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Effect of Direct-Fed Microbials on Performance, Diet Digestibility, and Rumen Characteristics of Holstein Dairy Cows[1]

M. L. Raeth-Knight,*[2] J. G. Linn,* and H. G. Jung*†
*Department of Animal Science, University of Minnesota, St. Paul 55108
†USDA-ARS, St. Paul, MN 55108

ABSTRACT

The objective of this study was to determine the effect of feeding direct-fed microbial (DFM) products containing *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* on the performance, nutrient digestibility, and rumen fermentation of Holstein dairy cows in midlactation. Experiments were conducted from February to May 2003. Cows were fed 1 of 3 dietary treatments: 1) 1×10^9 colony-forming units (cfu)/d of live *L. acidophilus* strain LA747 and 2×10^9 cfu/d of live *P. freudenreichii* strain PF24 (DFM1); 2) 1×10^9 cfu/d of live *L. acidophilus* strain LA747, 2×10^9 cfu/d of live *P. freudenreichii* strain PF24, and 5×10^8 cfu/d of *L. acidophilus* strain LA45 (DFM2); or 3) lactose (control). Treatments were administered by mixing 45 g of finely ground corn with 5 g of DFM products or lactose and top dressing on the total mixed rations once daily. All cows received the same total mixed ration: 12.7% alfalfa hay, 46.2% corn silage, and 41.1% concentrate on a dry matter (DM) basis. In study 1 (lactation study), 39 multiparous and 18 primiparous Holstein cows were blocked by parity and randomly assigned to treatments for 84 d. Starting on d 35, fecal grab samples were collected from each cow at 5- to 8-h intervals over 48 h for digestibility measurements. A rumen fermentation study (study 2) was conducted concurrently with the lactation study. Three rumen-fistulated, multiparous Holstein cows were randomly assigned to dietary treatments DFM1, DFM2, and control in a 3×3 Latin square design with 28-d periods. In study 1, there was no difference in average DM intake (23.9, 23.6, and 24.2 kg/d) or 4% fat-corrected milk (36.8, 35.3, and 36.2 kg/d) for treatments DFM1, DFM2, and control. Percentage or yield of milk components also did not differ among treatments. Feed efficiency averaged 1.52 kg of 4% fat-corrected milk/kg of DM intake and did not differ among treatments. There were no differences in apparent DM, crude protein, neutral detergent fiber, or starch digestibility among treatments. In study 2, there was no difference in rumen pH and concentrations of ammonia or total volatile fatty acids measured at 0, 1, 3, and 6 h after feeding. Under the conditions of these studies, supplementing midlactation cows with DFM products containing *L. acidophilus* and *P. freudenreichii* did not affect cow performance, diet digestibility, or rumen fermentation.

Key words: direct-fed microbial, dairy cow, midlactation

INTRODUCTION

Direct-fed microbials (**DFM**) are defined as a source of live, naturally occurring microorganisms (Krehbiel et al., 2003). They are utilized in dairy production to improve animal performance, feed efficiency, and health (Yoon and Stern, 1995). Due to rising public concern over the use of antibiotics in animal production, there continues to be interest in the feeding of "natural" feed additives such as DFM, and, according to Nocek and Kautz (2006), the inclusion of a DFM in dairy cow diets has become a generally accepted practice.

A definitive mode of action for bacterial or fungal DFM has not been established, although a variety of mechanisms have been suggested. These include the modification of rumen or lower gut microbial populations, alteration of rumen fermentation patterns, increased intestinal nutrient flow, improved diet digestibility, and immune system modulation (Yoon and Stern, 1995; Krehbiel et al., 2003).

