Propionibacteria Fed to Dairy Cows: Effects on Energy Balance, Plasma Metabolites and Hormones, and Reproduction¹

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ABSTRACT

To determine the effect of feeding Propionibacteria on energy balance, milk yield, and composition, metabolites and hormones of early-lactating dairy cows, multiparous Holstein cows were individually fed a total mixed ration from -2 to 12 wk postpartum with no addition (control, $n = 10$) or with an additional 17 g of Propionibacteria culture daily (Treated, $n = 9$). Daily feed intake and milk production were recorded. Plasma cholesterol, nonesterified fatty acids (NEFA), leptin, insulin, glucose, insulin-like growth factor-I (IGF-I), IGF-binding proteins (IGFBP), and progesterone concentrations were measured up to twice weekly. Cows fed supplemental Propionibacteria had improved energy balance at wk 1 of lactation and had lower DMI per kg of body weight than control cows on wk 3 to 7, 10, and 12. Cows fed Propionibacteria had a greater percentage of milk protein and solids-not-fat and plasma NEFA concentrations than did control cows only at wk 1 of lactation. Treatment did not affect milk production or percentage of milk fat and lactose. Leptin levels were greater in treated than control cows throughout the study. Plasma glucose, insulin, cholesterol, IGFBP-3, and IGF-I concentrations were not affected by feeding Propionibacteria, but those variables increased with week postpartum. Plasma IGFBP-2 and IGFBP-5 levels decreased with week postpartum. Measures of reproductive and ovarian function did not differ between Propionibacteria-treated and control cows. Feeding Propionibacteria culture to transition and early lactating dairy cows may hold potential for improved milk protein production and metabolic efficiency during early lactation, without affecting reproductive function.

(**Key words:** energy balance, propionibacteria, ovarian activity, insulin-like growth factor)

Abbreviation key: ADU = arbitrary densitometric units, DMV = daily milk yield, EB = energy balance, IGFBP = IGF binding protein(s), MUN = milk urea nitrogen, P₄ = progesterone, RIA = radioimmunoassay.

INTRODUCTION

Many high producing dairy cows are unable to consume enough feed to meet the energy demands of early lactation, resulting in a state of negative energy balance. Energy balance (EB) is quantified using measures of DMI, milk production (quantity and composition) and BW, and may be associated with reproductive efficiency. In lactating dairy cows, EB during the first few weeks postpartum is positively related to concentrations of plasma progesterone (P₄) during the first postpartum estrous cycle (Villa-Godoy et al., 1988; Spicer et al., 1990). Cows exhibiting estrus with subsequent formation of a functional corpus luteum that secretes a greater amount of P₄ have the best chance of maintaining pregnancy (Villa-Godoy et al., 1988). In addition, cows that express estrus before the first postpartum ovulation have greater EB than cows that do not express estrus (Spicer et al., 1990). Negative EB is, therefore, a likely cause for poor reproductive efficiency in lactating dairy cows (Kimura et al., 1987; Opsomer et al., 1996). Because plasma cholesterol (Carroll et al., 1990; Spicer et al., 1993), insulin (Koprowski and Tucker, 1973; Smith et al., 1976) and IGF-I (Spicer et al., 1990, 1993) increase, whereas plasma NEFA decrease (Staples et al., 1990; Beam and Butler, 1998) with increasing week of lactation, those hormones and metabolites are primary candidates for transmitting the metabolic status of a cow to its reproductive axis. Indeed, concentrations of cholesterol and IGF-I in blood of cattle are modified by variations in fat, protein, and (or) energy intake, and increase as EB increases (Grummer and Davis, 1984; Spicer et al., 1990). Moreover, insulin and IGF-I stimulate mitogenesis and steroidogenesis of bovine ovarian cells in vitro (for review see

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Spicer and Echternkamp, 1995), and thus, negative EB may affect ovarian activity by decreasing luteal P₄ production (Grummer and Carroll, 1988; Spicer et al., 1993). Recent studies also implicate leptin as a possible metabolic mediator of reproduction by inhibiting steroidogenesis of bovine granulosa and theca cells (Spicer and Francisco, 1997, 1998).

Propionate, a ruminal VFA, acts as a precursor for hepatic glucose production. Propionate infusion at 200 g in the abomasum of heifers for 21 d increased glucose concentration compared with control animals (Rutter et al., 1983). Similarly, intravenous infusion of propionate increased plasma glucose and insulin in sheep (Sano et al., 1993, 1995). Drenching lactating cows with calcium propionate (Jonsson et al., 1998) increased plasma glucose concentrations. Conversely, infusion of butyrate (a ruminal VFA that inhibits the use of propionate for gluconeogenesis into the rumen of lactating cows) decreased plasma glucose concentrations (Huhtanen et al., 1993). Whether plasma insulin, IGF-I, cholesterol, and other metabolites are altered by changes in ruminal propionate in lactating dairy cows is unknown.

Propionibacteria are natural inhabitants of the rumen that make up 1.4% of the ruminal microflora and produce propionic acid in the rumen (Oshio et al., 1987). Directly feeding Propionibacteria may increase hepatic glucose production via increased ruminal propionate production and absorption. Theoretically, the efficiency of propionate as a source of energy in the form of ATP is 109% compared with glucose (McDonald et al., 1987). The efficiency of utilization for maintenance of propionic acid is 0.86 versus 0.59 for acetate and 0.76 for butyrate (McDonald et al., 1987). Thus, the present study was conducted to test the hypothesis that manipulating rumen microflora by supplementing the diet with a direct-fed microbial, such as Propionibacteria culture, will increase plasma concentration of glucose, IGF-I, insulin, and (or) other metabolic modulators of reproductive function. This would then potentially increase plasma P₄ concentrations and shorten days to the first postpartum estrous cycle.

MATERIALS AND METHODS

Experimental Design and Sample Collection

Twenty Holstein multiparous cows were assigned randomly to one of two dietary groups: TMR without Propionibacteria (control, $n = 10$) or TMR plus Propionibacteria (treated, $n = 10$) from -2 to 12 wk postpartum. Cows calved between November 26, 1997, and March 26, 1998. Cows were allowed continuous access to water. Each treated cow received 17 g of Propionibacteria culture (Agtech Products Inc., Waukesha, WI) once daily, top-dressed into 1 to 2 kg of the TMR. Cows were

individually fed and housed in a stanchion barn and grouped by treatment to prevent potential transfer of Propionibacteria from treated to control cows. Half of each group of cows were placed facing across from each other, and treatment groups were separated from each other by two unoccupied stalls. Each day, control and treated cows were separated, and each group was placed in one of the two adjacent dry lots for two 4- to 5-h intervals (0900 to 1200 h and 2100 to 0300 h). One Propionibacteria-treated cow was taken out of the study due to a foot problem. Weekly BW were recorded and BCS of the cows were evaluated at wk 4 and 10 postpartum using a five-point scale: 1 = very thin to 5 = excessively fat (Sniffen and Ferguson, 1995).

