Effects of Feeding Propionibacteria to Dairy Cows on Milk Yield, Milk Components, and Reproduction¹

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ABSTRACT

Two weeks before parturition, 38 Holstein primiparous and multiparous cows were assigned to 1 of 3 treatment groups: control animals (n = 13) received regular total mixed rations (TMR), the low-dose group (n = 14) received the control TMR plus 6×10^{10} cfu/cow of *Propionibacterium* strain P169 (P169), and the high-dose group (n = 11) received the control TMR plus 6×10^{11} cfu/cow of P169 from -2 to 30 wk postpartum. Weekly milk samples were analyzed for percentage of milk fat, protein, lactose, and SNF, milk urea nitrogen, and somatic cell counts. Daily milk production expressed as 4% fat-corrected milk was affected by treatment and week $\times$ parity. High-dose and low-dose P169-treated cows exhibited 7.1 and 8.5% increases above controls in daily 4% fat-corrected milk, respectively. Treatment $\times$ parity and week significantly influenced percentage of milk fat, lactose, and protein, whereas treatment $\times$ parity and treatment $\times$ week influenced SNF. Ruminal propionate levels were influenced by treatment such that high-dose P169 cows had greater molar percentage of propionate than did low-dose P169 and control cows. Change in body weight postpartum was influenced by week $\times$ parity and treatment $\times$ parity such that high-dose and low-dose P169 multiparous cows exhibited a more rapid recovery of wk-1 body weight than did control multiparous cows. There was no treatment, parity, or interaction on days to first postpartum ovulation or on estrous behavior at 45 and 90 d postpartum. We concluded that P169 might have potential as an effective direct-fed microorganism to increase milk production in dairy cows.

Key words: propionibacteria, direct-fed microorganism, milk production, estrous detection

INTRODUCTION

After parturition, dairy cows are challenged with a sudden increase in nutrient requirements for lactation (Drackley, 1999; Lucy, 2001). Propionibacteria are natural inhabitants of the rumen and produce propionate, a major precursor for glucose production through hepatic gluconeogenesis (Sauer et al., 1989). Theoretical efficiency of propionate as a source of energy for ATP is 108% compared with glucose (McDonald et al., 2002), and thus directly feeding propionibacteria may be a natural way to increase hepatic glucose production and positively influence metabolism (Francisco et al., 2002). Postpartum dairy cows first partition metabolizable energy toward milk production and body condition gain before reproductive functions (Lucy, 2001); therefore, direct-fed microorganisms have potential to increase reproductive and metabolic efficiency during lactation (Yoon and Stern, 1995; Ghorbani et al., 2002). However, only one study has evaluated the effect of direct-fed propionibacteria (fed at a single amount) on the lactational performance of dairy cows in early lactation (Francisco et al., 2002). The present study was conducted to evaluate the effect of feeding 2 levels of the direct-fed microorganism, *Propionibacterium* P169, for 30 wk on milk production, milk components, and reproductive efficiency of dairy cows.

MATERIALS AND METHODS

Experimental Design and Sample Collection

Two weeks before parturition, 19 primiparous and 19 multiparous Holstein cows housed at the Oklahoma State University (OSU) Dairy Cattle Center were randomly assigned to 1 of 3 dietary treatment groups, based on age, expected calving date, and the previous year's lactation averages (for multiparous) or current PTA (for primiparous). The control group (n = 5 primiparous, n = 8 multiparous) received a lactation TMR (Table 1), the low-dose group (n = 8 primiparous, n = 6 multiparous) received the control TMR plus 6×10^{10} cfu/cow of *Propionibacterium* strain P169 (low-dose P169), and the high-dose group (n = 6 primiparous, n =

Received July 12, 2005.

Accepted September 6, 2005.

¹This work was approved for publication by the Director, Oklahoma Agricultural Experiment Station, and supported in part under projects H-2329 and H-2510 and a grant from Agtech Products, Inc.

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Table 1. Ingredient and nutrient composition of the control transition diet (TD) and lactation diet (LD). The TD was fed from d -14 to parturition and the LD was fed from parturition through 30 wk postpartum.

Ingredient, % (DM basis)	Transition diet	Lactation diet
Sorghum silage	28.53	16.44
Alfalfa (RFV 180)	0.00	16.94
Bermuda grass hay	13.95	6.53
Whole cottonseed	0.00	4.07
Corn gluten feed	21.87	6.60
Megalac-R ¹	0.00	0.91
Grain mix		
Ground corn	21.26	27.84
Wheat middlings	3.92	8.16
Soybean meal (SBM)	3.94	6.79
Extruded SBM	3.43	2.59
Calcium carbonate	1.28	0.94
Sodium bicarbonate	0.00	0.54
Diamond V yeast ²	0.45	0.55
Magnesium oxide	0.29	0.27
Salt, white	0.20	0.27
Zinpro 4-Plex ³	0.20	0.07
Vitamin-trace mineral pak	0.38	0.27
Calcium chloride	0.20	0.00
Nutrient		
DM, % (as fed)	60.8	55.9
CP, %	12.8	17.1
ADF, %	33.8	25.1
NDF, %	53.5	38.7
NE _L , Mcal/kg	1.43	1.67
Ca, %	0.80	0.97
P, %	0.31	0.41
Mg, %	0.39	0.36
K, %	1.08	1.44
Na, %	0.23	0.25
S, %	0.15	0.20
Zn, ppm	72.75	58.00
Fe, ppm	247.75	281.25
Cu, ppm	18.75	16.83
Mn, ppm	103.75	75.42
Mo, ppm	<1.0	1.20

¹Arm & Hammer Animal Nutrition Group, Princeton, NJ; Megalac-R contains: fat (as fatty acids), 82.5%; Ca, 8.5%; IOD (moisture), 3 to 4%.

²Diamond V-XP Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA.

³Zinpro Corp., Eden Prairie, MN; Zinpro 4-Plex contains: Zn, 2.58%; Mn, 1.43%; Cu, 0.90%; Co, 0.18%; Methionine, 8.21%; Lysine, 3.8%.

⁴Contents per kilogram: vitamin A (1,650,000 IU), vitamin D₃ (517,000 IU), vitamin E (8,800 IU), biotin (352 mg), Ca (15.4%), Mn (1.0%), Zn (8,600 ppm), Fe (6,000 ppm), Cu (1,500 ppm), iodine (250 ppm), and Se (110 ppm).

