Effect of Feeding *Propionibacteria* on Milk Production by Early Lactation Dairy Cows

W. P. Weiss,¹ D. J. Wyatt, and T. R. McKelvey

Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

This experiment was conducted to determine the effect of a direct-fed microbial agent, *Propionibacterium* strain P169 (P169), on rumen fermentation, milk production, and health of periparturient and early-lactation dairy cows. Starting 2 wk before anticipated calving, cows were divided into 2 groups and fed a control diet or the control diet plus 6×10^{11} cfu/d of P169. Cows were changed to a lactation diet at calving, and treatments continued until 119 d in milk. Rumen fluid samples were taken about 1 wk before calving, and at 1 and 14 wk after calving. Cows fed P169 had lower concentrations of acetate (mol/100 mol of total volatile fatty acids) at all time points, greater concentrations of propionate on the first and last sampling points, and greater concentrations of butyrate on the first 2 time points. Concentrations of glucose in plasma and milk and plasma concentrations of β -hydroxybutyrate were not affected by treatment. Cows fed P169 had greater concentrations of plasma nonesterified fatty acids on d 7 of lactation. The high nonesterified fatty acids at that time point was probably related to the high production of milk during that period by cows fed the additive. Cows fed P169 during the first 17 wk of lactation produced similar amounts of milk (44.9 vs. 45.3 kg/d, treatment vs. control) with similar composition as cows fed the control diet. Calculated net energy use for milk production, maintenance, and body weight change was similar between treatments, but cows fed the P169 consumed less dry matter (22.5 vs. 23.5 kg/d), which resulted in a 4.4% increase in energetic efficiency.

INTRODUCTION

The need for glucose and other energy-providing substrates increases dramatically as a dairy cow transitions from gestation to lactation. If these needs are not met, cow health and milk production can be compro-

mised. Ruminally derived propionate is the major precursor for gluconeogenesis in early-lactation dairy cows (Reynolds et al., 2003), and increasing ruminal synthesis of propionate may increase glucose supply, reduce ketosis, and provide increased substrate for lactose synthesis. Increasing ruminal propionate also can increase energetic efficiency via reduced fermentation losses (Rogers and Davis, 1982) and reduced heat increment (Orskov and Allen, 1966).

The most common method of increasing the production of propionate in the rumen is to feed more grain (i.e., starch), but it can also be increased by certain feed additives, such as propylene glycol and monensin. Oral administration of propylene glycol during the peripartum period often reduces concentrations of plasma ketones and NEFA, increases plasma glucose and insulin, and modestly increases milk yield with little effect on energetic efficiency (reviewed by Nielsen and Ingvarsen, 2004). Monensin, an ionophore, increases ruminal propionate, milk yield, and energetic efficiency in dairy cows (reviewed by McGuffey et al., 2001). Specific direct-fed microbial agents may provide alternatives to chemical modifiers of ruminal fermentation. For example, various strains of *Propionibacterium* have increased the molar proportion of ruminal propionate when fed to ruminants (Kim et al., 2000; Stein et al., 2006).

The effect of feeding *Propionibacterium* alone or in combination with other bacteria to dairy cows has been evaluated but results were inconsistent. In one study (Francisco et al., 2002), early-lactation cows fed 17 g of a *Propionibacterium* culture (strain identified as P169 and supplementation rate given as approximately 6×10^{10} cfu/d in Stein et al., 2006) consumed less DM and produced similar quantities of milk as control cows. A subsequent study (Stein et al., 2006) reported that early lactation, multiparous cows fed 6×10^{10} or 6×10^{11} cfu/d of *Propionibacterium* strain P169 produced about 8% more FCM than did control cows, but no difference was observed for primiparous cows. In that study, treatments were applied to a pen; pen and treatment were completely confounded, and DMI could not be statistically evaluated. In a more recent study (Raeth-Knight

Received September 14, 2007.

Accepted October 25, 2007.

¹Corresponding author: weiss.6@osu.edu

et al., 2007), feeding 2×10^9 cfu of *Propionibacterium freudenreichii* d in combination with a *Lactobacillus* strain did not affect any production measure in mid-lactation dairy cows.