The TMR was composed of sorghum/sudan silage, alfalfa hay, whole cottonseed, and concentrate (Table 1). Energy concentration of the diet was formulated to support daily milk production of 50 kg (NRC, 1989). Daily feed intake was recorded and the diet was sampled weekly and composited monthly for analysis.

Cows were milked twice daily (0300 and 1500 h) and milk yield was recorded. Milk samples were collected weekly during successive a.m. and p.m. milkings and analyzed for percentage milk fat, protein, lactose, and SNF, and milk urea nitrogen (MUN). The average interval to the first postpartum milk collection was 5 ± 1 d for control cows and 4 ± 1 d for treated cows. Milk production was corrected for milk fat and identified as FCM.

Blood samples (10 ml) were collected twice weekly at 3- to 4-d intervals via coccygeal venipuncture. After collection in tubes containing EDTA, blood was centrifuged at $1200 \times g$ for 20 min (5°C), and plasma was decanted and stored frozen at -20°C for subsequent analysis. The average interval to the first postpartum blood collection was 5 ± 1 d for control cows and 4 ± 1 d for treated cows.

Ovaries of cows were scanned weekly by transrectal ultrasonography using a linear array ultrasound scanner equipped with 7.5 MHz rectal probe (Corometrics Medical Systems, Inc., Wallingford, CT) starting at 3 wk and continuing through 12 wk postpartum to measure follicles and determine presence of a corpus luteum. Follicles on right and left ovaries were measured and categorized as largest, second largest, or third largest follicle per cow during 3 to 12 wk postpartum. Furthermore, follicles were categorized as small (3 to 5 mm), medium (6 to 9 mm), and large (≥ 10 mm) and counted as described by Lucy et al. (1991a).

Assays

Concentrations of IGF-I in plasma were determined in all samples by radioimmunoassay (RIA) after acid-

Table 1. Ingredient and nutrient composition of the control diet (DM basis).

Ingredient	%	Nutrient	%
Sorghum/Sudan silage	31.87	DM (as fed)	69.7
Alfalfa hay	21.25	CP	18.73
Cottonseed with lint	7.97	ADF	20.76
Rumofat ¹	0.86	NDF	30.94
Blood meal	0.86	NE ₁ , Mcal/kg	1.78
Mix 1	37.18	Ca	0.86
Corn, ground	23.39	P	0.47
Corn, distillers dehydrated	2.68	Mg	0.32
Soybean hulls	2.68	K	1.23
Soybean meal 48	6.69	S	0.22
Salt-white	0.13	Na	0.34
Sodium bicarbonate	0.52		ppm
Zinpro 4-plex ²	0.04	Fe	81.26
Limestone	0.20	Zn	54.66
Magnesium oxide	0.07	Cu	15.74
Ca 23%: P 18%	0.56	Mn	50.91
Minerals/Vitamins	0.19		

¹Robt Morgan, Inc., Paris, IL, contains: free fatty acids—93%, MIU—2.0% and cane molasses flavor—5.0%.

²Zinpro Corp., Eden Prairie, MN contains, Zinc—2.58%, Mn—1.43%, Cu—0.90%, Co—0.18%, Methionine—8.21%, Lysine—3.8%.

ethanol extraction (16 h at 4°C) as described previously (Echternkamp et al., 1990). Intraassay and interassay coefficients of variation were 13 and 15%, respectively.

Plasma P₄ concentrations were determined in all samples using a solid phase RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) without extraction, as previously described (Stewart et al., 1996). Intraassay and interassay coefficients of variations were 8.7 and 8.6%, respectively.

Plasma concentrations of insulin were determined in all samples by using a solid-phase insulin RIA kit (Micromedic Insulin Kit, ICN Biomedicals, Costa Mesa, CA), except that bovine insulin was used as a reference standard (25.7 IU/mg) as previously described (Simpson et al., 1994). Intraassay and interassay coefficients of variation were 12.8 and 7.8%, respectively.

Plasma concentrations of glucose were determined in all samples in one assay using a Glucose kit (Roche Diagnostic Systems, Inc., NJ) and a clinical analyzer (Cobas FARA II, Roche Analytical Instrument, Montclair, NJ). The intraassay coefficient of variation was 2.3%.

Concentrations of total plasma cholesterol were determined in all samples using an enzymatic method using a total cholesterol kit (Sigma, St. Louis, MO) as previously described (Spicer et al., 1993). Intraassay and interassay coefficients of variation were 2.2 and 1.4%, respectively.

Concentrations of NEFA were determined in plasma samples collected on wk 1, 6, and 12 postpartum by an enzymatic method using NEFA-C kits (Waco Chemicals USA, Inc., VA) and a clinical analyzer (Cobas FARA

II, Roche Analytical Instrument, Montclair, NJ). The intraassay coefficient of variation was 4.5%.

Concentrations of leptin were measured in plasma samples collected during wk 2, 4, 6, 8, 10, and 12 postpartum using a multi-species RIA kit (LINCO Research, Inc., St. Charles, MO) as previously described (Maciel et al., 2001). Briefly, on d 1, 100 µl of first antibody were added to all tubes, except total count and nonspecific binding tubes. Then tubes were vortexed, covered, and incubated for 24 h at 4°C. The standard curve was modified to include 1, 2, 3, 5, 10, and 20 ng/ml of human leptin standard. On the second day, 100 µl of the tracer (¹²⁵I-human leptin) was added to all tubes, which were then incubated for another 24 h at 4°C. On the third day, 1.0 ml of precipitating reagent was added to all tubes except total count tubes, and all tubes were incubated for 20 min at 4°C. Tubes were centrifuged at 3000 × *g* for 30 min, the supernatant was decanted, and the precipitate was counted using a gamma counter. The sensitivity of the assay as defined as 95% of total binding was 0.85 ± 0.08 ng/ml. The intraassay coefficient of variation was 9.8%.

