. (2007) showed no difference in DMI when feeding a DFM compared with a placebo. Raeth-Knight et al. (2007) reported no difference in DMI between 2 DFM dosing amounts of *L. acidophilus* and *P. freudenreichii* compared with control when fed to mid-lactation Holsteins. The authors suggested that no difference in DMI may be due to cows receiving the same TMR between lactation phases and consuming comparable amounts of DM. In contrast, others have reported a decrease in DMI when DFM was fed pre- and postpartum (Francisco et al., 2002; Nocek et al., 2003), or an increase in DMI (Nocek and Kautz, 2006). Variation in DMI in response to DFM is possibly due to different diets, different DFM, or the dosing amount of DFM, among other factors.

Milk Production and Composition

Route of DFM administration had no effect ($P > 0.10$) on milk production or components (Table 5). Similar results were reported by Oetzel et al. (2007) when administrating 2 strains of *Enterococcus faecium* (5×10^9 cfu) and a yeast, *S. cerevisiae* (5×10^9 cfu), compared with control. Raeth-Knight et al. (2007) detected no differences in milk production and milk components when the previously described treatments were fed to mid-lactation dairy cattle. Nocek et al. (2003) showed similar results with no difference in milk production between treatments when cows were fed the *S. cerevisiae* (Biomate yeast plus) and 2 strains of *Enterococcus* spp. (DFM fed at 90 g/cow per day); however, milk protein was greater for DFM-treated cows from wk 2 through 10 ($P < 0.05$). Nocek and Kautz (2006) reported different results, with an increase in milk yield over the control group when 2 g of DFM/cow per day (Probios TC), *S. cerevisiae* (1 g; 5×10^9 cfu), and 2 strains of *Enterococcus* spp. (5×10^9 cfu) were fed. They also reported that during the first 14 DIM, the fat percentage was lower for DFM-treated cows over the control. The DFM-administered cows had no differences in milk protein, 3.5% FCM, MUN, or SCC. Stein et al. (2006) reported an increase in 4% FCM milk production and a greater amount of milk fat in low-dose and control cows versus the high-dose multiparous cows. It is possible that some bacterial strains of DFM have the greatest affect when fed through the transition phase to peak lactation, as seen by the results by Stein et al. (2006), whereas others do not, such as the strains fed by Oetzel et al. (2007) and Nocek et al. (2003). The results reported by Raeth-Knight et al. (2007) suggest that propionic bacteria were not affected when fed during mid to late lactation, which is similar to the present study. A possible theory is that cows are not in a high negative energy balance during mid to late lactation and some ME is going to body reserves rather than body reserves being used to help drive milk production. Raeth-Knight et al. (2007) suggested that a greater concentrate diet may be needed to provide more ruminal lactic acid concentration, which could be used by lactic acid–using bacteria to produce propionate. This does not follow the present study because the forage:concentrate ratio for Nocek et al. (2003) was 40:60 and in the present study forage:concentrate was 36:64. Nocek et al. (2003) hypothesized that if too much DFM is fed, it can cause the level of ruminal acid to increase too high for the ruminant's ability to use the available acid.

The abomasal infusion of DFM increased ($P < 0.01$) C8:0 milk fatty acid concentration at 1.75 μg/100 g versus 0.89 μg/100 g (control), 0.99 μg/100 g (TD), and 0.77 μg/100 g (RuI) and percentage of total composition: 0.26% versus 0.13% (control), 0.11% (TD), and 0.09% (RuI). By comparison, AbI was statistically greater in

Table 5. Effects of type of administration of direct-fed microbials on milk production and components in lactating dairy cows[1]

						P-value[2]			
Item	Control	TD	RuI	AbI	SEM	TRT	C_1	C_2	C_3
Milk production, kg	29.37	29.19	27.91	29.58	1.53	0.87	0.79	0.81	0.44
Butterfat, %	3.54	3.70	3.31	3.73	0.21	0.36	0.86	0.48	0.17
Protein, %	3.32	3.19	3.20	3.31	0.13	0.83	0.56	0.67	0.57
SCC[3]	248.00	330.00	994.00	254.00	416.09	0.54	0.57	0.57	0.23
Lactose, %	4.61	4.64	4.64	4.73	0.13	0.91	0.66	0.80	0.62
SNF,[3] %	8.79	8.71	8.71	8.93	0.22	0.88	0.99	0.69	0.49
MUN, %	13.85	13.49	14.77	12.99	0.82	0.49	0.92	0.71	0.14

[1]Data presented are LSM; treatment, n = 4.