5 multiparous) received the control TMR plus 6×10^{11} cfu/cow of P169 (high-dose P169). This particular *Propionibacterium* strain (P169) was originally isolated from rumen fluid collected from fistulated dairy cows at the OSU Dairy Cattle Center (Davidson, 1998), and was manufactured by Agtech Products Inc. (Waukesha, WI) as a viable freeze-dried cell preparation containing strain P169 fermentation product and maltodextran as a carrier. The 305-d mature equivalent milk production for control, low-dose, and high-dose multiparous cows

was $10,265 \pm 716$ kg, $10,439 \pm 905$ kg, and $10,174 \pm 905$ kg, respectively, and did not differ ($P > 0.97$) among treatment groups. The PTA for control, low-dose, and high-dose primiparous cows was $+120 \pm 104$, $+236 \pm 78$, and $+187 \pm 95$ kg, respectively, and did not differ ($P > 0.67$) among treatment groups. The TMR was formulated to support daily milk production of at least 45 kg and comprised sorghum/sudan silage, alfalfa hay, Bermuda grass hay, whole cottonseed, corn gluten feed, Diamond V-XP yeast culture (Diamond V-XP Yeast Culture; Diamond V Mills Inc., Cedar Rapids, IA), and mineral concentrate (Table 1). The TMR was sampled weekly and composited monthly throughout the study for analysis by Dairy One Inc., Forage Testing Laboratory (Ithaca, NY). The lactation TMR analysis averaged $17.07 \pm 0.33\%$ CP, 1.67 ± 0.006 Mcal of NE_L/kg, $68.67 \pm 0.55\%$ total digestible nutrients, $25.06 \pm 0.87\%$ ADF, $38.73 \pm 1.0\%$ NDF, and $0.97 \pm 0.03\%$ Ca on a DM basis. The 2-wk prepartum feeding period consisted of the P169 treatments with substitution of the lactation TMR with the transition TMR (Table 1). The number of treatment days before parturition did not differ ($P > 0.28$) among control, low-dose P169, and high-dose P169 groups (13 ± 2 d, 14 ± 2 d, and 18 ± 2 d, respectively). Cows had free access to water and were housed in the same open-air free-stall barn, but divided into 3 separate free-stall and feeding areas according to treatment group. Cows were individually fed the P169 via topdress on 4.5 kg of TMR once a day (1700 h) while isolated in a free stall. Otherwise, cows were provided feed ad libitum in 2 allocations fed daily at 0900 and 1800 h.

Cows calved between August 26, 2002 and October 25, 2002, with no difference ($P > 0.36$) in date of birth among groups: average day of birth for the control, low-dose P169, and high-dose P169 groups were Julian d 270 ± 5 , 261 ± 5 , 269 ± 5 , respectively. Local climate data for the 30-wk experiment, collected 1.6 km from the OSU Dairy Cattle Center, were obtained from the Oklahoma Mesonet (Norman, OK). The highest weekly average maximum temperature ($24.9 \pm 1.6^\circ\text{C}$) occurred at wk 1 of lactation and lowest weekly average minimum temperature ($-4.3 \pm 0.7^\circ\text{C}$) occurred at wk 19 of lactation; no significant differences were observed among groups in weekly average minimum and maximum temperature during wk 1 to 25 or wk 25 to 30 (time of bST administration).

To assess the effects of feeding P169 during concomitant bST administration, bST [Posilac (sterile sometribove zinc suspension; 500 mg); Monsanto, St. Louis, MO] was administered every 2 wk to all cows from wk 25 to 30 of lactation; a total of 3 injections were given in the ischiorectal fossa (s.c.) and diet treatments continued during bST administration. Because all cows were injected with bST, measurements taken before

bST administration from each animal served as control values for bST effects.

Cows were milked twice daily at 0400 and 1600 h. The sum of daily a.m. and p.m. milk weights were averaged each week for daily milk production per cow, and then corrected for milk fat percentage to 4% FCM for statistical analysis. Milk samples were collected twice weekly during successive a.m. and p.m. milkings (the p.m. before and the a.m. of blood sample collection day) and analyzed for MUN, SCC, and percentage of fat, protein, lactose, and SNF. Milk component analysis was completed by Heart of America DHIA (Manhattan, KS). The number of days postpartum at first milk collection averaged 4.2 ± 0.3 d, and did not differ ($P > 0.37$) among groups.

Once weekly from wk 1 through 30 postpartum, between 0530 and 0730 h (after the a.m. milking), blood samples were collected via venipuncture of the coccygeal vein/artery in Vacutainer tubes (7 mL, Becton Dickinson Co., Franklin Lakes, NJ) containing EDTA. Blood was stored on ice, transported to the laboratory, and centrifuged at $1,200 \times g$ for 15 min (4°C); plasma was decanted and stored at -20°C . Days postpartum at first blood collection did not differ ($P > 0.50$) among groups and averaged 3.6 ± 0.6 d. Weekly BW were also recorded during the time of blood sample collection.

Plasma progesterone (P_4) concentrations were determined using a solid-phase ^{125}I radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) previously validated for bovine plasma (Stewart et al., 1996; Maciel et al., 2001). Sensitivity of the assay, defined as 95% of total binding, averaged 0.028 ng/mL (12 determinations). Intra- and interassay coefficients of variation averaged 6.8 and 11.7%, respectively.

The HeatWatch system (DDX Inc., Denver, CO) was used to monitor estrous behavior. Transmitters were set to be activated by a continuous pressure lasting ≥ 1 s from the weight of a mounting female. The number of days postpartum when the electronic estrous activity sensors were placed on cows averaged 17 ± 5 d. Cows were observed during the p.m. feeding period to monitor the stability of the patches and to evaluate if reattachment was needed. The HeatWatch system contained a daily supervisory check feature that reported the status of all transmitters, which was monitored daily. A computer recorded the date, time, and duration of all mounts. Cows were subjected to 2 separate synchronization periods involving 2 i.m. injections, 11 d apart, using $\text{PGF}_{2\alpha}$ (Estrumate, 2 mL; Schering-Plough, Union, NJ). Period 1 was implemented at 45 ± 1 d postpartum and period 2 was implemented at 90 ± 1 d postpartum. Depending on the response of the individual animal to the $\text{PGF}_{2\alpha}$ injection, data recorded for period 1 and period 2 were totals of either a single

observation (response to either the first or second $\text{PGF}_{2\alpha}$ injection) or an average of 2 observations (response to first and second $\text{PGF}_{2\alpha}$ injections). A response to $\text{PGF}_{2\alpha}$ in cows was defined as >1 ng/mL P_4 at time of $\text{PGF}_{2\alpha}$ administration and subsequent <1 ng/mL P_4 1 wk after $\text{PGF}_{2\alpha}$ administration; cows with a response to $\text{PGF}_{2\alpha}$ were considered cyclic (i.e., exhibiting luteal activity). The beginning of estrous behavior was defined as the first of 2 mounts within 4 h, and the end of estrus was defined as the last mount with at least 1 mount in the preceding 4 h and no mounts in the following 12 h (Floyd, 2001). Cows were subjected to the AI protocol of the OSU Dairy Cattle Center after period 2.

Rumen fluid samples were collected from all animals via intubation at d -30 , $+60$, $+120$, $+175$ (end of original study), $+204$ (end of wk-4 bST administration), and $+264$ (60 d after end of P169 feeding) postpartum. At each sampling time, approximately 100 to 150 mL of rumen fluid was collected and transferred into conical vials, placed on ice, and shipped to Agtech Products Inc. (Waukesha, WI) for VFA analyses. The animal experimentation described in this report was approved by the OSU Institutional Animal Care and Use Committee.