Currently available data on effects of propionibacteria on dairy cows are limited and inconsistent. Our objective was to test the hypothesis that feeding a specific *Propionibacterium* would increase ruminal propionate, and thereby improve energetic efficiency and reduce ketosis in early-lactation cows.

MATERIALS AND METHODS

Cows and Treatments

Fifty Holstein cows in their second or greater gestation (median and mean gestation number = 2 and 2.8, respectively) were assigned to 1 of 2 treatments during the dry period. Cows were blocked based on calving date, and within each block cows were ranked by milk yield during the previous lactation. Higher-producing cows within each block were alternately assigned to a treatment. Treatments consisted of a control diet and control diet plus 25 g/d of an additive that contained *Propionibacterium* strain P169 (Agtech Products, Inc., Waukesha, WI). The treatment diet is designated as **P169**. The additive was guaranteed by the manufacturer to contain 6×10^{11} cfu of viable bacteria per 25 g of material (dextrose was the carrier). The additive (25 g) was sealed into individual foil pouches by the manufacturer, shipped to our facility, and stored in the freezer (-20°C) until fed. Cows began on treatment 14 d before anticipated calving and ended treatment at 119 DIM. Two cows on the additive treatment died shortly after calving. Upon necropsy, one cow was found to have a rupture in the rumen and a severely torn uterus. The cause of death for the other cow was listed as undefined metabolic disturbance. A control cow was removed from the study because of a severe leg injury. Data from those 3 cows were not used; therefore, the experiment included data from 23 cows fed the treatment diet and 24 cows fed the control diet.

Before treatments started, all cows were housed in a common pen and fed a dry-cow diet (DM basis) of 15% corn silage, 25% medium maturity grass silage, 45% mature grass hay, and 15% concentrate consisting of ground corn, soybean meal, and minerals and vitamins. At 14 d before anticipated calving, cows were moved to individual box stalls and fed a prefresh diet (Tables 1 and 2) with or without the supplement until parturition. Three days after parturition, cows were moved into individual tie stalls and fed the lactation diet (Tables 1 and 2) with the same additive treatment that they received prepartum. During both gestation and lactation, the TMR was delivered to the feed bunk at approxi-

Table 1. Ingredient composition of diets (% of DM)

Item	Prefresh	Lactation
Mature orchardgrass hay, long	26.0	—
Corn silage	26.0	35.5
Alfalfa silage	13.0	18.0
Corn grain, ground	18.4	19.6
Soybean meal, 44% CP	9.0	4.8
Soypass ¹	—	2.8
Soybeans, roasted, whole	—	10.0
Soybean hulls	4.8	6.3
Animal-vegetable fat	0.35	0.37
Urea	—	0.07
Limestone	0.55	0.79
Dicalcium phosphate	0.16	0.35
Magnesium oxide	0.30	0.08
Trace mineral salt	0.39	0.46
Sodium bicarbonate	0.09	0.20
Trace mineral premix ²	0.23	0.18
Vitamin premix with biotin ³	0.73	0.50

¹Lignotech Inc., Rothschild, WI.

²Contained Na_2SeO_4 , CuSO_4 , ZnSO_4 , and Zn methionine (Zinpro, Inc., Eden Prairie, MN) and, based on DMI of 12.4 kg/d (prefresh) and 23.5 kg/d (lactation), provided 4.5 or 6.5 mg of Se, 125 or 175 mg of Cu, 400 or 700 mg of total Zn, and 200 and 170 mg of Zn from Zn methionine daily.

³Based on DMI of 12.4 kg/d (prefresh) and 23.5 kg/d (lactation) provided 13 or 19 mg of biotin, 97 or 87 kIU of vitamin A, 24 or 29 kIU of vitamin D, and 940 or 510 IU of vitamin E daily.

mately 0600 h. Then, for each treatment cow, a sealed packet of P169 (25 g) was opened and its contents mixed in the top portion of the TMR. Adequate TMR was fed to achieve approximately 5% orts. Some of the additive offered may not have been consumed but because of the procedures followed, actual intake of the additive was probably very close to the targeted intake of 25 g/d.

Sampling and Measurements

Feed offered and refused was measured daily to calculate DMI. During lactation, cows were milked twice daily, and milk weights were recorded electronically. Cows were weighed at the start of the experiment (14 d before anticipated calving), when moved to the tie stalls (3 DIM), and then every 2 wk.