Ligand Blots

Plasma samples of the cows collected on wk 1, 6, and 12 postpartum were analyzed for IGF binding proteins (IGFBP) using one-dimensional SDS-PAGE (Stewart et al., 1996; Maciel et al., 2001). Briefly, 4 µl of each sample was mixed with 21 µl of nonreducing denaturation buffer (62.5 mM Tris-HCl, 2% SDS, 25% glycerol, and 0.01% bromphenol blue without mercap-

toethanol) (BIORAD, Hercules, CA). Proteins were denatured at 100°C for 3 min and centrifuged at 4700 $\times g$ for 3 min and loaded to wells of 12% PAGE (15 lanes per gel). All plasma samples from 1 to 2 cows from each treatment group were run on each gel. Four gels were run at a time in a single electrophoresis chamber. Control lanes included either 25 μ l of wide range color marker (MW 6500 to 205,000, Sigma, St. Louis, MO) or a mixture of 4 μ l of bovine plasma pool and 21 μ l of the nonreducing denaturation buffer. Gels were run for 18 to 20 h at constant current and varying voltage, and bands were transferred using nitrocellulose paper (Midwest Scientific, St. Louis, MO) for 2.5 to 3.0 h and hybridized with 125 I-IGF-II (about 15,000 cpm/0.1 ml; total volume = 6 ml) at 4°C for 12 h in a platform shaker. Nitrocellulose blots were washed with Tris-buffered saline and 0.1% Tween, and then washed again with Tris-buffered saline. Nitrocellulose was dried and placed on X-ray film for 5 d at -80°C. X-ray films were developed and bands measured using a densitometer (Molecular Analyst, BIORAD). Variation among gels was monitored by running the same bovine plasma pool on each gel; IGFBP-2, -3 and -5 bands were scanned and the resultant arbitrary densitometric units (ADU) for each IGFBP was used to calculate inter-gel coefficient of variation, which averaged $19.4 \pm 4.5\%$.

Energy Balance Calculations

Daily EB was calculated each week as: NE_L intake—net energy required for maintenance—net energy secreted in milk. Net energy intake was calculated as the average daily DMI for the week, multiplied by the NE_L concentration of the diet. Net energy (Mcal) required for daily maintenance of the animals was derived using the equation $80 \times BW^{0.75}$ (kg)/1000 (NRC, 1989). Daily energy for milk production was calculated using the formula (Tyrell and Reid, 1965): $EB = \text{milk yield (kg)} \times [92.239857(\% \text{ milk fat}) + 49.140211(\% \text{ SNF}) - 56.393297]/1000$, where milk yield is the average daily yield for the week, and milk composition is based on weekly milk analysis of the a.m. and p.m. samples. Energy balance was expressed as megacalories of NE per day for each week and could be positive or negative.

Maximum Progesterone and Area Under Progesterone Curve Calculations

Days of first and second ovulations were defined as the days to first and second increase in plasma $P_4 \geq 1.0$ ng/ml that were maintained for three or more sampling days. Based on those criteria, 79.8% of the

cows (8 of 10 control and 7 of 9 treated cows) had a “first ovulation” and 57.8% of the cows (7 of 10 control and 4 of 9 treated cows) had a “second ovulation” during the 12-wk study. Data for the third postpartum ovulation were not included in the analysis because of the small number of cows that exhibited a third estrous cycle within 90 d postpartum (4 of 10 control and 2 of 9 treated cows). The maximum P_4 concentration and area under P_4 curve were used to evaluate luteal activity of the early postpartum cows. Peak P_4 concentration was defined as the maximum concentration of P_4 achieved during diestrus of the first and second postpartum estrous cycles of the cows. Multiple regression analysis was utilized to fit a curve through the initial and final nadirs of each estrous cycle, and a definite integral of the equation generated the P_4 area under curve.

Statistical Analyses

Milk production and composition, EB, BW, BCS, DMI, plasma hormones, metabolites, and IGFBP, and numbers and size of follicles were analyzed as a completely randomized design for repeated measures, utilizing the mixed model (Littell et al., 1996): $Y_{ijk} = U + D_i + C(D)_{ij} + W_k + (D \times W)_{ik} + e_{ijk}$ where U = overall mean, D = diet, $C(D)$ = cow within dietary group, W = week postpartum, $(D \times W)$ = diet $\times$ week-postpartum interactions, and e = residual error. Similarly, P_4 area under the curve, maximum P_4 concentration and intervals to first and second postpartum ovulations were analyzed using the same procedure and model but cycle number was used instead of week. The model of the covariate structure for repeated measurements was an autoregressive with lag equal to one (Littell et al., 1996). If the week $\times$ diet interaction was significant, simple effects of diet were analyzed using the slice option for the LSMEANS statement. Conversely, main effects were analyzed using LSMEANS with the DIFF option if the interaction was not significant. Relationships between EB, FCM, and milk composition, plasma metabolites and hormones were calculated using Pearson correlation coefficients (Ott, 1977).

RESULTS

Energy Balance, BW, and BCS

Week postpartum ($P < 0.001$) and treatment ($P < 0.10$) affected EB (Figure 1A), but treatment $\times$ week postpartum was not significant. Average EB on wk 1, 3, and 6 differed ($P < 0.05$) from their succeeding week by -3.1, -2.5, and -2.27 Mcal/d, respectively. Average EB tended to differ ($P < 0.10$) between cows fed Propionibacteria (-1.596 ± 0.72 Mcal/d) and control cows

Figure 1. Weekly changes in energy balance (panel A) and average daily DMI (g per kg of BW basis; panel B) of postpartum cows fed Propionibacteria ($n = 9$) and control ($n = 10$) diets during the first 12 wk postpartum. Panel A: Pooled SEM was 1.48 Mcal/d for control and 1.54 Mcal/d for Propionibacteria-treated cows. Panel B: Pooled SEM was 0.8 g/kg of BW per day for control and 0.8 g/kg BW per day for Propionibacteria-treated cows. Means within week indicated by an "a" are greater than (panel A, $P < 0.10$; panel B, $P < 0.01$) the respective weekly means.

(0.196 ± 0.69 Mcal/d) during the 12-wk feeding period, particularly on wk 1, 7, and 10 (Figure 1A). The first week that EB was significantly greater than zero was at wk 9 in control cows, and at wk 11 in treated cows.

During the first 12 wk of lactation, average BW of postpartum cows tended to differ ($P < 0.10$) between Propionibacteria-treated ($667.1 \text{ kg} \pm 19 \text{ kg}$) and control ($616.2 \pm 18 \text{ kg}$) cows, but BW was not affected ($P > 0.50$) by treatment $\times$ week postpartum. Also, weekly BW differed ($P < 0.001$) among weeks postpartum. In both groups of cows, BW decreased between wk 1 and 3, but did not change between wk 5 and 12 of lactation (data not shown).