Statistical Analyses

Milk production (corrected to 4% fat), milk components, and BW data were analyzed as repeated-measures ANOVA using the MIXED procedure of SAS (version 8, 1999; SAS Institute, Inc., Cary, NC). Cow nested within diet treatment and parity was considered random and all other effects in the model were considered fixed. The model of the covariate structure for repeated measurements was an autoregressive with a lag equal to one (Littell et al., 1996). If main effects or their interactions were significant, mean separation was accomplished using paired student's *t*-test.

The VFA and pH data were analyzed as repeated-measures ANOVA using the MIXED procedure of SAS (SAS Institute) with d -30 used as a covariable. The model included diet treatment, parity, and day as main effects with all interactions included. If main effects or their interactions were significant, mean separation was accomplished using paired student's *t*-test.

Interval to first ovulation, mounting activity, and mounting behavior were analyzed via ANOVA using the GLM procedure of SAS. The model included diet treatment and parity as the main effects, and if significant, mean separation was accomplished using paired student's *t*-test. Response to $\text{PGF}_{2\alpha}$ and pregnancy was analyzed by the FREQ procedure of SAS using a χ^2 statement. To make a direct comparison between results of the present study to those of Francisco et al.

Figure 1. Effect of feeding propionibacteria on milk production through 25 wk of lactation. Production of 4% FCM in multiparous cows (top panel; $n = 19$) fed high-dose ($n = 5$) and low-dose ($n = 6$) *Propionibacterium* strain P169 and control ($n = 8$) rations for wk 1 to 25, and in primiparous cows (bottom panel; $n = 19$) fed high-dose ($n = 6$) and low-dose ($n = 8$) *Propionibacterium* P169 and control ($n = 5$) rations for wk 1 to 25.

(2002), data were analyzed separately for wk 1 to 12 and wk 13 to 25 in addition to wk 1 to 25.

RESULTS

Milk Production

Daily milk production, calculated as 4% FCM, was affected by treatment ($P < 0.003$), parity ($P < 0.01$), week ($P < 0.01$), and week $\times$ parity ($P < 0.05$) with no other interaction being significant ($P > 0.15$). Daily 4% FCM production, averaged across primiparous and multiparous cows, was higher in both the high-dose (32.2 ± 0.6 kg/d) and low-dose (32.7 ± 0.6 kg/d) P169 cows than in control cows (29.9 ± 0.6 kg/d) during the 25-wk study. There was no significant difference between the high-dose P169 and low-dose P169 cows (Figure 1). During the 25-wk study, high-dose P169 cows exhibited a 7.1% increase in daily 4% FCM production

above controls, and low-dose P169 cows exhibited an 8.5% increase above controls. Similarly, uncorrected daily milk production was 8.1% higher in high-dose P169 (34.2 ± 1.0 kg/d) and 7.7% higher in low-dose P169 (34.0 ± 0.9 kg/d) cows than in control cows (31.4 ± 0.9 kg/d). Daily 4% FCM production in primiparous cows increased significantly from wk 1 through 3 and remained constant throughout the remainder of the 25-wk lactation period (Figure 1). Daily 4% FCM production in multiparous cows showed a significant increase from wk 1 through 5, after which a significant decrease in milk production was not seen until after wk 17 of lactation (Figure 1). The percentage decrease in milk production from peak production at wk 6 (40.4 kg/d) for multiparous cows and wk 7 (27.6 kg/d) for primiparous cows to wk 25 of lactation was 15.1 and 0.6%, respectively.

Daily 4% FCM production for wk 1 to 12 displayed a tendency to be affected by treatment ($P < 0.07$) such that

4% FCM, averaged across primiparous and multiparous cows, was higher in low-dose P169 cows (32.7 ± 0.7 kg/d) than either the high-dose P169 cows (31.9 ± 0.8 kg/d) or control cows (30.3 ± 0.8 kg/d). Daily 4% FCM production for wk 13 to 25 was affected by treatment ($P < 0.01$) such that 4% FCM, averaged across primiparous and multiparous cows, was greater in both the high-dose (32.5 ± 0.8 kg/d) and low-dose (32.8 ± 0.8 kg/d) P169 cows than in control cows (29.6 ± 0.8 kg/d). Expressed as a percentage increase above controls, high-dose P169 cows exhibited a 8.9% increase and the low-dose P169 cows exhibited a 9.7% increase in daily 4% FCM production during wk 13 to 25.

When bST was administered for 5 wk at the end of the study, 4% FCM production was affected by treatment ($P < 0.03$) and parity ($P < 0.001$) with no other significant main effects ($P > 0.40$) or interactions ($P > 0.30$). Daily 4% FCM production during the bST administration averaged 31.9 ± 1.2 , 33.7 ± 1.1 , and 29.6 ± 1.1 kg/d for the high-dose P169, low-dose P169, and control cows, respectively. During bST administration, daily 4% FCM production averaged 28.8 ± 0.9 kg/d for primiparous cows and 34.7 ± 0.9 kg/d for multiparous cows. Average 4% FCM production after 5 wk of bST administration (i.e., wk 30) for both primiparous and multiparous cows did not significantly differ from wk 25 (wk 0 of bST) milk production. Daily 4% FCM production was 12.1% higher ($P < 0.05$) in the low-dose P169 cows than control cows during the 5-wk bST administration. Milk production in high-dose P169 cows did not differ from either low-dose P169 or control cows.

Milk Components

Milk Fat. Milk fat percentage was altered by treatment $\times$ parity ($P < 0.02$), parity ($P < 0.01$), and week ($P < 0.001$) but not by other main effects ($P > 0.30$) or interactions ($P > 0.45$). Percentage milk fat was significantly greater in low-dose P169 and control multiparous cows than in high-dose P169 multiparous cows, but there was no difference among treatment groups in primiparous cows (Figure 2). Milk fat percentage decreased ($P < 0.05$) 18% from wk 1 ($4.4 \pm 0.1\%$) to wk 4 ($3.6 \pm 0.1\%$) and plateaued after wk 5 with no significant change between wk 5 and 25 (data not shown).

When milk fat data were analyzed separately for wk 1 to 12 and wk 13 to 25, treatment had no significant effect. However, milk fat percentage was altered by week ($P < 0.001$) during wk 1 to 12, such that milk fat percentage decreased significantly from wk 1 to 4. During wk 13 to 25, parity ($P < 0.03$) affected milk fat percentage such that multiparous cows ($3.7 \pm 0.1\%$) had

Figure 2. Milk fat percentage for treatment $\times$ parity interaction in multiparous and primiparous cows fed high-dose ($n = 11$) and low-dose ($n = 14$) *Propionibacterium* P169 and control ($n = 13$) rations during wk 1 to 25 of lactation. ^{a,b}Means ($\pm$ SEM) without a common superscript differ ($P < 0.02$).

higher percentage milk fat than did primiparous cows ($3.5 \pm 0.1\%$).