Samples of silages, hays, and concentrate mixes were taken weekly, stored in the freezer (-20°C), and composited by month. A subsample of each silage was analyzed for DM (100°C for 24 h) each week to adjust diets for changes in DM. Feed refusals were sampled from each cow every 2 wk and analyzed for DM (100°C oven for 24 h) to calculate DMI. Composited silage samples were lyophilized and all samples were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Ground samples ($n = 13$ for each feed) were analyzed for DM (100°C oven for 24 h), NDF (Ankom²⁰⁰ Fiber Analyzer, Ankom Technology, Fairport, NY) with sodium sulfite and amylase (Sigma A3306, Sigma Diag-

Table 2. Nutrient composition of forages and diets (DM basis)

Item	Prefresh TMR	Lactation TMR	Corn silage	Alfalfa silage	Grass hay
DM, %	70.9	66.6	44.9 (3.2) ²	52.4 (3.9)	84.8 (2.7)
CP, %	14.0	16.6	8.3 (1.1)	19.2 (1.5)	13.4 (3.5)
RDP ¹ , %	10.9	9.7	—	—	—
NE _L ¹ , Mcal/kg	1.59	1.63	—	—	—
NDF, %	39.1	31.4	36.5 (5.2)	47.1 (3.3)	63.3 (5.2)
Starch, %	27.7	31.1	43.7 (3.6)	2.6 (0.2)	3.3 (0.4)
Lignin (H ₂ SO ₄), %	—	—	2.0 (0.4)	6.9 (0.9)	—
Ca, %	0.92	0.99	0.14 (0.01)	1.19 (0.11)	0.58 (0.29)
P, %	0.32	0.36	0.22 (0.01)	0.31 (0.02)	0.33 (0.05)
Mg, %	0.32	0.24	0.13 (0.01)	0.27 (0.04)	0.25 (0.07)
K, %	1.50	1.33	0.73 (0.05)	3.14 (0.55)	2.33 (0.68)
pH	—	—	3.83	4.85	—
Acetic acid, %	—	—	0.93	0.86	—
Propionic acid, %	—	—	0.02	0.01	—
Lactic acid, %	—	—	3.61	3.02	—

¹Calculated using NRC (2001). Actual DMI and feed composition data were entered into the model except that library values were used for protein kinetic measures.

²Standard deviation in parentheses.

nostics, St. Louis, MO), CP (Kjeldahl N $\times$ 6.25), and ash (AOAC, 2000). Starch concentrations were measured (Weiss and Wyatt, 2000) in all composited corn silage samples and in 3 samples of all other feeds. The pH and concentrations of VFA (Weiss and Wyatt, 2000) and lactic acid (R-Biopharm cat. no. 11112821035, R-Biopharm, Marshall, MI) were measured in silage samples (undried and unground).

Milk (a.m. and p.m.) was sampled every Tuesday from all cows that were >3 DIM and analyzed for concentrations of milk fat, protein, lactose (B2000 Infrared Analyzer, Bentley Instruments, Chaska, MN), and MUN (Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH). An additional milk sample (p.m. only) was taken from each cow during wk 1, 2, 3, 4, 8, 12, and 16 of lactation, skimmed, and analyzed for glucose (Infinity Glucose Hexokinase Liquid Stable Reagent, Thermo Electron, Pittsburgh, PA). Skimmed milk was prepared by centrifuging at $17,000 \times g$ for 30 min (4°C). Rumen samples were taken from the first 8 blocks of cows on 7 d after cows began the experiment (i.e., 7 d before anticipated calving), at 7 DIM, and during wk 14 of lactation (coded as 100 DIM) via stomach tube. Samples were taken approximately 4 h after feeding and immediately acidified, filtered, and frozen until analysis for VFA by GLC. Blood was sampled from the tail vein of all cows on 14 d before anticipated calving (before treatments started), 7 d before anticipated calving, then every Monday, Wednesday, and Friday until the cow calved, and then at approximately 7, 14, and 28 DIM. Blood was drawn into heparinized tubes, kept on ice until centrifuged, and plasma was removed and frozen into separate vials for analysis. After all cows had calved, blood samples were chosen for analysis. The

pretreatment sample was taken an average of 16 d (SD = 3.9) before calving; samples closest to 7 d before actual calving (averaged -6.6 d with SD = 1.2), and samples closest to 2 d before calving (averaged -2.1 d with SD = 0.8) were analyzed. Because of weekends, average DIM for postcalving samples were 5.7 (SD = 0.8), 12.7 (SD = 0.8), and 26.7 (SD = 0.7). Samples were analyzed for NEFA (NEFA C, Wako Chemicals, Richmond, VA), glucose (Glucose Liquicolor, Stanbio Laboratory, Boerne, TX), and BHBA (β -Hydroxybutyrate Liquicolor, Stanbio Laboratory).