[2]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

[3]SCC = number times 100,000; SNF = percent solids-not-fat.

C8:0 milk fatty acid concentration ($P = 0.003$) versus RuI. As a percentage of total composition, only control versus TD, RuI, and AbI were different ($P = 0.01$) for C8:0. There tended to be a difference in the μg/100 g concentration of 17:0 (RuI vs. AbI, $P = 0.10$), 20:1 (RuI vs. AbI, $P = 0.08$), 20:2 (RuI vs. AbI, $P = 0.08$), and 20:3n-3 (TD differing from RuI and AbI, $P = 0.07$; and TD vs. RuI and AbI, $P = 0.02$) milk fatty acids (Table 6). There was no effect of DFM on total milk fatty acid classes (SFA, UFA, MUFA, PUFA, n-6 PUFA, n-3 PUFA, and conjugated linoleic acid) by concentration or percentage of composition (Table 7). Slight increases in the fatty acid profile of the milk could possibly be the effect of dosing the bacteria into the abomasum. However, no other studies could be found on the effect of bacterial DFM on milk fatty acid concentration or percentage of composition in lactating dairy cattle when the DFM was administered by abomasal infusion. As stated previously, the study started with cows at or reaching peak milk, suggesting that throughout the study the cows did not have a negative energy balance, and therefore, some ME is going to body reserves rather.

Digestibility

No significant differences were detected between the 4 treatments on apparent total-tract digestibility of DM, CP, NDF, or ADF (Table 4). Values for apparent total-tract digestibility for DM, CP, and NDF were within the range reported by previous research (Nennich et al., 2003). The effects of *L. acidophilus* and *P. freudenreichii* on nutrient digestibility has been previously determined by Raeth-Knight et al. (2007), where apparent total-tract digestibility of DM, NDF, CP, and starch did not differ, similar to the present results. Ruminal digestibility of DM from forage was increased in cows fed *Enterococcus faecium* with yeast for 21 d prepartum through 70 d postpartum (Nocek and Kautz, 2006). Raeth-Knight et al. (2007) explained that similar results could be observed when feeding *L. acidophilus* or *E. faecium* due to both being homofermentative lactic acid bacteria. However, in the Nocek and Kautz (2006) study, the combination with yeast did not allow the current study to be directly compared. The authors concluded that feeding *L. acidophilus* and *P. freudenreichii* without yeast does not affect DMI or total-tract apparent nutrient digestibility.

Ruminal Fluid Analysis

Ruminal fluid dilution rate, ruminal fluid volume, ruminal turnover time, and ruminal fluid flow rate of ruminal digesta (Table 8) did not differ between the control, TD, RuI, and AbI treatments. No previous research could be found which had evaluated the effect of bacterial DFM on rumen digesta kinetics in lactating dairy cattle. As stated earlier, no statistical difference was observed in DMI. It could be expected that cattle with similar DMI fed the same TMR may have similar rumen digesta kinetics. Lehloenya et al. (2008a) reported no difference in ruminal kinetics when *Propionibacterium* strain 169 was fed to Angus × Hereford steers, with similar DMI across treatments.