The effect of DFM supplementation on cow performance or rumen fermentation has been reviewed by several authors (Martin and Nisbet, 1992; Jouany, 1994; Newbold, 1995; Yoon and Stern, 1995; Krehbiel et al., 2003). Although DFM supplementation has improved milk production, component yield, feed effi-

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[2]Corresponding author: raeth003@umn.edu

ciency, and health, animal response to DFM have been inconsistent. In addition, results of DFM studies conducted with dairy cattle are difficult to compare because of the many different organisms, strains of organisms, and combinations of multiple organisms that have been supplemented. Other differences among studies include the DFM inclusion level in the diet, diet composition, feed intake, and feeding frequency, along with animal factors such as age, physiological stage, health, and stress status (Wagner et al., 1990).

Fungal DFM products have been studied to a greater extent in lactating dairy cows than have bacterial DFM products, and the effect of bacterial DFM products on cow performance has not been definitively demonstrated. *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* are the primary bacterial organisms fed to ruminants. Feeding these organisms together may be advantageous, because *L. acidophilus* is a lactate-producing bacteria and *P. freudenreichii* is a lactate-utilizing bacteria that produces propionate, a glucose precursor, as a product of fermentation. The objective of our study was to determine the effect of feeding a bacterial DFM containing *L. acidophilus* and *P. freudenreichii* on the performance, nutrient digestibility, and rumen fermentation of Holstein dairy cows in midlactation.

Table 1. Ingredient composition of diet

Ingredient	Amount
	% of DM
Corn silage	47.9
Protein mix	35.9
Alfalfa hay	12.9
Corn grain	3.3
	% of protein mix
Corn	28.3
Soybean meal, 46%	27.6
SoyPlus[1] (60% bypass protein)	8.40
Whole cottonseed	12.5
Distillers dried grains	8.4
Sodium bicarbonate	2.1
Ca	1.6
Choice white grease	2.1
Megalac[2] (bypass fat)	2.5
Bloodmeal	2.9
Mixing salt	1.1
Dicalcium phosphate	0.6
Urea, 278%	0.6
Vitamin-trace mineral premix	0.5
Dynamate[3] (S, K, and Mg)	0.4
Magnesium oxide	0.4

[1]Manufactured by West Central (Ralston, IA).

[2]Manufactured by a division of Church & Dwight Co., Inc. (Princeton, NJ).

[3]Manufactured by a division of IMC-Agrico Co. (Mundelein, IL).

MATERIALS AND METHODS

Two studies, a lactation study that included digestibility measurements and a rumen fermentation study with fistulated cows, were conducted at the University of Minnesota Dairy Research Facility. The studies were conducted from February through May 2003. All cows were housed in a tie-stall barn and cared for according to the University of Minnesota Institutional Animal Care and Usage Committee recommendations (animal subjects code 0212A38841).

Lactation Study

Cows and Diets. Thirty-nine multiparous and 18 primiparous lactating Holstein cows were blocked by parity and randomly assigned to 1 of 3 dietary treatments: 1) **DFM1**: 1×10^9 cfu/d of live *L. acidophilus* strain LA747 and 2×10^9 cfu/d of live *P. freudenreichii* strain PF24; 2) **DFM2**: 1×10^9 cfu/d of live *L. acidophilus* strain LA747, 2×10^9 cfu/d of live *P. freudenreichii* strain PF24, and 5×10^8 cfu/d of LA strain LA45; and 3) **Control**: Lactose

At the initiation of the study, DIM averaged 74 ± 32 (mean ± SD), 69 ± 32, and 69 ± 32 d, and milk production was 42 ± 7, 43 ± 7, and 43 ± 8 kg/d for treatments DFM1, DFM2, and control, respectively. Dietary treatments were fed for 84 d. Treatments were prepared by mixing 45 g of finely ground corn with 5 g of live microbial product (Nutrition Physiology Corp., Indianapolis, IN) or lactose. The treatments were stored in individual, daily-sized packages at –30°C until fed. All cows received the same TMR (Table 1), which was formulated to meet or exceed nutrient recommendations for 40 kg/d of milk production (NRC, 2001). The TMR was offered twice daily at 0600 and 1400 h, allowing for approximately 5% feed refusal. Treatment mixtures were top-dressed on the 1400 h feeding of the TMR. Dry matter of the corn silage was determined weekly, and dietary amounts were adjusted if silage DM varied more than 2 percentage units. Cows were milked twice daily at 0500 and 1600 h. Feed intake and milk production were recorded daily throughout the study.