Statistical Analyses

Daily DMI (postpartum) and milk production data were averaged for each week within a cow (17 observations per cow). Those data and milk composition and component yields were analyzed using Proc Mixed (SAS Institute, 2004) and models that included block (random with 24 df), treatment (fixed with 1 df), week (repeated within cow, fixed with 16 df), and time $\times$ treatment interaction (fixed, 16 df). The effect of treatment on DMI during the transition period (10 d before and after calving) was evaluated using the same model except day (19 df) replaced week. Blood, milk glucose, and rumen fluid data were analyzed with the same basic model (block, treatment, day of sampling, and interaction). Degrees of freedom for block were 7 for rumen fluid and 24 for blood data. Degrees of freedom for day of sampling were 2 for rumen fluid, 5 for blood data, and 6 for milk glucose. For all statistical models, when data suggested an interaction ($P < 0.15$), the SLICE option was used to compare treatments within each time period (SAS Institute, 2004). The covariance structure that resulted in the lowest Akaike's information

Table 3. Effect of feeding *Propionibacterium* P169 on BW and DMI¹

Item	Control	P169	SE	<i>P</i> < ²
Prepartum BW, kg	760	749	13	0.5
Postpartum BW, kg	660	643	10	0.3
Prepartum DMI, kg/d	12.4	12.0	0.4	0.6
Peripartum DMI, kg/d	13.5	13.2	0.36	0.6
Postpartum DMI, kg/d	23.5	22.5	0.5	0.02
Postpartum DMI, g/kg of BW	35.9	35.1	0.66	0.27

¹Prepartum BW measured once (average of 16 d before calving) and postpartum BW measured every 2 wk starting 3 d postcalving. Prepartum DMI includes the last 10 d of gestation, peripartum DMI includes the 10 d before and after calving, and postpartum DMI includes 1 to 119 DIM.

²Time (day for peripartum DMI, week for postpartum DMI, and every 2 wk for postpartum BW) affected all measures (*P* < 0.01), but no interactions between time and treatment were observed (*P* > 0.5).

criterion was used for each model. For production data, first-order autoregressive was usually used, and for rumen and blood data, compound symmetry was used. Disease prevalence data were compared using a χ^2 test.

RESULTS AND DISCUSSION

Production Measures

During the prepartum and transition periods (10 d before and after calving), DMI was affected by time but not by treatment (*P* > 0.5) or treatment by time interaction (Table 3). Daily DMI during the lactation phase (1 to 119 DIM) was lower (*P* < 0.02) for cows fed P169 than for control cows (Table 3), but treatment did not affect DMI when expressed per unit of BW (*P* > 0.25). In a previous experiment with early-lactation cows, P169 reduced DMI per unit of BW, but treatment did not affect DMI expressed as kilograms per day (Francisco et al., 2002). Mid-lactation cows fed a different *Propionibacterium* in combination with a *Lactobacillus* had similar DMI as control cows (Raeth-Knight et al., 2007). Cows on the control treatment weighed slightly more (2.6%, *P* > 0.5) than cows fed P169 (initial BW differed by similar magnitude so the difference was likely not caused by treatment). The difference in BW accounts for some, but likely not all, of the difference in DMI (kg/d) between groups. Cows fed the P169 consumed 4.3% less DM (kg/d) than cows fed the control diet, but the difference was 2.2% on a BW basis. In a statistical analysis that included BW as a continuous variable, the *P*-value for effect of treatment on DMI (kg/d) was still 0.06, suggesting that treatment had an effect on intake independent of any differences in BW. Treatment altered rumen VFA profile (discussed below), and changes in propionate supply can directly affect DMI in dairy cattle (Oba and Allen, 2003).