Ruminal pH did not differ between treatments, and there was no treatment × time interaction. Ruminal pH did not differ among routes of DFM delivery as evaluated in this study (Table 9). Raeth-Knight et al. (2007) showed similar results between all treatments when feeding *L. acidophilus* strain LA747 and *P. freudenreichii* strain PF24 at 2 different levels (1×10^9 cfu/d and 2×10^9 cfu/d, respectively; 1×10^9 cfu/d and 2×10^8 cfu/d respectively; or lactose control), with a high pH averaging 6.42 and a low averaging 5.98 across all treatments. Stein et al. (2006) showed different results with a decrease in ruminal pH when *Propionibacterium* strain 169 was fed at a high dose of 6×10^{11} cfu/d, when compared with low dose of 6×10^{10} cfu/d and control. Therefore, dose of *Propionibacterium*

Table 6. Effects of type of administration of direct-fed microbials on milk fatty acid contents (µg/100 g fatty acids concentration) in lactating dairy cows[1]

						P-value[2]			
Item	**Control**	**TD**	**RuI**	**AbI**	**SEM**	**TRT**	**C_1**	**C_2**	**C_3**
8:0	0.89[a]	0.99[a]	0.77[a]	1.75[b]	0.20	0.01	0.24	0.27	<0.01
10:0	10.31	9.82	8.08	11.19	1.24	0.37	0.68	0.90	0.10
12:0	17.63	18.03	15.67	18.21	1.70	0.70	0.87	0.61	0.30
12:1	0.55	0.58	0.49	0.57	0.04	0.47	0.89	0.38	0.20
13:0	0.61	0.61	0.62	0.66	0.07	0.47	0.80	0.69	0.68
14:0	62.16	64.09	56.64	64.23	4.90	0.67	0.93	0.55	0.29
14:1	3.78	3.95	3.39	3.70	0.30	0.61	0.78	0.28	0.47
15:0	5.98	5.92	5.69	6.36	0.44	0.76	0.98	0.84	0.30
16:0	180.43	185.71	164.24	188.33	10.19	0.76	0.93	0.46	0.11
16:1	6.41	6.63	5.79	6.76	0.47	0.48	0.97	0.54	0.16
17:0	4.03	3.97	3.59	4.22	0.25	0.39	0.73	0.84	0.10
17:1	1.00	1.00	0.94	1.09	0.07	0.58	0.92	0.91	0.18
18:0	119.84	119.53	100.78	121.06	8.42	0.30	0.54	0.42	0.11
18:1 *cis*-11	6.91	6.77	6.65	7.16	0.41	0.84	0.92	0.79	0.39
18:1 *trans*-11	12.27	12.39	11.74	12.15	0.95	0.97	0.87	0.71	0.76
18:1 *cis*-9	185.22	180.55	162.78	187.08	11.40	0.44	0.53	0.69	0.15
18:1 *trans*-9	6.54	6.49	5.81	6.65	0.36	0.37	0.58	0.56	0.12
18:2 *trans*-10,*cis*-12	0.14	0.14	0.12	0.18	0.03	0.59	0.85	0.84	0.19
18:2 *cis*-9,*trans*-11	4.69	4.69	4.26	4.70	0.37	0.80	0.75	0.65	0.41
18:2n-6	33.96	34.84	31.11	35.30	2.27	0.57	0.94	0.56	0.21
18:2 *trans*	6.82	6.57	6.23	6.88	0.36	0.59	0.55	0.98	0.22
18:3n-3	3.79	3.90	3.52	4.14	0.31	0.57	0.87	0.86	0.18
20:0	1.66	1.68	1.45	1.75	0.14	0.50	0.82	0.65	0.16
20:1	0.38	0.43	0.35	0.44	0.04	0.26	0.53	0.49	0.08
20:2	0.39	0.42	0.34	0.44	0.04	0.29	0.76	0.49	0.08
20:3n-3	0.37[yz]	0.41[y]	0.28[z]	0.28[z]	0.04	0.07	0.27	0.02	0.96
20:3n-6	1.99	1.97	1.78	2.08	0.28	0.89	0.89	0.90	0.46
20:4n-6	2.35	2.33	2.13	2.35	0.19	0.80	0.70	0.71	0.42
20:5n-3	0.52	0.55	0.61	0.52	0.08	0.85	0.70	0.92	0.44
22:0	0.81	0.82	0.70	0.83	0.08	0.62	0.77	0.56	0.25
22:1	0.24	0.40	0.26	0.32	0.07	0.41	0.31	0.22	0.57
22:6n-3	0.57	0.61	0.59	0.66	0.06	0.73	0.51	0.84	0.38
23:0	0.19	0.20	0.28	0.21	0.04	0.26	0.39	0.27	0.15
24:0	0.25	0.26	0.28	0.27	0.03	0.83	0.48	0.56	0.90

[a,b]Different superscripts within row indicate significance, $P < 0.05$.