Four cows were removed from the study for reasons not attributed to treatments. Two cows were removed from treatment DFM1; 1 cow because of severe mastitis and 1 cow died. One cow each was removed from the DFM2 and the control treatments because of severe mastitis and death, respectively.

Sample Collection and Analysis. Individual milk samples were taken once weekly during the 1400 and 0500 h milking of consecutive days. Samples were analyzed for fat, protein, lactose, MUN, and SCC (Minnesota DHIA, Sauk Center). The Gaines formula (NRC,

2001) was used to calculate 4% FCM (kg/d = 0.4 × milk, kg/d + 15 × fat, kg/d). Energy-corrected milk was calculated as [ECM, kg/d = milk yield × (0.0929 × fat %) + (0.0563 × true protein %) + (0.0395 × lactose %)] ÷ 0.749 (Krause and Combs, 2003).

Diet ingredient samples were taken weekly. A sample of feed refusal was taken daily and then composited by week for analysis. Feed and feed refusal samples were dried for 24 h in a 60°C forced-air oven to determine DM content and ground to pass through a 1-mm screen (Wiley mill, Thomas Scientific, Swedesboro, NJ). Organic matter was determined by ashing the samples in a muffle furnace at 500°C (AOAC, 1995). Samples were analyzed for CP (NA2100 protein nitrogen analyzer, ThermoQuest Italia SPA., Milan, Italy; AOAC, 1995), and ether extract (AOAC, 1995). The Ankom^{200} fiber system (Ankom Technology Corp., Fairport, NY) was used for sequential analysis of NDF, ADF, and acid detergent lignin (Hintz et al., 1996). Samples were analyzed for NDF using sodium sulfite and α-amylase (A3306, Sigma Chemical Co., St. Louis, MO). Acid detergent lignin was determined by digesting the ADF residue in 72% sulfuric acid (Van Soest et al., 1991). Neutral detergent insoluble CP and acid detergent insoluble CP were determined by Kjeldahl analysis (AOAC, 1995) using the NDF or sequential ADF residue, respectively. Starch was hydrolyzed with α-amylase and amyloglucosidase (3514, Sigma Chemical Co.) as described by Bal et al. (2000), and glucose was measured using a glucose kit (510-A, Sigma Chemical Co.) in a plate reader at 450 nm. Minerals were analyzed using the Applied Research Laboratories (Sunland, CA) model 3560 AES inductively coupled plasma spectrometer system.

Live bacterial numbers of DFM1 and DFM2 were enumerated with species specificity for *L. acidophilus* and genus specificity for *P. freudenreichii*. Two DFM packages were analyzed for total counts of *L. acidophilus*, and 1 DFM package was analyzed for total counts of *Propionibacteria*. All samples were analyzed in duplicate. Samples (1 g) were transferred to tubes containing buffered peptone water and serially diluted to 10^{-6}, and 0.1-mL aliquots of this dilution were plated onto Man, Rogosa, and Sharpe agar (Becton Dickinson, Sparks, MD) and sodium lactate agar (**SLA**) plates (Vedamuthu and Reinbold, 1967). Mann-Rogosa-Sharpe plates were incubated at 35°C for 24 h, and SLA plates were incubated anaerobically in a sealed jar under an O_2-free CO_2 atmosphere at 30°C for 7 d. After incubation, colonies were counted, and the live bacterial count was calculated using the dilution factors. Colonies in Man, Rogosa, and Sharpe were confirmed with species specificity as *L. acidophilus* using analytical profile index strips (Biomerieux, Marcy l'Etoile, France) specific for *Lactobacillus*. Colonies on SLA were identified with genus specificity as *Propionibacteria* with analytical profile index strips specific for anaerobic organisms.