Table 4. Effect of feeding *Propionibacterium* P169 on milk yield and composition

Item	Control	P169	SE	<i>P</i> < ¹	
				Trt	Int
Milk, kg/d	45.3	44.9	1.0	0.7	0.7
ECM, ² kg/d	47.7	46.6	1.3	0.4	0.02
ECM/DMI	2.10	2.15	0.06	0.6	0.9
Milk fat, %	4.12	4.00	0.11	0.17	0.05
Milk fat, kg/d	1.86	1.78	0.07	0.21	0.04
Milk protein, %	2.72	2.73	0.03	0.9	0.01
Milk protein, kg/d	1.22	1.21	0.03	0.8	0.01
Milk lactose, %	4.69	4.72	0.04	0.6	0.9
Milk lactose, kg/d	2.14	2.13	0.05	0.9	0.9
MUN, mg/dL	11.2	10.8	0.27	0.22	0.9

¹Trt = effect of treatment; Int = interaction between week of lactation and treatment. Week affected all measures (*P* < 0.01).

²ECM calculated according to Tyrrell and Reid (1965).

Yields of milk and milk components, and milk composition were not affected by treatment (Table 4), but several time $\times$ treatment interactions were observed. Concentrations and yields of milk fat and protein and yield of ECM (Tyrrell and Reid, 1965) were greater (*P* < 0.05) during the first week of lactation for cows fed P169, but then no treatment effects were observed until the last 2 wk of the experiment (wk 16 and 17 of lactation). During the last 2 wk, control cows had greater concentrations and yields of milk fat (*P* < 0.05) and tended (*P* < 0.10) to produce more ECM. Results from this study are similar to those reported by Francisco et al. (2002). Both studies found that feeding this strain of *Propionibacterium* did not affect milk production or milk composition but it reduced DMI. Both studies also reported a lactation week $\times$ treatment interaction for milk protein (greater concentration during the first week and then no difference). Conversely, Stein et al. (2006) reported that milk yield and concentration of lactose increased and milk fat concentration decreased when multiparous cows were fed this strain of *Propionibacterium*. In that experiment, treatments were applied to pens; therefore, DMI could not be evaluated. Another experiment (Raeth-Knight et al., 2007) with mid-lactation cows fed a different strain of *Propionibacterium* in combination with a *Lactobacillus* resulted in no production responses to treatment.

Calculated (NRC, 2001) NE_L expenditures for maintenance and lactation, energy associated with BW change (gain or loss), and total NE_L use did not differ (*P* > 0.3) between treatments (Table 5). As expected, energy expenditures were affected by time after calving but no interactions were observed except for NE_L expended for milk production. Energetic efficiency was calculated by summing NE_L used for maintenance, milk production, and BW gain (or subtracting NE_L provided by BW loss) and dividing that value by DMI. Cows fed

Table 5. Effect of feeding *Propionibacterium* P169 on calculated NE_L use and efficiency

Item	Control	P169	SE	$P <^1$	
				Trt	Int
Maintenance, Mcal/d	10.4	10.2	0.13	0.3	0.3
Milk production, Mcal/d	32.3	31.9	1.0	0.80	0.02
BW change, Mcal/d	-1.9	-1.4	0.5	0.5	0.9
Total NE _L use, Mcal/d	40.8	40.9	0.77	0.9	0.5
NE _L use/DMI, Mcal/kg	1.81	1.89	0.03	0.06	0.6

¹Trt = effect of treatment; Int = interaction between week of lactation and treatment. Week affected all measures ($P < 0.01$).

P169 were 4.4% more efficient ($P < 0.06$) in converting DMI to NE_L than control cows. Previous experiments did not report energetic efficiency; however, Francisco et al. (2002) reported that cows fed this strain of *Propionibacterium* ate less DM, but produced similar amounts of FCM and had similar changes in BCS suggesting improved energetic efficiency. The actual mode of action for improved efficiency cannot be determined from the data, but at least 2 possible reasons exist: 1) the P169 could have increased digestibility; however, nutrient digestibility was not altered when dairy cows were fed another strain of propionibacteria (Raeth-Knight et al., 2007); or 2) improved efficiency might have been a result of increased production of propionic acid in the rumen and the concomitant reduction in methane (Rogers and Davis, 1982). Additional gains in efficiency could occur by increased use of propionate for synthesis of milk components; that is, lower heat increment (Dado et al., 1993).