During the 5-wk bST administration, there was no significant effect of treatment, parity, week, or their interactions on milk fat percentage ($P > 0.13$; data not shown).

Milk Lactose. Milk lactose percentage was influenced by treatment $\times$ parity ($P < 0.03$), treatment ($P < 0.001$), and week ($P < 0.001$) but not by other main effects ($P > 0.45$) or interactions ($P > 0.35$). Milk lactose percentage was significantly greater in the high-dose P169 ($5.12 \pm 0.04\%$) than low-dose P169 ($4.91 \pm 0.04\%$) multiparous cows, with lactose levels in low- and high-dose P169 multiparous cows significantly higher than in control ($4.77 \pm 0.03\%$) multiparous cows (Figure 3). Milk lactose levels in high-dose P169 primiparous cows ($5.04 \pm 0.04\%$) did not differ from control primiparous cows ($4.93 \pm 0.04\%$), but were higher ($P < 0.05$) than in low-dose P169 primiparous cows ($4.89 \pm 0.03\%$). Averaged across groups, milk lactose levels increased from wk 1 to 7 of lactation, after which milk lactose levels did not change.

When milk lactose data were analyzed separately for wk 1 to 12 and wk 13 to 25, milk lactose percentage was influenced by treatment ($P < 0.002$) and week ($P < 0.0001$) during wk 1 to 12, such that percentage of milk lactose was significantly greater in the high-dose P169 ($5.04 \pm 0.04\%$) than in low-dose P169 ($4.88 \pm 0.04\%$) and control ($4.82 \pm 0.04\%$) cows. During wk 13 to 25, milk lactose percentage was influenced by treatment $\times$ parity ($P < 0.0001$) similar to that observed in wk 1 to 25.

Milk lactose levels during 5 wk of bST administration were influenced by treatment $\times$ parity ($P < 0.06$) and followed a pattern very similar to lactose levels during

Figure 3. Effect of feeding propionibacteria on milk lactose percentage during 25 wk of lactation. Milk lactose percentage in multiparous cows (top panel; $n = 19$) fed high-dose ($n = 5$) and low-dose ($n = 6$) *Propionibacterium* P169 and control ($n = 8$) rations for wk 1 to 25, and in primiparous cows (bottom panel; $n = 19$) fed high-dose ($n = 6$) and low-dose ($n = 8$) *Propionibacterium* P169 and control ($n = 5$) rations for wk 1 to 25.

wk 1 to 25 (data not shown). No other main effects or interactions ($P > 0.30$) were significant.

Milk Protein. Milk protein percentage was influenced by week ($P < 0.001$) with a tendency to be influenced by treatment $\times$ parity ($P < 0.11$) with no other significant main effects ($P > 0.40$) or interactions ($P > 0.20$). Milk protein percentage significantly decreased from wk 1 to 4, and then gradually increased through wk 15, with no change thereafter (Figure 4). High-dose ($3.11 \pm 0.06\%$) and low-dose P169 ($3.11 \pm 0.06\%$) treatments tended to increase ($P < 0.11$) milk protein percentage in multiparous cows vs. control ($3.00 \pm 0.04\%$) multiparous cows (Figure 4); these differences were not evident in primiparous cows (data not shown).

When milk protein data were analyzed separately for wk 1 to 12 and wk 13 to 25, milk protein percentage was influenced by week ($P < 0.001$) during wk 1 to 12, such that weekly milk protein percentage exhibited a significant decrease from wk 1 to 4. There was no sig-

nificant difference in milk protein percentage due to treatment, parity, week, or their interactions during wk 13 to 25.

Milk protein percentage during the 5-wk bST administration was influenced by week $\times$ parity ($P < 0.002$) and treatment $\times$ week ($P < 0.09$) with no other main effects ($P > 0.45$) or interactions ($P > 0.15$). Milk protein percentage in multiparous cows showed no significant change between wk 25 and wk 30, whereas milk protein percentages in primiparous cows increased after each biweekly bST injection (data not shown).

Milk SNF. Percentage of milk SNF was influenced by treatment $\times$ parity ($P < 0.02$), treatment ($P < 0.01$), week ($P < 0.008$), and treatment $\times$ week ($P < 0.10$) but not by other main effects ($P > 0.35$) or interactions ($P > 0.15$). Percentage of SNF was greater in both high-dose ($9.16 \pm 0.09\%$) and low-dose ($8.94 \pm 0.09\%$) P169 multiparous cows than in control ($8.67 \pm 0.07\%$) multiparous cows (Figure 5). However, differences were not

Figure 4. Milk protein percentage during wk 1 to 25 of lactation in multiparous cows ($n = 19$) fed high-dose ($n = 5$) and low-dose ($n = 6$) *Propionibacterium* P169 and control ($n = 8$) rations.

evident among high-dose P169, low-dose P169, and control primiparous cows (9.08 ± 0.08 , 8.87 ± 0.06 , and $9.01 \pm 0.09\%$, respectively) during wk 1 to 25 (data not shown). Averaged across all groups, percentage SNF significantly decreased from wk 1 to 4, slowly increased from wk 4 to 12, and then remained constant through wk 25 (Figure 5).

When milk SNF data were analyzed for wk 1 to 12, percentage of milk SNF was influenced by treatment $\times$ parity ($P < 0.05$) and week ($P < 0.0001$); percentage SNF followed a similar profile of response as the wk 1 to 25 analysis. The wk 13 to 25 analysis revealed a significant treatment effect ($P < 0.01$), with high-dose P169 cows ($9.15 \pm 0.07\%$) having a greater percentage of SNF than

Figure 5. Milk SNF percentage during wk 1 to 25 of lactation in multiparous cows ($n = 19$) fed high-dose ($n = 5$) and low-dose ($n = 6$) *Propionibacterium* P169 and control ($n = 8$) rations.

low-dose P169 ($8.91 \pm 0.06\%$) and control cows ($8.85 \pm 0.07\%$).

Milk SNF percentage during the 5-wk bST administration was affected by week ($P < 0.05$) and week $\times$ parity ($P < 0.04$) with no other significant main effects ($P > 0.15$) or interactions ($P > 0.13$). Primiparous cows exhibited a greater increase in percentage SNF than did multiparous cows in response to the first bST injection, after which percentage SNF response to bST varied with the time of administration within parity groups (data not shown).

MUN. Levels of MUN were influenced by week ($P < 0.001$), parity ($P < 0.07$), and week $\times$ parity ($P < 0.08$) but not by other main effects ($P > 0.30$) or interactions ($P > 0.12$). The general trend was an increase in MUN levels over the first 15 wk of lactation, but specific effects (i.e., increases and decreases) varied depending on the week of lactation and parity of the animal. In particular, the first significant increase in MUN above wk 1 occurred on wk 3 in multiparous (wk 1 = 11.8 ± 0.5 mg/dL) cows and on wk 4 in primiparous (wk 1 = 11.7 ± 0.5 mg/dL) cows; further significant increases occurred between wk 3 and 9 in both groups such that MUN increased similarly (i.e., 29%) between wk 1 and 9 in multiparous and primiparous cows (data not shown).