Digestibility Measurements

Starting on d 35 of the lactation study, fecal grab samples were collected from cows. Six fecal samples were collected per cow at 5- to 8-h intervals over a 48-h period. Samples were composited by cow. Total mixed ration and feed refusal samples were collected once daily for 5 d beginning 3 and 2 d, respectively, before the start of the fecal sampling. Analysis of TMR, feed refusals, and fecal samples included DM, NDF, CP, and starch as previously described. Samples were also analyzed for acid insoluble ash (Van Keulen and Young, 1977) to calculate DM, NDF, CP, and starch digestibilities.

Rumen Fermentation Study

Cows and Diets. A rumen fermentation study was conducted concurrently with the lactation study. Three rumen-cannulated multiparous Holstein cows were randomly assigned to dietary treatments DFM1, DFM2, and control in a 3 × 3 Latin square design with 28-d periods. Periods consisted of 21 d for adaptation, followed by a 7-d collection period. The compositions of the TMR and of DFM treatments were as previously described for the lactation study. Cows were milked and fed as in the lactation study. At the beginning of the rumen fermentation study, the 3 cows averaged 48 ± 8 DIM and 43 ± 1 kg/d of milk.

Sample Collection and Analysis. Rumen fluid was sampled on d 1, 2, 6, and 7 during the last week of each period. Samples were taken just before feeding (0 h) and 1, 3, and 6 h after feeding twice daily starting at approximately 0600 and 1400 h, for the morning and evening feedings, respectively. Following collection, rumen samples were strained through 4 layers of cheesecloth and immediately analyzed for pH. Twenty-five milliliters of rumen fluid was acidified with 1 mL of 50% sulfuric acid, vortexed, and frozen until analyzed for ammonia using the Kjeldahl procedure (AOAC, 1995). Five milliliters of 25% metaphosphoric acid was added to 25 mL of rumen fluid, vortexed, and frozen for VFA and lactic acid analysis. Volatile fatty acids and lactic acid were determined by gas chromatography (Hewlett-Packard 6890, Hewlett-Packard Co., Palo Alto, CA; Erwin et al., 1961) using a 4% Carbowax 20M/80/120 Carbopack B-DA column (Supelco, Bellefonte, PA). Samples were run at 175°C with a flow rate of 24, 40, and 450 mL/min for N, H, and air, respectively.

Statistical Analysis

Production data from the lactation study were analyzed as a repeated measures randomized complete block design using the PROC MIXED procedures of SAS (SAS Institute, 1999). Parity was the blocking factor, and cow was included as a random effect. Average milk yield 1 wk before the start of the study was utilized as a covariate, and the first-order autoregressive covariance structure was used. Nonorthogonal contrasts were used to compare DFM1 vs. DFM2 and DFM1 vs. control. The model used was

$$Y_{ijk} = \mu + C_i + B_j + T_k + W_l + (BT)_{jk} + (TW)_{kl} + \beta + e_{ijkl},$$

where Y_{ijk} = observed response; μ = overall mean; C_i = random effect of cow; B_j = effect of block (parity); T_k = effect of treatment; W_l = effect of week; $(BT)_{jk}$ = interaction of block and treatment; $(TW)_{kl}$ = interaction of treatment and week; β = effect of covariate; and e_{ijkl} = residual error.

Because PROC MIXED failed to converge for the digestibility data, these data were analyzed as a randomized block design using the PROC GLM procedures of SAS. Cow was assumed to be a fixed effect, and parity was the blocking factor. Nonorthogonal contrasts were used to compare DFM1 vs. DFM2 and DFM1 vs. control. The model used was

$$Y_{ij} = \beta + B_i + T_j + e_{ij},$$

where Y_{ij} = value of observation; μ = general mean; B_i = effect of block (parity); T_j = effect of treatment; and e_{ij} = error ($B_i \times T_j$).