Efficiency of converting DMI to NE_L (including an adjustment for BW change) decreased ($P < 0.01$) as lactation progressed (averaged 2.1 Mcal/kg at 14 DIM vs. 1.6 Mcal/kg at 84 DIM, data not shown), but no interaction between treatment and time was observed. Theoretically, efficiency of energy use should not change during lactation, which means that either some of our measurements were incorrect or one or more of the equations (NRC, 2001) used to estimate NE_L expenditures were incorrect. Ellis et al. (2006) suggested that maintenance requirements increase as lactation progresses, reaching an approximate plateau at about 22 wk of lactation. When their maintenance equation was used, efficiency was still affected by time ($P < 0.01$), but the numerical difference was slightly less. The treatment effect remained ($P < 0.06$) and no interaction was observed. A likely reason for the time effect is errors associated with estimating changes in body energy reserves. For example, the increase in DMI in early lactation causes measured BW loss to be less than the true loss. If this is occurring, our data may underestimate the difference in efficiency between treatments because DMI was less for cows fed P169.

Table 6. Effect of feeding *Propionibacterium* P169 on concentrations of rumen VFA (mol/100 mol total VFA)¹

VFA	Control	P169	$P <^2$	
			Treatment	Int
Acetate (SE = 1.07)			0.01	0.67
-7 d	68.5	64.9		
7 d	63.0	60.7		
100 d	62.4	58.8		
Propionate (SE = 1.17)			0.09	0.15
-7 d	16.3	18.7	0.10	
7 d	20.7	20.4	0.80	
100 d	21.2	24.8	0.05	
Acetate:Propionate (SE = 0.20)			0.06	0.11
-7 d	4.22	3.58	0.03	
7 d	3.05	3.14	0.75	
100 d	2.98	2.44	0.09	
Butyrate (SE = 0.64)			0.13	0.07
-7 d	10.7	12.0	0.11	
7 d	12.7	14.7	0.03	
100 d	12.9	11.9	0.30	
Valerate (SE = 0.23)			0.06	0.25
-7 d	1.48	1.67		
7 d	1.72	2.18		
100 d	1.60	2.40		

¹Days expressed relative to calving; treatment started on d -14.

²Day affected ($P < 0.05$) all measures. The P -values in the row with the VFA name are from the repeated-measures model. When an interaction of time $\times$ treatment (Int) was suggested ($P < 0.15$), the SLICE option (SAS Institute, 2004) was used to compare treatments at each time period. The SE shown is associated with time $\times$ treatment cell means.

Rumen VFA

Treatment effects on the major rumen VFA varied depending on day of sampling (Table 6). For the prepartum sample (-7 d), cows fed P169 had greater concentrations of both propionate ($P < 0.10$) and butyrate ($P < 0.11$) with a concomitant decrease in acetate. At the early postpartum time point (7 DIM), cows fed the additive had greater butyrate ($P < 0.03$) and lower acetate ($P < 0.01$). At the established lactation time point (100 DIM), cows fed P169 had greater propionate and lower acetate. Most previous studies found greater propionate (as a proportion of total VFA) when propionibacteria were fed (Kim et al., 2000; Stein et al., 2006; Raeth-Knight et al., 2007). Reasons why P169 increased propionate concentration at some time points and butyrate at other time points in this study is not clear, but Stein et al. (2006) also reported variable changes in ruminal propionate and butyrate when *Propionibacterium* P169 was fed. In that study, a low feeding rate of *Propionibacterium* (6×10^{10} cfu/d) tended to increase butyrate concentration and a high feeding rate (6×10^{11} cfu/d) tended to increase propionate concentration. In our study, P169 increased butyrate concentrations during time points with low DMI (average DMI at -7, 7, and 100 d was 12.6, 15.4, and 25.7 kg/d). Whether DMI is related to responses is unclear, but because of the way the

additive was fed, intake of P169 likely did not differ greatly among time points. The time point when treatment did not affect ruminal propionate (7 DIM) corresponds to a time when the rumen was in flux. Cows underwent a substantial diet change (prefresh to lactation diet) 1 wk before this sample was taken and DMI was changing rapidly (approximately 0.8 kg/d). Whether this variability influences the effect of P169 is not known.