[y,z]Different superscripts within row indicate a trend, $P < 0.10$.

[1]Data presented are LSM; treatment, n = 4.

[2]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

may affect ruminal pH. Nocek et al. (2002) reported even lower ruminal pH values when a combination of bacterial and yeast DFM containing *Enterococcus faecium* at 1×10^5 cfu/mL of rumen fluid, *Lactobacillus plantarum* at 1×10^6 cfu/mL of rumen fluid, and *S. cerevisiae* at 1×10^7 cfu/mL of rumen fluid were supplemented via a ruminal cannula. The ruminal pH was below 5.5 for 13.1 h for the cows dosed at 10^5 or 10^7 cfu/mL of ruminal fluid versus 16.1 h for the cows dosed at 10^6 cfu/mL of ruminal fluid. Cattle receiving the 10^5 treatment had a higher daily average pH than those receiving the 10^6 or 10^7 treatment (pH 5.8 vs. 5.6 and 5.5, respectively).

Route of administration of *L. acidophilus* and *P. freudenreichii* had no effect on ruminal concentrations of acetate, propionate, or ratios of acetate and propionate (Table 9). Though not significant, the molar proportions of the 3 primary VFA were greater with 4.36% more acetate, 7.75% more propionate, and 7.59% more butyrate when the DFM was administer by a route that bypassed the rumen (AbI) compared with treatment routes that entered the rumen (TD and RuI). Stein et al. (2006) reported an

Table 7. Effects of type of administration of direct-fed microbials on total milk fatty acid classes (µg/100 g fatty acids concentration and percentage of total composition of fatty acids) in lactating dairy cows[1]

						P-value[3]			
Item[2]	Control	TD	RuI	AbI	SEM	TRT	C_1	C_2	C_3
SFA, µg/100 g	404.80	411.61	358.81	419.08	23.09	0.28	0.76	0.43	0.08
SFA, Total %	58.99	59.75	59.28	59.35	0.92	0.95	0.95	0.67	0.70
UFA, µg/100 g	278.91	275.60	249.16	283.45	15.65	0.43	0.88	0.62	0.20
UFA, Total %	40.82	40.27	41.20	40.11	1.00	0.58	0.58	0.88	0.62
MUFA, µg/100 g	223.31	219.18	198.20	225.92	12.72	0.43	0.55	0.66	0.14
MUFA, total %	32.74	32.05	32.85	31.91	0.94	0.85	0.85	0.67	0.78
PUFA, µg/100 g	55.60	56.43	50.96	57.53	3.51	0.58	0.88	0.62	0.20
PUFA, Total %	8.08	8.22	8.35	8.19	0.25	0.90	0.90	0.56	0.88
n-6 PUFA, µg/100 g	38.30	39.14	35.02	39.73	2.63	0.60	0.91	0.59	0.22
n-6 PUFA, Total %	5.56	5.71	5.76	5.67	0.21	0.93	0.93	0.56	0.99
n-3 PUFA, µg/100 g	5.26	5.46	4.99	5.59	0.38	0.70	0.84	0.72	0.28
n-3 PUFA, Total %	0.75	0.79	0.79	0.80	0.04	0.77	0.77	0.31	0.99
CLA, µg/100 g	4.83	4.83	4.38	4.88	0.37	0.75	0.77	0.67	0.35
CLA, total %	0.71	0.70	0.71	0.69	0.04	0.95	0.95	0.78	0.93

[1]Data presented are LSM; treatment, n = 4.