Treatment and treatment $\times$ week postpartum did not affect ($P > 0.50$) BCS, measured at wk 4 and 10 postpartum; BCS ranged from 2.5 to 3.75 and aver-

aged 2.69 ± 0.08 for control cows and 2.68 ± 0.06 for Propionibacteria-treated cows. Average weekly BCS increased ($P < 0.01$) from wk 4 to 10 (2.53 ± 0.07 vs. 2.86 ± 0.06) in both groups of cows.

DMI and Daily Milk Yield

Dry matter intake (g/kg of BW) per day was lower ($P < 0.01$) in Propionibacteria-treated (35.3 ± 0.8 g/kg) than control (38.9 ± 0.9 g/kg) cows starting from wk 3 and continuing through wk 7, 10, and 12 postpartum (Figure 1B). DMI consistently increased ($P < 0.05$) from wk 1 to 12 of lactation in both groups of cows but treatment $\times$ week postpartum did not affect ($P > 0.50$) DMI expressed as grams per kilogram of BW (Figure 1B). The DMI at wk 1, 2, 3, and 4 postpartum were less ($P < 0.01$) than their succeeding week (Figure 1B). DMI expressed as kilograms per day was not affected ($P > 0.10$) by treatment (control = 23.9 ± 0.5 kg/d; Propionibacteria = 23.4 ± 0.5 kg/d) or treatment $\times$ week but increased ($P < 0.05$) between wk 1 (15.9 ± 0.6 kg/d) and 6 (24.7 ± 0.6 kg/d) (data not shown).

Daily milk yield (DMY) and FCM production did not differ ($P > 0.50$) between the control and treated cows over the 12-wk study. Also, the interaction of treatment and week postpartum did not affect ($P > 0.50$) DMY or FCM (data not shown). Daily milk yield and FCM averaged 40.9 ± 1.4 and 34.49 ± 0.86 kg/d for control cows and 39.9 ± 1.4 and 35.16 ± 0.89 kg/d for Propionibacteria-treated cows, respectively. There were weekly changes ($P < 0.001$) in FCM production of the cows. Between wk 1 and 3 of lactation, FCM production increased ($P < 0.05$) from 29.0 ± 1.3 to 36.0 ± 1.3 kg/d, whereas between wk 9 and 11, FCM production decreased from 36.6 ± 1.3 to 31.9 ± 1.3 kg/d. Postpartum changes in DMY were similar to changes in FCM production (data not shown).

Milk Protein and SNF

There was a significant interaction ($P < 0.001$) between treatment and week postpartum on percentage of milk protein. Propionibacteria-treated cows had higher ($P < 0.001$) percentage milk protein than control cows on wk 1 of lactation but not in the subsequent weeks (Figure 2A). Percentage milk protein decreased ($P < 0.05$) from wk 1 to 3 in both groups of cows, remained unchanged between wk 4 and 12 in control cows, but decreased ($P < 0.05$) between wk 3 and 12 in Propionibacteria-treated cows (Figure 2A).

A significant interaction between treatment and week postpartum ($P < 0.05$) on percentage milk SNF existed such that Propionibacteria-treated cows had a higher ($P < 0.001$) percentage SNF than control cows

Figure 2. Weekly changes in percentage of milk protein (panel A) and milk SNF (panel B) of postpartum cows fed Propionibacteria ($n = 9$) and control ($n = 10$) diets during the first 12 wk of lactation. Panel A: Pooled SEM = 0.15% for control and 0.16% for Propionibacteria-treated cows. The mean with an "a" (wk 12) within Propionibacteria-treated cows, differs ($P < 0.05$) from means on wk 3 and 4. Panel B: Pooled SEM = 0.14% for control and 0.15% for Propionibacteria-treated cows; Means with a "b" within control cows, differ ($P < 0.05$) from week 3. The "c" indicates differences ($P < 0.05$) from respective control means in both panels A and B.

on wk 1 of lactation but not during the following weeks (Figure 2B). Percentage SNF decreased ($P < 0.05$) from wk 1 to 3 in both groups of cows, remained unchanged between wk 3 and 12 in Propionibacteria-treated cows, but decreased ($P < 0.05$) between wk 3 and 10 in control cows (Figure 2B).

Milk Fat and Lactose

There was no treatment effect ($P > 0.10$) nor interaction ($P > 0.50$) between treatment and week postpartum

on percentages of both milk fat and lactose. Milk fat percentage averaged $3.2 \pm 0.08\%$ in Propionibacteria-treated cows and $3.02 \pm 0.08\%$ in control cows ($P > 0.10$). Averaged across treatment groups, milk fat percentage decreased ($P < 0.001$) 24% between wk 1 and 12 postpartum (data not shown).

There was no treatment effect ($P > 0.10$) nor interaction ($P > 0.50$) between treatment and week postpartum on the percentage of lactose in milk. Percentage of lactose averaged $5.0 \pm 0.02\%$ and $4.98 \pm 0.02\%$ in Propionibacteria-treated and control cows, respectively, over the 12-wk period and did not differ ($P > 0.10$) between the two groups of cows. However, week affected ($P < 0.001$) percentage of lactose in milk such that percentage of lactose in milk increased ($P < 0.05$) 5% from wk 1 to 2, remained unchanged between wk 3 and 5, and decreased ($P < 0.05$) 3% between wk 5 and 10 (data not shown).

Milk Urea Nitrogen

There was no treatment effect ($P > 0.50$) or interaction ($P > 0.50$) between treatment and week postpartum on MUN. Concentrations of MUN averaged 20.15 ± 0.42 and 20.26 ± 0.66 mg/dl in control and Propionibacteria-treated cows, respectively, and did not differ ($P > 0.50$). However, MUN concentrations changed ($P < 0.001$) with week postpartum. There was a 28% increase ($P < 0.001$) in MUN levels between wk 2 and 3 postpartum from 15.5 ± 1.1 to 19.9 ± 1.1 mg/dl. After wk 3, concentrations of MUN were constant (data not shown).

Glucose and Insulin

Neither plasma glucose ($P > 0.10$) nor plasma insulin ($P > 0.50$) concentrations were affected by treatment or treatment $\times$ week postpartum. Cows fed Propionibacteria had an average plasma glucose concentration of 60.0 ± 0.9 mg/dl compared with 60.0 ± 0.9 mg/dl control cows ($P > 0.50$). Concentrations of glucose in plasma increased ($P < 0.01$) with week postpartum such that glucose concentration at wk 2 was 4% greater ($P < 0.01$) than at wk 1, and wk 5 was 7% greater ($P < 0.01$) than wk 2 (Figure 3). Plasma glucose levels did not significantly change after wk 5 postpartum.