Rumen pH, VFA, lactic acid, and ammonia data from the rumen fermentation study were analyzed as a Latin square. Data were analyzed using a repeated measures model and the PROC MIXED procedures of SAS. Due to unequally spaced repeated measurements, spatial power law was used as the covariance structure. Nonorthogonal contrasts were used to compare DFM1 vs. DFM2 and DFM1 vs. control. The model used was

$$Y_{ijkl} = \mu + P_i + C_j + T_k + H_l + (TH)_{kl} + e_{ijkl},$$

where Y_{ijkl} = value of observation; μ = general mean; P_i = effect of period; C_j = effect of cow; T_k = effect of treatment; H_l = effect of time; and e_{ijkl} = residual error.

Milk yield and DMI data taken during the rumen fermentation study were analyzed using the same model, except data were reduced to a period mean and, therefore, time was not included in the model. All treatment results are reported as least square means, and significance was declared at $P < 0.05$.

Table 2. Nutrient composition of diet

Item	Amount
DM, %	42.9
	— % DM —
CP	17.2
NDF	28.8
Forage NDF	23.8
ADF	15.6
Starch	24.1
Ether extract	4.70
NFC[1]	45.0
OM	94.1
Ca	0.50
Ps	0.39
TDN_{1X}[2]	76.5
NE_{L3X}[3] (Mcal/kg)	1.70

[1]NFC = [100 – (NDF – NDICP) + CP + ash + EE], where EE = ether extract and NDICP = neutral detergent insoluble CP.

[2]TDN_{1X} = total digestible nutrients at maintenance DMI as calculated by the 2001 Dairy NRC model (NRC, 2001).

[3]NE_{L3X} = net energy for lactation as calculated by the 2001 Dairy NRC model (NRC, 2001).

RESULTS AND DISCUSSION

The nutrient composition and energy value of the TMR is shown in Table 2. Using a species-specific test for *L. acidophilus* and a genus-specific test for *P. freudenreichii*, enumeration analysis confirmed the presence of viable *L. acidophilus* and *Propionibacteria* in DFM1 and DFM2 and the absence of *L. acidophilus* or *Propionibacteria* in the control treatment. The *L. acidophilus* concentrations in the DFM1 and DFM2 treatments were 6.20×10^8 and 5.8×10^8 cfu/g compared with expected values of 2.0×10^8 and 3.0×10^8 cfu/g, respectively. *Propionibacterium* counts for the DFM1 and DFM2 treatments were 5.0×10^7 and 4.0×10^7 cfu/g, respectively, compared with an expected value of 4.0×10^8 cfu/g. Counts of *L. acidophilus* were very close to expected values, whereas *Propionibacterium* counts were slightly lower. However, due to complications with the enumeration of *Propionibacterium*, this analysis was only conducted on 1 sample per treatment. The enumeration analysis results verify the microorganisms being fed were viable and at concentrations approximating target values.

Lactation Study

Supplementing dairy cows in midlactation with *L. acidophilus* and *P. freudenreichii* did not affect cow performance (Table 3). Feed intake of cows supplemented with *L. acidophilus* and *P. freudenreichii* did not differ from control cows. Milk yield and 4% FCM were not different between treatments with cows producing 41.6 kg of milk/d and 36.1 kg of 4%FCM/d across treatments. Feed efficiency was similar across treat-

Table 3. Dry matter intake, milk production, and milk composition for the DFM1, DFM2, and control treatments during the 84-d study.