When MUN data were analyzed separately for wk 1 to 12, MUN levels were influenced by week ($P < 0.001$) with changes similar to that observed in the wk 1 to 25 analysis. For wk 13 to 25, MUN levels were altered by treatment ($P < 0.05$) with a tendency to be influenced by parity ($P < 0.07$). Levels of MUN were greater in high-dose P169 (15.6 ± 0.3 mg/dL) and low-dose P169 cows (15.8 ± 0.3 mg/dL) than in control cows (14.9 ± 0.3 mg/dL). Levels of MUN in multiparous cows tended to be greater than in primiparous cows ($P < 0.07$) during wk 13 to 25.

During the 5-wk bST administration, MUN levels were influenced by week ($P < 0.05$) but not by other main effects ($P > 0.90$) or interactions ($P > 0.15$). Levels of MUN were lowest at wk 29 (14.4 ± 0.4 mg/dL; 2 wk after the second bST injection) and highest at wk 28 (15.7 ± 0.3 mg/dL; 1 wk after the second bST injection at wk 27).

SCC. During wk 1 to 25, SCC were influenced by week $\times$ parity ($P < 0.001$) and week ($P < 0.001$) but not by other main effects ($P > 0.25$) or interactions ($P > 0.75$). Primiparous cows exhibited a greater SCC at wk 1 than multiparous cows (1871.0 ± 193.1 and $370.0 \pm 204.7 \times 10^3/\text{mL}$, respectively) and SCC in multiparous cows were greater than in primiparous cows at wk 3 (167.7 ± 189.8 and $504.3 \pm 196.9 \times 10^3/\text{mL}$ respectively). Between wk 1 and 4, SCC decreased dramatically, and from wk 4 to 25 no significant differences in SCC were observed between parity groups (data not shown).

When SCC data were analyzed separately for wk 1 to 12, week $\times$ parity ($P < 0.001$) but not diet ($P > 0.10$) influenced SCC, with primiparous cows ($256.3 \pm 80.6 \times 10^3/\text{mL}$) having a greater SCC than multiparous cows ($148.7 \pm 82.9 \times 10^3/\text{mL}$). For wk 13 to 25, SCC were altered by diet ($P < 0.007$), with both high-dose P169 ($36.0 \pm 29.8 \times 10^3/\text{mL}$) and low-dose P169 ($92.0 \pm 27.5 \times 10^3/\text{mL}$) cows exhibiting a significantly lower SCC than control cows ($168.5 \pm 28.0 \times 10^3/\text{mL}$).

During the 5-wk bST administration, SCC tended to be altered by treatment ($P < 0.09$) with no other main effects ($P > 0.20$) or their interactions ($P > 0.60$) being significant. The SCC of high-dose P169 cows ($38.6 \pm 28.0 \times 10^3/\text{mL}$) were lower ($P < 0.03$) than control ($123.4 \pm 26.3 \times 10^3/\text{mL}$) cows. Low-dose P169 ($95.6 \pm 26.3 \times 10^3/\text{mL}$) cows did not differ ($P > 0.10$) from high-dose P169 or control cows.

Ruminal Acetate

The molar percentage of ruminal acetate was affected by day such that ruminal acetate was greater at d 120 ($67.2 \pm 0.9\%$) and 175 ($66.7 \pm 0.9\%$) than at d 60 ($63.6 \pm 0.9\%$). The molar percentage of ruminal acetate was not affected by treatment ($P > 0.30$) and averaged 64.4 ± 1.2 , 66.4 ± 1.1 , and $66.7 \pm 1.1\%$ for high-dose P169, low-dose P169, and control cows, respectively, between d 60 and 175. The molar percentage of ruminal acetate at d -30 was 71.5 ± 1.5 , 74.0 ± 1.3 , and $72.8 \pm 1.3\%$ for high-dose P169, low-dose P169, and control cows, respectively.

At the end of the 5-wk bST administration (d 204), the molar percentage of ruminal acetate was not affected by treatment, parity, or their interaction with molar percentages of 64.3 ± 1.7 , 66.2 ± 1.2 , and $66.6 \pm 1.2\%$ for high-dose P169, low-dose P169, and control cows, respectively.

At d 265 (60 d after end of P169 feeding), the molar percentage of ruminal acetate was influenced by treatment ($P < 0.04$) such that high-dose P169 ($67.0 \pm 1.0\%$) cows exhibited a lower molar percentage of acetate than either the low-dose P169 ($70.0 \pm 1.0\%$) or control ($70.7 \pm 0.9\%$) cows.

Ruminal Propionate

Ruminal propionate levels were influenced by treatment ($P < 0.05$) such that high-dose P169 cows, averaged across d 60, 120, and 175, had an 18.5 and 17.0% increase in molar percentage of rumen propionate over the low-dose P169 and control cows, respectively (Figure 6). The molar percentage of ruminal propionate at d -30 was 15.7 ± 1.4 , 14.1 ± 1.2 , and $13.5 \pm 1.3\%$ for high-dose P169, low-dose P169, and control cows, respectively.

Figure 6. Effect of feeding propionibacteria on ruminal VFA. A) Molar percentage of ruminal propionate (as % of acetate, propionate, and butyrate) by treatment of multiparous ($n = 19$) and primiparous ($n = 19$) cows fed high-dose ($n = 11$) and low-dose ($n = 14$) *Propionibacterium* P169 and control ($n = 13$) rations for d 60, 120, and 175. ^{a,b}Means ($\pm$ SEM) without a common superscript differ ($P < 0.05$). B) Acetate/propionate ratio for multiparous ($n = 19$) and primiparous cows ($n = 19$) fed high-dose ($n = 11$) and low-dose ($n = 14$) *Propionibacterium* P169 and control ($n = 13$) rations for d 60, 120, and 175. ^{a,b}Means ($\pm$ SEM) without a common superscript differ ($P < 0.06$).

At the end of the 5-wk bST administration (d 204), molar percentage of ruminal propionate was not affected by treatment, parity, or their interaction, and averaged 22.9 ± 1.0 , 23.8 ± 0.9 , and $22.6 \pm 0.9\%$ for high-dose P169, low-dose P169, and control cows, respectively.

At d 265 (60 d after end of P169 feeding), the molar percentage of ruminal propionate was not affected by treatment, parity, or treatment $\times$ parity interaction, with molar percentages of 21.7 ± 1.1 , 19.7 ± 1.0 , and $19.6 \pm 1.0\%$ for high-dose P169, low-dose P169, and control cows, respectively.