[2]Total SFA: all fatty acids without any double bonds (8:0 to 24:0); total UFA: all fatty acids with double bond(s) (12:1 to 22:6n-3); total MUFA: all fatty acids with a single double bond (12:1 to 22:1); total PUFA: all fatty acids with 2 or more double bonds (18:2 *trans*-10,*cis*-12 to 22:6n-3); total n-6 PUFA: 18:2n-6, 20:3n-6, and 20:4n-6; total n-3 PUFA: 18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3; total conjugated linoleic acid (CLA): 18:2 *trans*-10,*cis*-12 and 18:2 *cis*-9,*trans*-11.

[3]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

increase in the molar percentage of ruminal propionate, with cows fed the high dose of *Propionibacterium* strain 169 averaging 18.5% greater than the low dose and 17.0% greater than control. The greater propionate percentages affected the acetate/propionate ratio resulting in the high dose having a ratio that was 15.4% lower than that of the low dose and 13.3% lower than that of the control. Route of administration of DFM had an effect on millimolar concentration of valerate, with TD having a higher concentration than RuI and AbI but not different from

Table 8. Effects of type of administration of direct-fed microbials on ruminal digesta kinetics in lactating dairy cows[1]

						P-value[3]			
Item[2]	Control	TD	RuI	AbI	SEM	TRT	C_1	C_2	C_3
FDR, %/h	0.14	0.13	0.14	0.14	0.01	0.90	0.81	0.55	0.73
RFV, L	77.82	74.97	79.86	85.47	16.68	0.97	0.91	0.71	0.81
TT, h	7.41	7.53	7.17	7.39	0.38	0.93	0.91	0.61	0.68
FFR, L/h	10.55	9.73	11.01	11.35	2.11	0.95	0.95	0.58	0.91

[1]Data presented are LSM; treatment, n = 4.

[2]FDR = ruminal fluid dilution rate: calculated from the slope of sample time by cobalt EDTA concentration at time of sampling; RFV = ruminal fluid volume: calculated by dose of cobalt EDTA (mg) divided by the antilog of cobalt EDTA (mg/L) concentration at sampling time 0; TT = ruminal turnover time: calculated as 1/FDR; FFR = ruminal fluid flow rate: outflow from the rumen in liters per hour = RFV × FDR.

[3]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

Table 9. Effects of type of administration of direct-fed microbials on ruminal pH, millimolar and molar proportions of VFA, and millimoles per liter of ruminal NH_3 in lactating dairy cows[1]

						P-value[2]			
Item	Control	TD	RuI	AbI	SEM	TRT	C_1	C_2	C_3
pH	6.07	6.09	6.13	6.13	0.05	0.77	0.37	0.56	0.92
Acetate, mmol/L	43.88	41.88	43.17	40.66	2.06	0.70	0.41	0.99	0.39
Acetate, %	50.89	49.48	50.55	50.22	0.71	0.54	0.33	0.30	0.74
Propionate, mmol/L	17.89	18.35	18.42	16.96	0.91	0.66	0.99	0.56	0.26
Propionate, %	20.97	21.74	21.84	21.15	0.41	0.37	0.21	0.63	0.25
Isobutyrate, mmol/L	3.49	3.44	3.45	3.42	0.04	0.57	0.21	0.92	0.51
Isobutyrate, %	4.37	4.48	4.33	4.68	0.17	0.45	0.53	0.90	0.14
Butyrate, mmol/L	11.85^{y}	11.63^{yz}	11.40^{yz}	10.61^{z}	0.37	0.10	0.14	0.17	0.13
Butyrate, %	14.15^{yz}	14.36^{y}	13.84^{yz}	13.64^{z}	0.22	0.09	0.42	0.02	0.52
Isovalerate, mmol/L	3.58^{y}	3.42^{z}	3.44^{yz}	3.49^{yz}	0.05	0.10	0.02	0.43	0.50
Isovalerate, %	4.45	4.46	4.31	4.79	0.17	0.24	0.73	0.67	0.05
Valerate, mmol/L	4.13^{ab}	4.27^{a}	4.09^{b}	4.04^{b}	0.06	0.03	0.97	0.00	0.52
Valerate, %	5.16	5.48	5.13	5.51	0.19	0.34	0.32	0.50	0.16
Acetate:propionate	2.45	2.35	2.35	2.43	0.06	0.62	0.35	0.62	0.42
NH_3, mmol/L	4.81^{a}	4.29^{ab}	3.51^{b}	3.35^{b}	0.41	0.04	0.02	0.09	0.79

^{a,b}Different superscripts within row indicate significance, $P < 0.05$.