Average concentration of insulin in plasma of cows fed Propionibacteria was similar ($P > 0.10$) to that for control cows (0.39 ± 0.02 vs. 0.42 ± 0.02 ng/ml, respectively). Concentrations of insulin in plasma increased ($P < 0.001$) with week postpartum, such that insulin concentrations at wk 4 were 50% greater ($P < 0.01$) than wk 1, and wk 10 was 38% greater ($P < 0.01$)

Figure 3. Weekly changes in plasma glucose and insulin concentration of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled ($n = 19$) because treatment $\times$ week interaction and treatment were not significant. Pooled SEM = 0.034 ng/ml for insulin and 1.22 mg/dl for glucose. The “a” on the graph indicates the first mean that differs from wk 1 mean ($P < 0.01$). The “b” indicates the first mean that differs from wk 2 (glucose) or wk 4 (insulin) means ($P < 0.05$).

than wk 4 (Figure 3). Plasma insulin concentrations increased gradually over the 12-wk period such that, plasma insulin concentration at wk 10 was double that of wk 1 (0.25 ± 0.03 ng/ml at wk 1 vs. 0.51 ± 0.03 ng/ml at wk 10); plasma insulin concentration did not change ($P > 0.10$) after wk 10 (Figure 3).

Cholesterol

Plasma cholesterol was not affected ($P > 0.50$) by treatment or by the interaction of treatment $\times$ week postpartum. Plasma cholesterol concentrations averaged 179.7 ± 7.1 mg/dl in Propionibacteria-treated and 178.5 ± 6.9 mg/dl in control cows. However, plasma cholesterol concentrations increased ($P < 0.001$) with week postpartum, such that concentrations of cholesterol more than doubled ($P < 0.01$) between wk 1 (85.6 ± 6.6 mg/dl) and 7 (211.7 ± 6.6 mg/dl) and remained unchanged ($P > 0.10$) between wk 8 (215.4 ± 6.6 mg/dl) and 12 (211.1 ± 6.6 mg/dl).

NEFA and Leptin

There was a significant ($P < 0.01$) treatment $\times$ week postpartum interaction on plasma NEFA concentrations. Plasma NEFA concentrations in Propionibacteria-treated cows at wk 1 postpartum were greater ($P < 0.01$) than in control cows (Figure 4A). Plasma NEFA concentrations decreased ($P < 0.001$) with week postpartum for both groups of cows, although NEFA concentrations decreased by a greater ($P < 0.05$) percentage in Propionibacteria-treated than control cows (Figure 4A).

Figure 4. Changes in plasma NEFA (panel A) and insulin-like growth factor-binding proteins (IGFBP-3, IGFBP-2, and IGFBP-5; Panel B) concentrations of postpartum cows fed Propionibacteria ($n = 9$) and control ($n = 10$) diets during the first 12 wk of lactation. Panel A: means without a common superscript differ ($P < 0.01$). Panel B: Data from cows fed Propionibacteria and control diets were pooled ($n = 19$) because treatment $\times$ week interaction was not significant. Within specific IGFBP, means at week one as indicated by an “a” differed ($P < 0.05$) from respective means on wk 6 and 12.

Plasma leptin concentrations tended to be greater ($P < 0.10$) in Propionibacteria-treated cows (8.10 ± 1.0 ng/ml) compared to control cows (5.25 ± 1.0 ng/ml) over the 12-wk study (Figure 5). However, treatment $\times$ week interaction ($P > 0.50$) and week postpartum ($P > 0.50$) did not affect plasma leptin concentrations (Figure 5).

Figure 5. Weekly changes in plasma leptin concentrations of postpartum cows fed Propionibacteria ($n = 9$) and control ($n = 10$) diets during the first 12 wk of lactation. Pooled SEM = 1.1 ng/ml for control and Propionibacteria-treated cows. Leptin means within week in the control group marked with an "a" tend to be lower ($P < 0.10$) than respective Propionibacteria means.

Insulin-like Growth Factor-I

There was no treatment ($P > 0.50$) or treatment $\times$ week postpartum effect ($P > 0.50$) on plasma IGF-I concentrations, which averaged 111.5 ± 14.0 ng/ml for control cows and 101.6 ± 14.5 ng/ml for Propionibacteria-treated cows. However, IGF-I concentrations increased ($P < 0.001$) with week postpartum such that concentrations of IGF-I on wk 2, 5, and 12 were greater than in the preceding weeks ($P < 0.05$; data not shown). Concentrations of IGF-I increased to nearly fourfold between wk 1 and 12 of lactation (49.1 ± 19.5 ng/ml at wk 1 to 195.8 ± 19.4 ng/ml at wk 12).

IGFBP

Plasma IGFBP-3 (40 to 44 kDa), IGFBP-2 (32 kDa), IGFBP-5 (30 kDa), a 28-kDa IGFBP, a 26-kDa IGFBP, and IGFBP-4 (22-kDa) concentrations were not influenced ($P > 0.50$) by treatment or treatment $\times$ week postpartum ($P > 0.50$). However, concentrations of plasma IGFBP-3, IGFBP-2, and IGFBP-5 changed ($P < 0.05$) with week postpartum (Figure 4B). Plasma IGFBP-3 concentration was 15 to 16% lower ($P < 0.001$) at wk 1 than wk 6 and 12, whereas both plasma IGFBP-2 and IGFBP-5 were 19 to 33% greater ($P < 0.001$) at wk 1 than wk 6 and 12 (Figure 4B). Plasma concentrations of the 28-kDa IGFBP, 26-kDa IGFBP, and IGFBP-4 did not vary with week postpartum ($P > 0.50$) and averaged 1.09 ± 0.1 , 0.95 ± 0.1 , and 1.16 ± 0.1 ADU/ μ l for Propionibacteria-treated cows and 1.24 ± 0.1 , 1.1 ± 0.1 , and 1.23 ± 0.1 ADU/ μ l for control cows.