No treatment effects or treatment by time interactions were observed for isobutyrate and isovalerate (data not shown). However, isobutyrate and isovalerate comprised a larger percentage ($P < 0.05$) of total VFA (on a molar basis) in rumen fluid collected during the prepartum period (1.09 and 1.71% of total VFA for isobutyrate and isovalerate, respectively) than that collected during the postpartum period (0.81 and 1.26% for isobutyrate and isovalerate). Cows fed P169 had greater ($P < 0.06$) concentrations of valerate across all time points (Table 3). In relative terms, treatment had a large effect on valerate (average increase was 30%), but in absolute terms, the effect was quite modest (0.5 percentage units). Valerate was not affected when *Propionibacterium* (different strain than used in this study) was fed to feedlot cattle receiving a high concentrate diet (Ghorbani et al., 2002). Ruminal valerate can increase when cows are fed diets with reduced RDP (Seymour et al., 1992) but based on the NRC (2001) model, RDP was the same in both diets (Table 2).

Blood Metabolites and Health

Similar to results of Francisco et al. (2002), plasma glucose concentrations were affected by time ($P < 0.01$)

but not by treatment or their interaction (data not shown). The time effect was caused by the difference between prepartum and postpartum samples (averaged 3.4 and 2.8 mM for pre- and postpartum samples, respectively). Milk glucose concentrations were also affected by time but not by treatment or the interaction (data not shown). Concentrations for treatment and control averaged 0.52 and 0.53 mM (SE = 0.03). The greatest concentrations were observed during the first week of lactation (0.60 mM), and the lowest concentrations were found during the eighth week of lactation (0.47 mM). Faulkner (1999) suggested that the concentration of milk glucose reflects intracellular concentrations of glucose (at least in the goat). The lack of a treatment effect on both plasma and milk glucose suggests that treatment did not greatly influence glucose supply.

Concentrations of plasma NEFA were affected by time ($P < 0.01$) and the time by treatment interaction ($P < 0.03$), but not by treatment (Figure 1). Cows fed P169 had greater ($P < 0.05$) plasma NEFA concentrations at 6 DIM but treatment means were similar at other time points. The interaction and lack of treatment effect observed in this experiment was identical to effects observed by Francisco et al. (2002). Cows fed P169 produced more ECM ($P < 0.05$) during the first week of lactation; therefore, calculated NE_L for maintenance and milk production was 3.7 Mcal/d higher ($P < 0.05$; data not shown). Because DMI was equal during the first week of lactation, cows fed P169 likely mobilized more body fat, resulting in greater NEFA concentrations during the first week of lactation.

Figure 1. Effect of feeding control diet (□, △) or *Propionibacterium* P169 (■, ▲) on concentrations of NEFA (squares, ---, mEq/L) and BHBA (triangles, —, mM). Treatment did not affect concentrations except for NEFA on d 6 ($P < 0.01$).

Plasma BHBA was affected by time ($P < 0.01$) but not by treatment or the time by treatment interaction. Concentrations of BHBA peaked at 6 DIM but remained high throughout the first 4 wk of lactation (Figure 1). Using a threshold concentration of >1.2 mM of BHBA (Duffield et al., 1998), 44, 30, and 31% of all cows had subclinical ketosis on 7, 14, and 28 DIM, respectively. Treatment did not affect prevalence of subclinical ketosis on d 7 (42 vs. 48% for control and treatment cows), but cows fed P169 tended to have less subclinical ketosis on d 14 (18 vs. 40%; $P < 0.08$) and d 28 (17 vs. 40%, $P < 0.07$). The significance of less subclinical ketosis may be minor because no difference was observed between treatments in the number of cows treated for ketosis during the first 30 DIM (13 and 14 cows for control and treatment), and no difference was observed in milk yield during the first 28 d of lactation. For unknown reasons, the prevalence of retained fetal membranes and metritis was reduced ($P < 0.03$) when P169 was fed (4 vs. 24% for treatment and control, respectively, for both ailments). The mode of action is not clear, and additional validation of the results is needed.