^{y,z}Different superscripts within row indicate a trend, $P < 0.10$.

[1]Data presented are LSM; treatment, n = 4.

[2]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

control (Table 9). A trend was also detected for butyrate in which butyrate concentrations for AbI cows (13.64%) tended to be less than those for control cows (14.15%); there were no differences between AbI and TD (14.36%) or RuI (13.84%). In addition, a trend ($P = 0.09$) was observed for molar proportion of butyrate (Table 9), with TD (11.63 mmol/L) differing from AbI (10.61 mmol/L) but not differing from the control (11.85 mmol/L) and RuI (11.40 mmol/L) treatments. Isovalerate concentrations for control (3.58 mmol/L) and TD (3.42 mmol/L) cows tended to be different from each other ($P = 0.10$) but were not different from RuI (3.44 mmol/L) or AbI (3.49 mmol/L) cows. No treatment × time interactions were detected for VFA. Stein et al. (2006) also reported a treatment effect on the molar proportion of butyrate, with cows fed the low dose having a greater proportion (13.9%) than control cows (12.7%) and cows fed the high dose (12.3%). In the present experiment, a trend was detected for control cows to have a higher millimolar concentration of butyrate than TD, RuI, and AbI cows, suggesting a potential shift in fermentation pathways when DFM are fed or infused. In contrast, Raeth-Knight et al. (2007) observed no differences in total VFA concentration among previously described DFM treatments.

No treatment × time interactions ($P > 0.10$) were observed for ammonia. The method of DFM administration affected millimoles per liter of ruminal NH_3 ($P = 0.04$, Table 9), with control cows having a greater concentration of ammonia (4.81 mmol/L) than cows on the TD (4.29 mmol/L), RuI (3.51 mmol/L), and AbI (3.31 mmol/L) treatments. The orthogonal contrast indicated that control cows had greater ($P = 0.02$) ruminal NH_3 compared with cows on the TD, RuI, and AbI treatments. Raeth-Knight et al. (2007) also reported no treatment effect on ruminal ammonia. Few studies could be found that had evaluated the effects of supplementing bacterial DFM to lactating dairy cows on ruminal VFA and ammonia concentrations. In the present experiment, it is unclear why control cows had greater ruminal ammonia concentrations than TD, RuI, and AbI cows or why TD cows tended to have greater ruminal ammonia than RuI and AbI cows. However, it may suggest that feeding a DFM decreases ruminal protein degradation.

Blood Metabolites

Differing applications of DFM had no effect ($P > 0.10$) on pre- or postprandial blood metabolites: glucose, total protein, BUN, BHB, NEFA, IGF-1, or lactate. Therefore, results are presented as the means of pre- and postprandial blood metabolites (Table 10). With collection of blood by coccygeal venipuncture, it is possible to collect a mixture of blood from the vein and artery, potentially skewing the results. A trend was observed ($P = 0.06$) for

Table 10. Effects of type of administration of direct-fed microbials on blood metabolites taken by coccygeal venipuncture in lactating dairy cattle[1]