Ruminal Acetate/Propionate Ratio

Changes in acetate/propionate ratio from pretreatment (-30 d) status were affected by treatment ($P < 0.06$). The high-dose P169 cows, averaged across d 60, 120, and 175, had 15.4 and 13.3% lower acetate/propionate ratios compared with the low-dose P169 and control cows, respectively (Figure 6). At the end of the 5-wk bST administration (d 204) and at d 265 (60 d after end of P169 feeding), acetate/propionate ratio was not affected by treatment, parity, or treatment $\times$ parity interaction (data not shown).

Ruminal Butyrate

The molar percentage of ruminal butyrate was affected by day ($P < 0.001$) such that the molar percentage of butyrate was greater at d 60 ($15.7 \pm 0.6\%$) compared

with d 120 ($11.8 \pm 0.6\%$) and d 175 ($11.4 \pm 0.6\%$). There was a tendency for the molar percentage of ruminal butyrate to be affected by treatment ($P < 0.11$) with levels of 12.3 ± 0.6 , 13.9 ± 0.5 , and $12.7 \pm 0.5\%$ for the high-dose P169, low-dose P169, and control cows, respectively. The molar percentage of ruminal butyrate at d -30 was 12.8 ± 0.9 , 11.9 ± 0.8 , and $13.3 \pm 0.9\%$ for high-dose P169, low-dose P169, and control cows, respectively.

At the end of the 5-wk bST administration (d 204), the molar percentage of ruminal butyrate was not affected by treatment or treatment $\times$ parity interaction averaging 11.1 ± 0.3 , 11.0 ± 0.3 , and $10.6 \pm 0.3\%$ for high-dose P169, low-dose P169, and control cows, respectively. The molar percentage of ruminal butyrate was influenced by parity ($P < 0.07$) with multiparous cows ($11.3 \pm 0.3\%$) exhibiting a greater molar percentage of ruminal butyrate than primiparous cows ($10.6 \pm 0.2\%$).

At d 265 (60 d after end of P169 feeding), the molar percentage of ruminal butyrate was not affected by treatment, parity, or treatment $\times$ parity interaction, averaging 10.5 ± 0.6 , 10.7 ± 0.6 , and $9.8 \pm 0.5\%$ for high-dose P169, low-dose P169, and control cows, respectively.

Ruminal pH

Analysis for changes in rumen pH from the (-30 d) pretreatment day status revealed that pH was influenced by treatment ($P < 0.02$) such that high-dose P169

Figure 7. Effect of feeding propionibacteria on BW during 25 wk of lactation. Changes in BW between wk 1 and 25 of lactation in multiparous cows (top panel; $n = 19$) fed high-dose ($n = 5$) and low-dose ($n = 6$) *Propionibacterium* P169 and control ($n = 8$) rations. Data are expressed as percentage of wk 1 BW, which averaged 739.4 ± 31.1 kg, 691.3 ± 28.2 kg, and 703.7 ± 24.0 kg for multiparous high-dose, low-dose, and control groups, respectively; and for primiparous cows ($n = 19$) fed high-dose ($n = 6$) and low-dose ($n = 8$) *Propionibacterium* P169 and control ($n = 5$) rations. Data are expressed as percentage of wk 1 BW, which averaged 554.3 ± 28.2 kg, 522.0 ± 24.4 kg, and 528.9 ± 30.9 kg for primiparous high-dose, low-dose, and control groups, respectively.

cows averaged across d 60, 120, and 175 had a lower pH than low-dose P169 and control cows with pH values of 6.65 ± 0.07 , 6.94 ± 0.06 , and 6.86 ± 0.06 , respectively. At the end of the 5-wk bST administration (d 204) and d 265 (60 d after end of P169 feeding), ruminal pH was not affected by treatment, parity, or treatment $\times$ parity interaction (data not shown).

Change in Weekly BW

Body weight expressed as percentage of wk 1 BW was influenced by week $\times$ parity ($P < 0.02$) and treatment $\times$ parity ($P < 0.004$) such that high-dose P169 and low-dose P169 multiparous cows exhibited a greater recovery of wk 1 BW than did control multiparous cows, recovering 98.4 ± 1.3 , 100.0 ± 1.1 , and $95.3 \pm 1.0\%$, respectively, by wk 25 (Figure 7). Nadir BW occurred at wk 5 for the multiparous cows with a percentage weight loss of 4.0 ± 2.4 , 2.9 ± 2.2 , and $7.5 \pm 1.7\%$ for

the high-dose P169, low-dose P169, and control multiparous cows, respectively. Control primiparous cows exhibited a greater recovery of wk 1 BW than did high-dose P169 primiparous cows, recovering 100.5 ± 1.2 and $97.2 \pm 1.13\%$, respectively, by wk 25. Body weight in low-dose P169 primiparous cows ($98.7 \pm 1.0\%$) did not differ from either the control or the high-dose primiparous cows (Figure 7). Nadir BW also occurred at wk 5 for the primiparous cows with a percentage BW loss of 9.0 ± 2.0 , 7.2 ± 1.7 , and $7.1 \pm 2.1\%$, respectively, for the high-dose P169, low-dose P169, and control primiparous cows. Nadir percentage BW loss occurred at wk 5 for both primiparous and multiparous cows, and by wk 25, primiparous cows had recovered $104.2 \pm 1.1\%$ of wk 1 BW vs. $100.0 \pm 1.2\%$ for multiparous cows.

During the 5-wk bST administration, change in BW was influenced by parity ($P < 0.006$) and week ($P < 0.05$) such that primiparous cows (averaged across treatment and week) recovered $105.7 \pm 1.30\%$ of wk 1 BW vs.

multiparous cows recovering $101.9 \pm 1.30\%$. During the 5-wk bST administration, averaged across treatment and parity, cows continued to gain BW, with BW increasing from $102.0 \pm 1.03\%$ at wk 25 to $105.3 \pm 1.1\%$ at wk 30.

Reproductive Measures

Interval to First Ovulation. There was no treatment, parity, or treatment $\times$ parity interaction on days to first ovulation after parturition. Days to first ovulation averaged 32.4 ± 6.0 , 30.8 ± 5.3 , and 32.9 ± 5.6 d for the high-dose P169, low-dose P169, and control groups, respectively.

Period 1 and Period 2 Estrous Behavior and Pregnancy. No significant differences among treatment groups existed in the percentage of cows that were cyclic during estrous synchronization period 1 (89.6%) or period 2 (97.4%) or in the percentage of cows responding (overall mean = 87%) to PGF_{2 α} . In addition, there were no significant differences between high-dose P169, low-dose P169, and control cows for number of mounts (5.1 ± 2.8), mount duration (3.2 ± 1.2), or duration of estrus (13.3 ± 4.2) for period 1 or period 2 synchronization. The percentage of cows demonstrating observable estrus (as detected by the HeatWatch system) during period 1 (45 d) and period 2 (90 d) averaged 57.5 and 60.2%, respectively. No significant difference existed in pregnancy rates at 30 wk postpartum for control (23.0%), low-dose P169 (21.4%), and high-dose P169 (36.3%) groups.