	Treatment				
Item	DFM1[1]	DFM2[2]	Control[3]	SE	*P*-value
N	17	18	18		
DMI, kg/d	23.9	23.6	24.2	0.73	0.71
Milk, kg/d	42.2	41.5	41.1	1.16	0.58
4% FCM, kg/d	36.8	35.3	36.2	1.19	0.49
ECM, kg/d	36.3	35.1	36.2	1.10	0.48
FE[4]	1.54	1.52	1.51	0.05	0.74
Fat, kg/d	1.32	1.28	1.33	0.07	0.80
Protein, kg/d	1.19	1.18	1.18	0.04	0.97
Lactose, kg/d	2.03	1.99	1.99	0.06	0.79
Fat, %	3.16	3.08	3.27	0.18	0.54
Protein, %	2.78	2.82	2.86	0.06	0.34
Lactose, %	4.78	4.80	4.84	0.06	0.53
MUN, mg/mL	18.2	18.5	18.2	0.51	0.74
Log_{10} SCC/mL	5.10	5.08	5.06	0.16	0.97

[1]DFM1 = 1×10^9 cfu/d of live *Lactobacillus acidophilus* strain LA747 and 2×10^9 cfu/d of live *Propionibacterium freudenreichii 1* strain PF24.

[2]DFM2 = 1×10^9 cfu/d of live *L. acidophilus* strain LA747, 2×10^9 cfu/d of live *P. freudenreichii* strain PF24, and 5×10^8 cfu/d of *L. acidophilus* strain LA45.

[3]Control = 5 g/d of lactose.

[4]FE = feed efficiency, kg of 4% FCM/kg of DMI.

ments, averaging 1.52 kg of 4% FCM/kg of DMI. Component yields were also similar across treatments, averaging 1.31, 1.18, and 2.0 kg/d for milk fat, protein, and lactose, respectively. There was also no treatment effect on the MUN concentration or SCC.

There was no significant effect of treatment on milk fat concentration (Table 3). Across treatments, concentration of milk fat decreased 13.3% ($P < 0.05$) from wk 1 to 5 (3.49 to 3.02%). During wk 5 to 10, milk fat remained constant, averaging 3.01%, and increased ($P < 0.05$) slightly to 3.19% at wk 12. The reason for the decline in milk fat from wk 1 to 5 is unknown. Concentration of milk protein and lactose remained constant throughout the study, with no significant differences ($P > 0.10$) between treatments for milk protein and lactose, averaging 2.82 and 4.81%, respectively.

The effect of *L. acidophilus* plus *P. freudenreichii* supplementation on dairy cow performance was previously investigated by West et al. (2005). Cows were supplemented with *L. acidophilus* (1×10^9 cfu/d) plus *P. freudenreichii* (2×10^9 cfu/d) for 70 d, and increases were reported for ECM (36.9 vs. 34.5 kg/d) and feed efficiency (1.40 vs. 1.30 kg of ECM/kg of DMI) for cows receiving the DFM compared with the control cows. The difference between our study and that of West et al. (2005) was that a higher-concentrate diet was fed (ratio of 40:60 compared with our 60:40 forage:concentrate). Therefore, cows in the West et al. (2005) study may have had a greater rumen lactic acid concentration and decreased rumen pH as compared with our study. Nocek et al. (2003) suggested that supplementing cows with lactate-producing bacteria may decrease total lactic acid concentrations and increase rumen pH by providing a more constant production or conversion of feed substrates into lactic acid in the rumen than with natural native microbial populations.

Digestibility Study

Milk yield and DMI of cows fed DFM1, DFM2, or control treatments during the digestibility study (wk 5 of lactation study) were similar (Table 4). Because all cows received the same TMR across treatments and consumed similar amounts of DM, there were no differences in CP, NDF, or starch intake, averaging 4.02, 6.65, and 5.57 kg/d, respectively. Apparent total tract digestibilities of DM, NDF, CP, and starch did not differ among treatments (Table 4). Apparent total tract digestibilities of DM, NDF, CP, and starch were within the normal range (Nennich et al., 2003; Oba and Allen, 2003).