Peak Progesterone and Area Under the Progesterone Curve

Area under the P_4 curve did not differ ($P > 0.50$) between Propionibacteria-treated (45.8 ± 7.9 ng·d/ml) and control (50.2 ± 5.9 ng·d/ml) cows, was not influenced ($P > 0.10$) by interaction of treatment $\times$ postpartum cycle or differ ($P > 0.50$) between the first and second postpartum estrous cycle (49.0 ± 5.9 vs. 46.9 ± 7.8 ng·d/ml, respectively). Area under the P_4 curve during first postpartum estrous cycle was not correlated ($P > 0.50$) with average EB during wk 1 ($r = -0.13$), wk 1 to 3 ($r = -0.11$) or wk 1 to 12 ($r = -0.03$).

Treatment, postpartum estrous cycle, or their interaction had no effect ($P > 0.10$) on peak P_4 concentrations. Peak luteal-phase P_4 concentration averaged 4.0 ± 0.2 ng/ml in control and 3.5 ± 0.3 ng/ml in Propionibacteria-treated cows. Peak P_4 concentration averaged 3.8 ± 0.2 ng/ml and 3.7 ± 0.3 ng/ml in the first and second postpartum estrous cycles, respectively ($P > 0.50$).

Postpartum Interval to Ovulation

Average days to first and second ovulations (i.e., first and second rise in plasma $P_4 \geq 1.0$ ng/ml) did not differ ($P > 0.10$) between control (30.1 ± 7.4 d and 57.7 ± 7.4 d, respectively) and Propionibacteria-treated (44.8 ± 7.7 d and 68.1 ± 8.4 d, respectively) cows. Average days to first postpartum ovulation were not correlated with average EB during wk 1 ($r = 0.40$, $P > 0.10$), wk 1 to 3 ($r = 0.18$, $P > 0.50$) or wk 1 to 12 ($r = -0.09$, $P > 0.50$).

Postpartum Follicular Growth

Average diameters of first, second, and third largest follicles were not influenced ($P > 0.50$) by treatment or treatment $\times$ week postpartum ($P > 0.50$). Diameter of the first and second largest follicles did not change ($P > 0.10$) with week postpartum, whereas diameter of the third largest follicles tended to increase ($P < 0.10$) with week postpartum (Figure 6). Diameters of the third largest follicles on wk 3, 4, and 5 were less ($P < 0.05$) than on wk 7, 8, 9, 10, and 11 postpartum (Figure 6).

The numbers of small, medium, and large follicles were not influenced ($P > 0.50$) by treatment, week postpartum, or their interaction ($P > 0.50$). Numbers of small, medium, and large follicles averaged 8.0 ± 0.9 , 1.6 ± 0.2 , and 0.7 ± 0.2 , respectively, in Propionibacteria-treated cows, and 7.7 ± 0.9 , 1.4 ± 0.2 , and 1.1 ± 0.2 in control cows, respectively.

Figure 6. Changes in follicle diameter classified as largest (F1), second largest (F2) and third largest (F3) of postpartum cows during the first 12 wk of lactation. Data from cows fed *Propionibacteria* and control diets were pooled ($n = 19$) because treatment $\times$ week interaction was not significant. Pooled SEM for F1 = 1.0 mm, F2 = 0.7 mm, and F3 = 0.4 mm. Means of wk 7, 8, 9, 10, and 11 as indicated with an "a" are greater than means of wk 3, 4, and 5 ($P < 0.05$).

Correlations

Simple correlation coefficients among weekly averages ($n = 228$) of EB, FCM, DMI, milk fat and protein, plasma hormones, and metabolites are shown in Table 2. Significant correlations associated with EB and its components are as follows: EB was positively correlated ($r = 0.68$, $P < 0.001$) with DMI but negatively correlated with FCM ($r = -0.38$, $P < 0.001$), milk fat ($r = -0.64$, $P < 0.001$), and milk protein ($r = -0.35$, P

< 0.001). Also, EB was positively correlated with IGF-I concentrations ($r = 0.47$, $P < 0.001$) and plasma P_4 concentrations ($r = 0.36$, $P < 0.001$). Dry matter intake was positively correlated with MUN ($r = 0.28$, $P < 0.001$), P_4 (0.40 , $P < 0.001$), cholesterol ($r = 0.69$, $P < 0.001$), IGF-I ($r = 0.43$, $P < 0.001$), insulin ($r = 0.37$, $P < 0.001$), and glucose ($r = 0.44$, $P < 0.001$).

DISCUSSION

Feeding *Propionibacteria* 1) increased plasma NEFA concentration, and percentages of milk protein and SNF during wk 1 postpartum; 2) decreased DMI per kilogram of BW on wk 3 to 6, 10, and 12 postpartum, while tending to increase plasma leptin concentrations between wk 2 and 12 postpartum; 3) did not affect BCS, FCM, percentage of milk fat and lactose, or concentrations of plasma cholesterol, insulin, glucose, IGF-I, and IGFBP-2, -3, -4, and -5; and 4) did not influence reproductive measures such as average days to ovulation, progesterone peak and area under the curve, and follicle size and numbers.

Several dietary supplements including fat (Carroll et al., 1990; Spicer et al., 1993; Beam and Butler, 1998) have been given to postpartum dairy cows in early lactation to increase EB, but have yielded inconsistent results. We found that EB of lactating cows tended to improve during wk 1 of lactation but tended to decrease during wk 7 and 10 of lactation with feeding *Propionibacteria*. Also, NEFA levels were significantly greater only during wk 1 postpartum in *Propionibacteria*-treated cows, which may indicate that these cows were in better BCS and thus had more fat to mobilize than control cows (Fronk et al., 1980;

Table 2. Correlation coefficients among average weekly ($n = 228$) energy balance (EB), DMI, FCM, BW, milk fat (FAT), milk protein (PROT), lactose (LAC), milk urea nitrogen (MUN), progesterone (P_4), cholesterol (CHOL), insulin-like growth factor-I (IGF-I), insulin (INS), glucose (GLU) and leptin (LEP, $n = 114$) during the first 12 wk of lactation in dairy cows ($n = 19$).

Variable	Milk component							Plasma component					
	FCM	DMI	BW	FAT	PROT	LAC	MUN	P_4	CHOL	IGF-I	INS	GLU	LEP
EB	-0.38**	0.68**	-0.18**	-0.64**	-0.35**	0.22**	0.14*	0.36**	0.44**	0.47**	0.32**	0.23**	-0.11
FCM		0.37**	0.17**	0.48**	-0.22**	-0.02	0.21**	-0.01	0.29**	-0.08	0.08	0.20**	0.04
DMI			0.08	-0.33**	-0.44**	0.22**	0.28**	0.40**	0.69**	0.43**	0.37**	0.44**	-0.03
BW				0.06	0.11	-0.18**	-0.09	0.07	0.07	0.14*	0.16*	0.17*	-0.06
FAT					0.20**	-0.23**	-0.16*	-0.26**	-0.29**	-0.22**	-0.13*	-0.08	-0.11
PROT						-0.33**	-0.43**	-0.19**	-0.45**	-0.24**	-0.26**	-0.18**	-0.08
LAC							0.26**	0.06	0.30**	0.20**	-0.02	0.21**	-0.05
MUN								0.08	0.43**	0.00	0.11	-0.04	0.21*
P_4									0.38**	0.37**	0.08	0.35**	0.06
CHOL										0.42**	0.31**	0.41**	-0.03
IGF-I											0.31**	0.43**	-0.17 ⁺
INS												0.35**	0.05
GLU													0.08

* $P < 0.05$.