CONCLUSIONS

Dairy cows fed 6×10^{11} cfu of *Propionibacterium* strain P169 daily during late gestation and the first 17 wk of lactation produced similar amounts of milk with similar composition as did cows not fed the additive. Calculated energy expenditures for maintenance, milk production, and BW changes over the first 17 wk of lactation were similar between treatments, but cows fed the additive had lower DMI resulting in a 4.4% increase in the efficiency of converting dietary DM into NE_L . The most likely mode of action was altered ruminal fermentation.

ACKNOWLEDGMENTS

Salaries and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Additional funds were provided by DSM Nutritional Products, Parsippany, NJ. Manuscript 27-07AS.

REFERENCES

- AOAC. 2000. Official Methods of Analysis. Vol. 1 and 2. 17th ed. AOAC International, Gaithersburg, MD.
- Dado, R. G., D. R. Mertens, and G. E. Shook. 1993. Metabolizable energy and absorbed protein requirements for milk component production. *J. Dairy Sci.* 76:1575–1588.
- Duffield, T. F., D. Sandals, K. E. Leslie, K. Lissemore, B. W. McBride, H. Lumsden, P. Dick, and R. Bagg. 1998. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. *J. Dairy Sci.* 81:2866–2873.
- Ellis, J. L., F. Qiao, and J. P. Cant. 2006. Evaluation of net energy expenditures of dairy cows according to body weight changes over a full lactation. *J. Dairy Sci.* 89:1546–1557.
- Faulkner, A. 1999. Changes in plasma and milk concentrations of glucose and IGF-1 in response to exogenous growth hormone in lactating goats. *J. Dairy Res.* 66:207–214.
- Francisco, C. C., C. S. Chamberlain, D. N. Waldner, R. P. Wettemann, and L. J. Spicer. 2002. Propionibacteria fed to dairy cows: Effects on energy balance, plasma metabolites and hormones, and reproduction. *J. Dairy Sci.* 85:1738–1751.
- Ghorbani, G. R., D. P. Morgavi, K. A. Beauchemin, and J. A. Z. Leedle. 2002. Effects of direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. *J. Anim. Sci.* 80:1977–1986.
- Kim, S. W., D. G. Standorf, H. Roman-Rosario, M. T. Yokoyama, and S. R. Rust. 2000. Potential use of *Propionibacterium acidipropionici*, strain DH42 as a direct-fed microbial for cattle. *J. Dairy Sci.* 83(Suppl. 1):292. (Abstr.)
- McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for dairy cattle: Current status and future outlook. *J. Dairy Sci.* 84(E Suppl.):E194–E203.
- Nielsen, N. I., and K. L. Ingvarsen. 2004. Propylene glycol for dairy cows—A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. *Anim. Feed Sci. Technol.* 115:191–213.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Oba, M., and M. S. Allen. 2003. Dose-response effects of intraruminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. *J. Dairy Sci.* 86:2922–2931.
- Orskov, E. R., and D. M. Allen. 1966. Utilization of salts of volatile fatty acids by growing sheep. 1. Acetate, propionate, and butyrate as sources of energy for young growing lambs. *Br. J. Nutr.* 20:295–317.
- Raeth-Knight, M. L., J. G. Linn, and H. G. Jung. 2007. Effect of direct-fed microbial on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. *J. Dairy Sci.* 90:1802–1809.
- Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.* 86:1201–1217.
- Rogers, J. A., and C. L. Davis. 1982. Rumen volatile fatty acid production and nutrient utilization in steers fed a diet supplemented with sodium bicarbonate and monensin. *J. Dairy Sci.* 65:944–952.
- SAS Institute. 2004. SAS/STAT User's Guide, Version 9. SAS Institute Inc., Cary, NC.
- Seymour, W. M., C. E. Polan, and J. H. Herbein. 1992. In vivo degradation of protein in diets formulated for two degradabilities. *J. Dairy Sci.* 75:2447–2453.
- Stein, D. R., D. T. Allen, E. B. Perry, J. C. Bruner, K. W. Gates, T. G. Rehberger, K. Mertz, D. Jones, and L. J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. *J. Dairy Sci.* 89:111–125.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. *J. Dairy Sci.* 48:1215–1223.
- Weiss, W. P., and D. J. Wyatt. 2000. Effect of oil content and kernel processing of corn silage on digestibility and milk production by dairy cows. *J. Dairy Sci.* 83:351–358.