						P-value[2]			
Item	Control	TD	RuI	AbI	SEM	TRT	C_1	C_2	C_3
Glucose, mg/dL	48.76	48.29	50.55	47.52	1.66	0.62	0.99	0.72	0.20
Total protein, mg/dL	5.04	5.19	4.94	5.09	0.17	0.77	0.86	0.41	0.53
BUN, mg/dL	14.63	16.74	15.51	17.03	1.53	0.66	0.31	0.80	0.49
BHB, m*M*	0.57	0.62	0.60	0.66	0.05	0.61	0.30	0.91	0.40
NEFA,[3] mEq/L	175.99	202.09	150.25	174.69	35.36	0.78	0.99	0.37	0.63
Insulin, μIU/mL	3.15	3.05	4.72	3.19	0.55	0.12	0.43	0.19	0.06
IGF-1, ηg/mL	121.90	148.35	131.00	186.08	36.37	0.61	0.43	0.82	0.29
Lactate, mmol/L	2.17	1.76	1.90	1.67	0.23	0.44	0.14	0.95	0.48

[1]Data presented are LSM; treatment, n = 4.

[2]Treatments (TRT) were analyzed by LSM. Compared by orthogonal contrast: C_1 = control vs. top dress (TD), ruminal infusion (RuI), and abomasal infusion (AbI); C_2 = TD vs. RuI and AbI; C_3 = RuI vs. AbI.

[3]NEFA = nonesterified fatty acids.

insulin when comparing RuI (4.72 μIU/mL) versus AbI (3.19 μIU/mL). Direct-fed microbial treatments had no treatment × time interaction ($P > 0.10$) for blood metabolites. Similar results were reported by Francisco et al. (2002) when cows were fed 17 g/d of a *Propionibacteria* culture (Agtech Products Inc.) with no effect on plasma glucose (60.0 mg/dL) or IGF-1 (111.5 ng/mL). Francisco et al. (2002) reported that after wk 1, NEFA concentration decreased faster in DFM cattle than control cattle, suggesting that the cattle were not using as much body reserves as the control cattle. However, the current study started with cows at or approaching peak milk yield with an average of 73.25 DIM and therefore cannot be fairly compared with Francisco et al. (2002), who evaluated during the transitional phase. Lehloenya et al. (2008b), Nocek et al. (2003), and Oetzel et al. (2007) also reported no DFM treatment effect on glucose, insulin, BHB, or NEFA. In the present experiment, there was a tendency for RuI cows to have a greater insulin concentration (4.72 μIU/mL) than AbI cows (3.19 μIU/mL). This is similar to results reported by Lehloenya et al. (2008b), where plasma insulin was greater for steers supplemented with bacterial DFM and yeast culture than for steers supplemented with control or yeast culture alone. Nocek et al. (2003) suggested that when evaluating blood parameters in transitioning dairy cattle, the best situation is to have a treatment increase blood glucose and insulin and decrease BHB and NEFA concentrations. By doing so the needs of the cows with high energy demand during early lactation would be better met by the diet, suggesting the reduction of body reserve mobilization with more complete oxidation of fatty acids. In the present study no differences in DMI, nutrient digestibility, and VFA among treatments may explain the lack of response on blood metabolites. Lack of response on blood metabolites may also be due to the cows starting the trial in a later stage of lactation that progressed well into the tail end of lactation while consuming a consistent TMR.

APPLICATIONS

The administration of the combination of *L. acidophilus* and *P. freudenreichii* to lactating dairy cows had no effects on DMI, milk production, or milk components. Apparent total-tract digestibility of DM, OM, CP, NDF, and ADF were similar across treatments. No differences were observed for rumen kinetics; rumen pH; rumen fermentation of acetate, propionate, butyrate, and acetate/propionate ratio; or NH_3. Last, the majority of milk fatty acids and blood metabolites glucose, total protein, BUN, BHB, NEFA, insulin, IGF-1, and lactate were similar across treatments. In conclusion, under the conditions of this study, the route of administration of *L. acidophilus* and *P. freudenreichii* had no effect on performance in lactating dairy cows, diet digestibility, rumen fermentation, milk fatty acids composition, or blood metabolites. We conclude based on previous literature that DFM might have their greatest effects during the transition period and early lactation. However, experiments are needed to determine whether DFM could be fed with decreasing forage:concentrate in late lactation to sustain milk production.

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LITERATURE CITED

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