The effect of *L. acidophilus* or *P. freudenreichii* on DM or nutrient digestibility of dairy cows has not been previously investigated. Nocek and Kautz (2006) reported increased ruminal digestibility of DM from forage when cows were fed *Enterococcus faecium* in conjunction with yeast for 21 d prepartum through 70 DIM. *Enterococcus faecium* and *L. acidophilus* are both homofermentative lactic acid bacteria and therefore might be expected to affect rumen fermentation similarly. However, because *E. faecium* was fed in com-

Table 4. Milk yield, nutrient intake, and apparent digestibility for DFM1, DFM2, and control treatments during the digestibility period[1]

	Treatment				
Item	DFM1[2]	DFM2[3]	Control[4]	SE	*P*-value
N	17	18	18		
Milk, kg/d	40.3	40.6	39.9	1.67	0.77
DMI, kg/d	23.1	23.1	23.3	0.69	0.98
CP intake, kg/d	4.01	4.01	4.03	0.12	0.98
NDF intake, kg/d	6.64	6.63	6.68	0.20	0.98
Starch intake, kg/d	5.56	5.56	5.60	0.17	0.98
DM digestibility, %	66.0	65.4	65.7	0.88	0.78
CP digestibility, %	70.8	70.7	70.3	0.91	0.79
NDF digestibility, %	34.7	32.7	33.9	1.71	0.61
Starch digestibility, %	96.9	96.3	96.7	0.43	0.63

[1]Fecal samples collected at d 35 to 36 and feed samples and production data collected at d 32 to 38.

[2]DFM1 = 1×10^9 cfu/d of live *Lactobacillus acidophilus* strain LA747 and 2×10^9 cfu/d of live *Propionibacterium freudenreichii* strain PF24.

[3]DFM2 = 1×10^9 cfu/d of live *L. acidophilus* strain LA747, 2×10^9 cfu/d of live *Propionibacterium freudenreichi* strain PF24, and 5×10^8 cfu/d of *L. acidophilus* strain LA45.

[4]Control = 5 g/d of lactose.

bination with yeast, the digestibility effect cannot be attributed to 1 organism.

Rumen Fermentation Study

Production parameters, rumen pH, VFA, and ammonia concentrations for the rumen fermentation study are presented in Table 5. Significant interactions of treatment with hour of sampling were not observed. There was no treatment difference in milk yield or DMI, averaging 41.2 kg/d and 25.8 kg/d, respectively, across treatments. Rumen pH was also similar ($P > 0.10$) among treatments. Rumen pH was highest ($P < 0.05$) at 0 h (just before feeding), averaging 6.42, and lowest at 3 h postfeeding, decreasing to 5.98 across treatments.

Other research has reported a decrease in rumen pH with the feeding of a DFM. Stein et al. (2006) reported rumen pH was lower in cows fed *Propionibacteria* at 6×10^{11} cfu/d compared with cows fed *Propionibacteria* at 6×10^{10} cfu/d or control-fed cows (pH 6.65, 6.94, and 6.86, respectively). This was a greater amount of *Propionibacteria* fed per day than in our study and may indicate a greater concentration needed to decrease pH. Nocek et al. (2002) reported cows supplemented with a DFM containing *E. faecium*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* at 10^5 or 10^7 cfu/mL of rumen fluid, via a rumen cannula, had a rumen pH below 5.5 for 13.1 h as compared with 16.1 h for cows supplemented at 10^6 cfu/mL of rumen fluid. The 10^5 treatment also significantly increased the mean daily pH to 5.8, compared with 5.6 and 5.5 for cows supplemented at 10^6 and 10^7, respectively, of the DFM product.

Total VFA concentration did not differ among the DFM1, DFM2, and control treatments and averaged 84.7 m*M* (Table 5). Average VFA concentration peaked 1 h postfeeding at 92.0 m*M* and declined to 81.3 m*M* at 6 h postfeeding across treatments. Although *L. acidophilus* is a homofermentative lactic acid bacteria fermenting carbohydrates solely to lactic acid (Axelsson, 2004), in our rumen samples, the concentration of lactic acid was below the detection limit (Table 5). There was no treatment effect on rumen ammonia concentrations (Table 5), with concentrations peaking 1 h postfeeding (58.8 mg of NH_3-N/L) and then declining to prefeeding concentrations by 6 h postfeeding (52.2 mg of NH_3-N/L). The main end products of *Propionibacteria* fermentation are propionic acid and acetic acid, along with CO_2 and water (Vorobjeva, 1999). However, supplementation with *L. acidophilus* plus *P. freudenreichii* did not result in any difference ($P > 0.05$) in the molar proportions of individual VFA. There was also no difference ($P > 0.05$) in the acetate-to-propionate ratio for the DFM1, DFM2, and control treatments. The lack of differences for lactic acid and VFA concentrations among treatments suggests the DFM treatments may not have resulted in metabolically active *L. acidophilus* or *P. freudenreichii* populations in the rumen.