** $P < 0.01$.

⁺ $P < 0.06$.

Nachtoml et al., 1986). In support of this suggestion, plasma leptin levels tended to be greater in Propionibacteria-treated than control cows between wk 2 and 12 postpartum. Because leptin is produced by adipocytes, and leptin concentrations rise in parallel with BCS (Delavaud et al., 2000; Ehrhardt et al., 2000), treated cows may have had higher body fat and therefore have had a higher fat mobilization capability, as indicated by higher plasma NEFA in these cows. This may explain why Propionibacteria-treated cows had greater EB and percentage milk protein and SNF than their control counterparts during wk 1 of lactation (Hart et al., 1979; Bauman et al., 1988; Tyrell et al., 1988). In the present study, systemic concentrations of leptin were unchanged between wk 2 and 12 of lactation. Kadokawa et al. (2000) and Block et al. (2001) reported that plasma leptin concentrations decrease dramatically during the periparturient period, increase slightly during the first 4 wk postpartum, and remain relatively unchanged after wk 4 postpartum in dairy cows. Both treated and control cows improved EB as week postpartum progressed, but control cows attained a more positive EB at wk 7 and 10 postpartum than treated cows. Because both groups of cows produced comparable FCM, Propionibacteria-treated cows in the succeeding weeks postpartum probably were not able to compensate for higher maintenance requirements because they had greater BW than control cows. Dry matter intake and FCM affect EB, and the results in this study concur with other studies that report positive correlations between DMI and EB ($r = 0.68$) (Coppock et al., 1974; De Kruif and Mitjen, 1992) and negative correlations between FCM and EB ($r = -0.38$) (Butler et al., 1981; Spicer et al., 1990; Villa-Godoy et al., 1988). Interestingly, DMI per kilogram of BW for Propionibacteria-treated cows was lower than for control cows. Similarly, early lactating Holstein cows fed monensin, an ionophore that increases ruminal propionate, had reduced DMI when expressed per kilogram of BW (Ramanzin et al., 1997). Previous studies have implicated leptin as a modulator of feed intake (for review see, Ingvarsen and Andersen, 2000; Meister, 2000). Whether the greater levels of leptin in the Propionibacteria-treated cows inhibited DMI will require further study.

Percentages of milk protein and SNF on wk 1 and milk fat on wk 9 and 10 of lactation were higher in cows fed Propionibacteria versus control cows, whereas percentages of milk fat and lactose did not differ between treatments. Similarly, others have found that infusion of propionate aimed to increase glucose in lactating cows resulted in higher percentage milk protein (Huhtanen et al., 1993, 1998). Higher percentages of milk protein and SNF in treated cows

during wk 1 postpartum is likely due in part to their higher plasma NEFA concentrations, because several studies showed that NEFA during early postpartum is primarily used for milk synthesis (Hart et al., 1979; Bauman et al., 1988; Tyrell et al., 1988). Fat mobilization in early lactation is a mechanism for dairy cows to cope with higher metabolic demands of lactation (Coppock et al., 1974; De Kruif and Mitjen, 1992) and greater plasma NEFA concentrations at early lactation (i.e., wk 1) compared with the succeeding weeks, as observed by others (Staples et al., 1990), may indicate that dietary intake of neither group of cows was meeting their energy requirement. In contrast to the present study, increasing the ratio of propionate to butyrate via 2-wk ruminal infusions caused a decrease in the percentage of milk fat and an increase in percentage of milk lactose in Ayrshire cows at 8 wk of lactation (Miettinen and Huhtanen, 1996). A change in milk fat and lactose percentage may depend on whether systemic glucose levels are elevated (Bauman and Grinari, 2001), the dose and (or) duration of Propionibacteria fed, and the stage of lactation when treatments are applied.

In the present study, plasma glucose and insulin concentrations increased with week postpartum. After 5 wk postpartum, insulin concentrations continued to increase while glucose concentrations remained constant. Similar trends were observed by other workers (Koprowski and Tucker, 1973; Smith et al., 1976). The lower insulin concentration during the first few weeks of lactation may be reflective of the amount of glucose in blood plasma as well as DMI of cows. In support of this statement, positive relationships between insulin and glucose ($r = 0.35$) and between DMI and insulin ($r = 0.37$) or glucose ($r = 0.44$) were observed in the present study. Theoretically, supplementation of Propionibacteria should increase propionate and thus increase glucose concentration via gluconeogenesis (Miettinen and Huhtanen, 1996; Sano and Terashima, 1998). Because supplemental Propionibacteria in the present study did not significantly affect glucose concentrations, the amount or timing of the Propionibacteria in the ration may not have been optimal. Alternatively, any increase in plasma glucose due to increased propionate production may have gone undetected if the mammary gland utilized it immediately. In sheep, increases in plasma glucose after venous propionate infusion are short-lived (i.e., <30 min) and followed by decreased plasma glucose (Sano et al., 1995) and, may explain, in part, why twice weekly blood collection was not frequent enough to detect treatment differences in plasma glucose levels in the present study. Stephenson et al. (1997) observed that monensin-treated cows had greater

plasma glucose concentrations before but not after calving, indicating metabolic status of an animal may impact whether glucose concentrations are altered by increased ruminal propionate. Because VFA and rumen microbes were not measured during the study, the absence of an increase in glucose cannot be fully explained. The lack of effect of feeding *Propionibacteria* on plasma insulin concentration is consistent with an absence of increased plasma glucose. Previously, ruminal infusion of propionate had no effect on plasma insulin concentrations (Miettinen and Huh-tanen, 1996). Because intravenous infusion of propionate acutely increases plasma glucose and insulin concentrations in early lactating dairy cows (Subiyatno et al., 1996) and yearling rams (Sano et al., 1993), further studies are needed to determine whether increased doses of *Propionibacteria* feeding will have similar effects in lactating dairy cows.