DISCUSSION

In the present study, supplemental P169 feeding increased 4% FCM production by 7.1 to 8.5%. With the administration of bST, daily 4% FCM production was significantly greater in the low-dose P169 cow than in control cows, indicating that the positive effects of supplemental P169 feeding on milk production can also be realized with concomitant bST administration. Previously, Francisco et al. (2002) reported that 4% FCM production was not significantly affected by a 12-wk P169 supplementation at a dosage comparable to the low-dose P169 level in the present study. When multiparous Holstein cows were fed an energy supplement containing 78% propionic acid from 3 wk prepartum to 3 wk postpartum, no effect on milk yield was observed (Mandebvu et al., 2003). Milk production data of the current study were analyzed separately for wk 1 to 12 to relate to the data of Francisco et al. (2002); only a tendency was found for the high-dose and low-dose P169 feeding to alter 4% FCM production. When analyzed separately for wk 13 to 25, 4% FCM production

was significantly increased by P169 feeding with an 8.9 and 9.7% increase in 4% FCM in the high-dose and low-dose P169 groups, respectively. Therefore, the Francisco et al. (2002) and Mandebvu et al. (2003) studies may not have been carried out over a period long enough to observe the persistency of lactation that occurred with P169 feeding in the present study. Alternatively, because live yeast or yeast culture supplementations have been reported to increase milk production in several studies (Williams et al., 1990; Piva et al., 1993; Putman et al., 1997), the presence of yeast culture in the TMR of the present study may have potentiated the effect of P169. Yeast supplementation has been shown to provide soluble growth factors (Harrison et al., 1988), stimulatory peptides (Rossi et al., 2004), and other substances (van Gylswyk et al., 1992) that stimulate the growth of specific groups of ruminal bacteria. However, other studies indicate no significant response of milk production to yeast supplementation (Erdman and Sharma, 1989; Kung et al., 1997). Thus, further research is warranted to investigate the potential interactions between supplemental yeast and P169 in regards to improving milk yield.

Consistent with the results of the present study in which ruminal propionate levels increased in the high-dose P169 group, use of propionibacteria as a direct-fed microorganism to feedlot cattle has been reported to increase propionate levels, and decrease acetate/propionate ratios (Kim et al., 2001a). Previous studies feeding 60 times less propionibacteria than the high-dose P169 treatment found no effect on ruminal propionate levels (Kim et al., 2001b; Ghorbani et al., 2002). Thus, the dose of propionibacteria may explain ruminal fermentation discrepancies among studies. Moreover, time of rumen fluid collection relative to feeding time may have a profound effect on rumen VFA, because ruminal propionate transiently increases after feeding and may also account for differences observed among studies.

In the present study, milk fat percentage was lower in high-dose P169 than low-dose P169 and control multiparous cows, whereas no differences were seen in primiparous cows. Francisco et al. (2002) reported no changes in percentages of milk fat with P169 supplementation in multiparous cows during the first 12 wk of lactation. However, decreased fat percentage in dairy cows has been observed after ruminal infusion of propionic acid for 14 d (Rigout et al., 2003), and after feeding an energy supplement containing 78% propionic acid from 3 wk prepartum to 3 wk postpartum (Mandebvu et al., 2003). Because several studies have shown increases in milk fat percentage after live yeast or yeast culture supplementation (Piva et al., 1993; Putman et al., 1997), perhaps presence of yeast enhanced the effect

of P169 in the present study. Because ruminal percentages of acetate did not differ among groups and the increase in ruminal propionate percentage (with subsequent decrease of acetate/propionate ratio) was observed only in the high-dose P169 group, it is possible that the milk fat depression observed in the present study resulted from insulin-induced shortages of glucogenic precursors (Bauman and Griinari, 2003). The molar percentage of ruminal butyrate tending to be lower in high-dose P169 than in low-dose P169 could help explain the differences in the reduction of milk fat percentage between the 2 treatment levels (Rigout et al., 2003).

Both high-dose and low-dose P169 feeding tended to increase milk protein percentage by 3 to 4% above control multiparous cows during wk 1 to 25. Francisco et al. (2002) reported a 25% increase in percentage of milk protein only during wk 1 of lactation in P169 supplementation vs. controls. Similarly, ruminal infusion of propionic acid in dairy cows for 2 wk increased percentage milk protein (Rigout et al., 2003). In multiparous Holstein cows fed an energy supplement containing 78% propionic acid from 3 wk prepartum to 3 wk postpartum there was no effect on milk protein percentage (Mandebvu et al., 2003). As described earlier for milk fat and milk production, the presence of yeast may have influenced P169 effects on milk protein levels (Erdman and Sharma, 1989; Williams et al., 1990; Piva et al., 1993; Putman et al., 1997). Moreover, changes in an animal's energy supply and flow of microbial protein to the lower intestinal tract could result in increased milk protein levels (Emery, 1978; Doepel et al., 2004) with variations dependent on blood flow to the mammary gland and the efficiency of amino acid use within the mammary gland (McDonald et al., 2002). Propionate spares glucogenic amino acids in gluconeogenesis (Seal and Parker, 1995; Rulquin et al., 2004) and consequently reduces the maintenance cost of metabolizable protein and possible heat increment (Van Soest, 1994). Whether the P169-induced increases in milk protein levels in the present study were due to improved rumen function, amino acid sparing, increased DMI, or a combination of these factors will require further evaluation.

Francisco et al. (2002) reported no changes in percentage of milk lactose with 12 wk of P169 feeding to multiparous cows, and their results are in agreement with the present study in which milk lactose data analyzed separately for wk 1 to 12 showed no difference in the percentage of milk lactose between low-dose P169 and control cows. However, the percentage of milk lactose was greater in the high-dose P169 than in low-dose P169 and control cows for wk 1 to 12. During wk 13 to 25 as well as wk 1 to 25, milk lactose percentage was significantly greater in high-dose P169 than in low-dose

P169 multiparous cows, and low-dose P169 multiparous cows had significantly greater milk lactose than control cows. Mandebvu et al. (2003) supplemented rations with propionic acid from 3 wk prepartum to 3 wk postpartum, and showed no effect on milk lactose levels, whereas Rigout et al. (2003) reported that ruminal infusion of propionic acid for 2 wk increased percentage milk lactose. Theoretically, P169 supplementation with increased ruminal propionate should increase blood glucose concentration via gluconeogenesis and subsequently increase milk lactose (Sauer et al., 1989; McDonald et al., 2002). The lack of response in the study of Francisco et al. (2002) suggests that the dose of propionibacteria was not high enough or measured over a period long enough to observe significant effects on milk lactose as seen in the present study.