There is very limited dairy cow research reporting the effect of bacterial DFM supplementation on ruminal VFA, lactic acid, or ammonia concentrations. Kung and Hession (1995) reported that high (8.0×10^6 cfu/mL of culture fluid) and low (8.7×10^5 cfu/mL of culture fluid) doses of *Megasphaera elsdenii* in vitro increased total VFA concentrations through 6 h of fermentation (126.9 vs. 63.3 m*M*). At 6 h of fermentation, there was

Table 5. Average production parameters, rumen pH, VFA, and ammonia concentrations for the DFM1, DFM2, and control treatments during the rumen fermentation study[1]

Item	Treatment			SE	*P*-value
	DFM1[2]	DFM2[3]	Control[4]		
N	3	3	3		
Milk, kg/d	40.7	42.2	40.7	0.60	0.34
DMI, kg/d	25.9	26.0	25.6	1.15	0.96
pH	6.20	6.15	6.15	0.03	0.80
Ammonia, mg/L	127.4	120.6	122.1	7.41	0.81
Total VFA, m*M*	84.6	85.1	84.3	4.20	0.99
Lactic acid	ND[5]	ND	ND		
Molar proportion of VFA, mol/100 mol					
Acetate	65.5	63.9	64.8	0.51	0.27
Propionate	21.7	24.1	22.4	0.36	0.07
Butyrate	9.81	9.16	9.95	0.30	0.33
Isobutyrate	0.72	0.69	0.68	0.03	0.61
2-Methylbutyrate	0.65	0.54	0.63	0.04	0.36
Isovalerate	0.56	0.53	0.53	0.02	0.67
Valerate	1.06	1.09	1.08	0.05	0.90
Acetate:propionate	3.07	2.71	2.96	0.07	0.13

[1]Data collected during the last week of each 28-d period.

[2]DFM1 = 1×10^9 cfu/d of live *Lactobacillus acidophilus* strain LA747 and 2×10^9 cfu/d of live *Propionibacterium freudenreichii* strain PF24.

[3]DFM2 = 1×10^9 cfu/d of live *L. acidophilus* strain LA747, 2×10^9 cfu/d of live *P. freudenreichii* strain PF24, and 5×10^8 cfu/d of *L. acidophilus* strain LA45.

[4]Control = 5 g/d of lactose.

[5]ND = not detectable.

no difference in acetate concentrations. However *M. elsdenii* significantly increased concentrations of propionate, isobutyrate, butyrate, isovalerate, and valerate. Fungal DFM have been reported to increase total and individual VFA concentrations and decrease rumen ammonia concentrations; however, these responses have been inconsistent (Yoon and Stern, 1995).

CONCLUSIONS

Supplementing dairy cows in midlactation with DFM products containing *L. acidophilus* and *P. freudenreichii* for 84 d did not affect DMI or milk production and components. There was also no effect of DFM supplementation on apparent diet digestibility or rumen fermentation. Apparent digestibilities of DM, NDF, CP, and starch were similar across treatments. Finally, there were no differences in ruminal pH, concentrations of total or individual VFA, or ammonia among treatments. In conclusion, under the conditions of this study, supplementing midlactation cows with *L. acidophilus* and *P. freudenreichii* did not affect cow performance, diet digestibility, or rumen fermentation.

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