In the present study, plasma cholesterol and IGF-I concentrations were not affected by *Propionibacteria* feeding and increased with week postpartum. Similar increases in systemic cholesterol (Carroll et al., 1990; Spicer et al., 1993; Petit et al., 2001) and IGF-I (Spicer et al., 1990; Hoshino et al., 1991) levels have been reported and may be used as possible indicators of the energy status of the animals. In support of this suggestion, plasma cholesterol ($r = 0.41$ to 0.44) and IGF-I ($r = 0.43$ to 0.47) concentrations are positively correlated with EB in the present and previous studies (Spicer et al., 1990; 1993). Because cholesterol is utilized by ovarian cells for steroidogenesis (Savion et al., 1982; Grummer and Carroll, 1988; Carroll et al., 1992) and IGF-I stimulates steroidogenesis (for review see Spicer and Echternkamp, 1995), it is likely that the increase in P_4 as time postpartum progresses is due in part to increases in plasma cholesterol and IGF-I concentrations (Spicer et al., 1993); the positive correlation between P_4 and cholesterol ($r = 0.38$) and between P_4 and IGF-I ($r = 0.37$) supports this suggestion.

Systemic IGFBP-2 and IGFBP-5 levels were greater in wk 1 of lactation and subsequently decreased with week postpartum in both *Propionibacteria*-treated and control cows of the present study. Previously, Vicini et al. (1991) reported that in early lactation, systemic IGFBP-2 levels are higher in animals that are in negative EB. Also, 2-d feed deprivation in lactating cows increases serum IGFBP-2 concentrations (McGuire et al., 1995a). Conversely, lactating Holstein cows under hyperinsulinemic-euglycemic clamps for 4 d have 73% lower IGFBP-2 (McGuire et al., 1995b). Our study is the first to show that systemic IGFBP-5 levels change during early postpartum period in dairy cows. Because concentrations of IGFBP-

5 and IGFBP-2 both decrease as EB increases, perhaps both are under similar control systems. Why the presumed minor form of IGFBP-5 (i.e., 28-kDa IGFBP; Stanko et al., 1994; Stewart et al., 1996) did not change with week postpartum is uncertain.

In the present study, IGFBP-3 increased with week postpartum and is probably regulated by EB. However, systemic IGFBP-3 levels did not change in 1) midlactation cows after 2 d of feed deprivation (McGuire et al., 1995a), 2) postpartum beef cows with low BCS (Roberts et al., 1997), and 3) Holstein heifers under negative EB (VandeHaar et al., 1995). The difference may be due to the longer period of observation that enabled us to track changes of IGFBP-3 with week postpartum. The other IGFBP identified in this study included IGFBP-4 (22- and 26-kDa IGFBP; Carr et al., 1994; Stanko et al., 1994), which was a minor IGFBP, and did not differ between treatments or change with week postpartum.

Feeding *Propionibacteria* had no significant effect on reproductive function in the present study. In previous studies (Villa-Godoy et al., 1988; Spicer et al., 1990, 1993), luteal function assessed by area under the P_4 curve and peak P_4 increased between the first and second postpartum estrous cycles but not in this study. These discrepancies may be because the first ovulation did not occur until 37.4 ± 5.3 d postpartum in the present study, whereas in the previous studies mentioned, first postpartum ovulation occurred much earlier (i.e., 17 to 24 d). Because P_4 was positively correlated with cholesterol and first ovulation occurred after the maximum plasma cholesterol level was achieved, it is likely that P_4 concentrations (i.e., luteal function) after the first and second postpartum ovulations in the present study were already maximal. Spicer et al. (1993) found that average EB during the first 4 wk of lactation was negatively correlated to postpartum interval to first ovulation ($r = -0.52$). Butler et al. (1981) found a similar correlation ($r = -0.60$). The absence of a significant correlation in the present study may be because cows exhibited longer average interval to first postpartum ovulation and thus were already moving towards positive EB at this time.

Diameter of the first and second largest follicles and number of follicles did not change between wk 3 and 12 postpartum in the present study. However, diameter of the third largest follicle increased between wk 3 and 7 postpartum. Consistent with this latter observation, cows supplemented with lipids in an attempt to improve EB have follicles with larger diameters (Lucy et al., 1991b). Lucy et al. (1991a) found that number of small follicles (<5 mm) decreased while number of large follicles (>10 mm) increased between d 0 and 25 postpartum, and EB was related to changes

in follicular populations. Because follicular populations change with EB, the difference in the magnitude of EB between groups of cows among studies as well as the frequency and timing of when measurements were made may have contributed to the discrepancy in the results.

CONCLUSION

Collectively, supplemental feeding of *Propionibacteria* may hold potential for improved milk protein production and metabolic efficiency in transition and early lactating dairy cows without affecting reproductive function. Supplemental feeding of *Propionibacteria* culture to dairy cows starting 2 wk before parturition and continuing through 12 wk of lactation increased plasma concentrations of leptin, decreased DMI on a per kilogram BW basis but did not affect FCM. Percentages of milk protein and SNF were higher in *Propionibacteria*-treated cows than control cows during wk 1 postpartum. This increase is possibly due to higher plasma NEFA concentration during wk 1 of lactation in *Propionibacteria*-treated cows. However, supplemental feeding of *Propionibacteria* did not significantly alter percentages of milk fat or lactose or concentrations of plasma cholesterol, a necessary precursor for ovarian P_4 production. Similarly, P_4 peak and area under the curve and interval to first and second postpartum ovulations were similar in both treated and control cows.

Plasma glucose, insulin, and IGF-I concentrations were similar between *Propionibacteria*-treated and control cows, and thus, our original hypothesis must be rejected. Subsequent studies using supplemental *Propionibacteria* need to evaluate the effect of dose of *Propionibacteria* fed as well as the timing and duration of treatment on those metabolic variables. Also, future studies should determine whether rumen and (or) plasma propionate levels increase with *Propionibacteria* feeding. Rumen samples should also be taken to verify the establishment of and an increase in *Propionibacteria*.

Propionibacteria supplementation did not affect IGFBP levels, but systemic concentrations of IGFBP-2 and IGFBP-5 decreased while IGFBP-3 increased with week postpartum. What regulates the levels of IGFBP of postpartum cows will require further study but likely depend on the stage of lactation and EB. Once an optimal dose and duration of supplemental *Propionibacteria* feeding is determined for affecting metabolism, herd trials using a greater number of cows should be conducted to evaluate its potential for improving production efficiency.

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