Francisco et al. (2002) reported that the percentage of SNF, like milk protein levels, was greater in cows fed P169 vs. control TMR at wk 1 of lactation. The present study indicated the percentage SNF was greater in both high- and low-dose P169 multiparous cows than in control multiparous cows, but no differences in SNF existed among high-dose P169, low-dose P169, and control primiparous cows. These patterns follow closely the patterns of the individual milk components (lactose and protein) that are used in the calculation of milk SNF.

Similar to the present study, Francisco et al. (2002) reported no effect of P169 supplementation on MUN levels in multiparous cows during wk 1 to 12 of lactation, and that changes in MUN levels paralleled milk yield. The tendency for MUN concentrations to be higher in multiparous than primiparous cows is likely due to production levels and intake differences (Godden et al., 2001). However, for wk 13 to 25, MUN levels were greater in high- and low-dose P169 cows than in control cows. Increased MUN could reflect increased DMI in the P169 groups or be due to increased ammonia incorporation into microbial protein in the rumen, which increases protein supply for milk protein synthesis and decreases N loss (Nousiainen et al., 2004). Additional work will be required to ascertain the role of supplemental P169 on nitrogen balance in lactating dairy cows.

In agreement with previous studies (Sordillo et al., 1997; Barkema et al., 1999), the largest reductions in SCC occurred during the first 2 wk postpartum in the present study. During wk 13 to 25, SCC levels were significantly altered by treatment, with both high-dose P169 and low-dose P169 cows exhibiting a significantly lower SCC than control cows, and this treatment effect was also observed during the 5 wk of bST administration. Whether the P169-induced reduction in SCC is

due to increased efficiency of the animal's immune system will require further study.

Consistent with Francisco et al. (2002), no significant differences in the average days to first ovulation in P169-fed vs. control cows were observed in the present study. Thus, increased milk production from P169 supplementation in the present study had no adverse effect on days to first ovulation and indicates that increased milk production may have been compensated for by altered metabolism, increased nutrient intake, improved nutrient use, or a combination of these.

The estrous behavior response (i.e., number of mounts, mount duration, and duration of estrus) did not significantly differ among groups and data recorded by the HeatWatch system are consistent with previously published data (Walker et al., 1996; Lopez et al., 2004). However, the efficiency of the HeatWatch system for detecting estrus in the present study (57 to 62%) was lower than previously reported values of 72 to 95%, and may be due to the fact that the total number of animals for each group was low (i.e., $n = 11$ to 14 per group) and the cows remained on concrete throughout the study. In addition, only 1 to 3 cows per group were synchronized at any given week during the study so that the 45-d and 90-d postpartum average could be attained. When the numbers of animals in estrus simultaneously increase, a proportional increase in the number of mounts per hour and increased mounting activity is recorded (Hurnik et al., 1975; Helmar and Britt, 1985; Floyd, 2001). Thus, if the number of cycling, nonpregnant animals in a group is low, regardless of the total group size, accurate detection of estrus may be difficult. The average total number of mounts in the present study (i.e., 5 to 6) was similar to the total attempted mounts recorded (i.e., 6.1) by Helmar and Britt (1985) when only one estrual cow was present in a pen, but lower than 11 mounts recorded when one estrual cow was present in a free-stall barn setting (Hurnik et al., 1975). Moreover, mounting activity of dairy cows is significantly less when cows are housed on concrete vs. dirt (Britt et al., 1986; Vailes and Britt, 1990; Lopez and Shipka, 2003). Therefore, the low number of cows synchronized each week per treatment group, the stage of the estrous cycle of the nonsynchronized cows in each group, and the concrete flooring surface likely combined to reduce the estrus detection efficiency of the HeatWatch system in the present study. The increased number of detected estruses from period 1 to period 2 supports Spicer et al. (1990, 1993) in which the percentage of cows detected in estrus increased from the first to second postpartum ovulations.

With the onset of lactation, most dairy cows are not able to meet the energy requirements for maintenance and milk production from the diet due to insufficient

feed intake and experience a postpartum negative energy balance (De Vries et al., 1999; Stevenson, 2001; Jorritsma et al., 2003), which results in BW loss. In the present study, nadir BW occurred at wk 5 for both the multiparous and primiparous cows in all groups. In contrast to Harrison et al. (1990), control multiparous cows (lower producers) exhibited a 7.5% loss of wk 1 BW at wk 5 than the low-dose P169 group (2.9%) and high-dose P169 (4.0%). High-dose P169 and low-dose P169 multiparous cows (higher producers) exhibited a significantly greater recovery of wk 1 BW than did control multiparous cows (lower producers). In contrast to multiparous cows, control primiparous cows exhibited a greater recovery of wk 1 BW than high-dose P169 primiparous cows, with low-dose P169 primiparous cows not differing from either control or high-dose primiparous cows. During the 5-wk bST administration, averaged across diet and parity, cows continued to gain BW. Postpartum dairy cows first partition metabolizable energy toward milk production, then body condition gain, and finally to reproductive functions (Lucy, 2001). It appears that the increased milk production from P169 supplementation had no adverse effect on changes in BW, indicating that the increased milk production was compensated for by altered metabolism, increased nutrient intake, or nutrient supply, as previously mentioned.

The most commonly accepted theory of changes in ruminal propionate altering metabolism in lactating cows is that increases in ruminal propionate production result in a greater supply of this glucogenic precursor to the liver, thereby increasing glucose production (Sauer et al., 1989). Why milk production, percentage milk protein, SNF, MUN, and changes in percentage BW were similar between the high-dose P169 the low-dose P169 groups while percentage ruminal propionate, milk fat, and milk lactose levels were significantly different is unclear. Perhaps transient changes in rumen VFA were not detected with a single rumen fluid collection, particularly if the increases were short-lived. The increased milk production in the high-dose P169 groups follows Sauer's theory (Sauer et al., 1989) of increased glucogenic precursors (i.e., propionate) increasing gluconeogenesis and is further supported by the increased percentage of milk lactose, increased ruminal propionate percentages, decreased acetate/propionate ratio, and depressed milk fat percentages. The low-dose P169 group lacking the measurable effects of increased gluconeogenesis as observed in the high-dose P169 group may follow a different (or combination) mode of action(s) other than increased gluconeogenesis, such as those categorized by Yoon and Stern (1995): 1) stimulation of desirable microbial growth in the rumen, 2) stabilization of rumen pH, 3) altered ruminal fermentation pat-

tern and end-product production, 4) increased nutrient flow postruminally, 5) increased nutrient digestibility, or 6) alleviation of stress through enhanced immune response. Regardless of the mechanism, these studies provide evidence for potential use of P169 as a direct-fed microbe for improving milk production in the dairy industry. Further studies should be conducted involving a larger number of cows in commercial settings to determine if P169 should be developed for commercial use.

ACKNOWLEDGMENTS

The authors thank Alan Miller and other members of the OSU Dairy Cattle Center for care and management of the cows; Heart of America DHIA (Manhattan, KS) for milk composition analysis; Forage Testing Laboratory (Ithaca, NY) for feed analysis; and Mark Payton for statistical advice.

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