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Effects of feeding two levels of propionibacteria to dairy cows on plasma hormones and metabolites

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To determine the effect of feeding propionibacteria on metabolic indicators during lactation, multiparous and primiparous Holstein cows were fed one of three dietary treatments in a 2×3 factorial design from 2 weeks *prepartum* to 30 weeks *post partum*: (1) Control (primiparous $n=5$, multiparous $n=8$) fed a total mixed ration (TMR); (2) high-dose group (primiparous $n=6$, multiparous $n=5$) fed TMR plus 6×10^{11}cfu/head daily (high-dose P169) of propionibacterium strain P169; or (3) low-dose group (primiparous $n=8$, multiparous $n=6$) fed TMR plus 6×10^{10} cfu/head daily (low-dose P169) of P169. Blood samples were collected weekly and analysed for plasma concentrations of glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, nonesterified fatty acids (NEFA) and cholesterol. Between weeks 25 and 30, all groups received bovine somatotropin (bST) every 2 weeks. Low-dose P169 multiparous cows had lower ($P<0{\cdot}05$) plasma insulin and glucose concentrations than high-dose P169 multiparous cows, whereas high-dose P169 primiparous cows had lower glucose but greater insulin concentartions than low-dose P169 primiparous cows ($P<0{\cdot}05$). Plasma insulin : glucose molar ratios were 13–18% lower ($P<0{\cdot}05$) in low-dose P169 cows than in control or high-dose P169 cows. Plasma IGF-I, NEFA and leptin levels did not differ among diet groups between weeks 1 and 25. Low-dose P169 multiparous cows had 25% greater plasma cholesterol levels than high-dose P169 and control multiparous cows, but cholesterol levels in primiparous cows did not differ. During bST treatment, high-dose P169 multiparous cows and low-dose P169 primiparous cows had lower IGF-I levels than their respective controls and, regardless of parity, high-dose P169 cows had greater NEFA than control cows. Although supplemental feeding of P169 altered plasma hormones and metabolites, the particular effects were dependent on dose of P169 and parity of cows.

Keywords: Propionibacteria, direct-fed microbial, insulin, glucose, leptin, insulin-like growth factor-I.

During the periparturient period and in early lactation in high producing dairy cows, maintaining energy balance is difficult and involves metabolic and hormonal changes (Church, 1993; Chilliard et al. 2005). In particular, negative energy balance has been associated with decreased plasma concentrations of cholesterol, insulin, insulin-like growth factor 1 (IGF-1) and leptin (Spicer et al. 1990, 1993; Chilliard et al. 2005) and increased concentrations of non-esterified fatty acids (NEFA) and somatotropin (bST) (Sartin et al. 1985; Francisco et al. 2002). Insulin increases hepatic response to bST and increases plasma IGF-I concentrations in dairy cows (Butler et al. 2003) illustrating the potential complex interplay among these metabolic hormones.

In ruminants, propionibacteria are natural propionate-producing inhabitants of the rumen and comprise 1·4–4·3% of the total microbial population (Mead & Jones, 1981; Oshio et al. 1987). Propionate is the only major VFA that is gluconeogenic (Bergman, 1990), and is an efficiently utilized source of energy (Church, 1993). Thus, feeding supplemental propionibacteria may help alleviate the metabolic demand of lactation by providing glucogenic precursors. Indeed, feeding glucose precursors such as propionate has been used as a management strategy to improve metabolic status in lactating dairy cows (Oba & Allen, 2003; Overton & Waldron, 2004). Supplemental feeding of propionibacteria to lactating dairy cows during

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a 12-week period did not significantly alter plasma glucose, insulin, cholesterol or IGF-I concentrations; however, leptin concentrations tended to be greater in cows fed propionibacteria (Francisco et al. 2002). Additional research is needed to clarify the role of propionate and direct-fed micro-organisms in the regulation of hormone and metabolite secretion particularly in dairy cows. Direct-fed microbials may be a natural way to increase milk production without using exogenous hormones. Because bST has become a widely used tool to increase milk production in several countries outside the EU (Bauman, 1999), it is important to ascertain potential interactions between bST and feed supplements.

During their first lactation, cows are still growing and produce less milk in response to bST treatment than multiparous cows (Thomas et al. 1991). This suggests that because of their metabolic differences, their response to feed supplements may also differ. Therefore, we hypothesized that long-term (⩾25 weeks) feeding of propionibacteria P169 could promote metabolic and hormonal changes that enhance overall metabolism in lactating Holstein cows and that this response would differ in primiparous *v.* multiparous cows particularly during bST treatment. The objective of the present study was to determine the effect of feeding two levels of propionibacteria P169 on key metabolic indicators such as plasma glucose, insulin, IGF-I, leptin, NEFA and cholesterol concentrations in multiparous and primiparous cows. Furthermore, the impact of bST on the effect of propionibacteria was assessed. This was a continuation of a study in which qualitative and quantitative milk characteristics and reproductive parameters were evaluated (Stein et al. 2006).

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 one of three dietary treatment groups, based on age, expected calving date and the previous year's lactation averages (for multiparous) or current PTA (for primiparous) as previously described (Stein et al. 2006). Briefly, the control group ($n=5$ primiparous, $n=8$ multiparous) received a lactation total mixed ration (TMR) of sorghum/sudan silage, alfalfa hay, bermuda hay, whole cottonseed, corn gluten, yeast (Diamond V-XP Yeast culture) and mineral concentrate (Stein et al. 2006); the low-dose group ($n=8$ primiparous, $n=6$ multiparous) received the control TMR plus 6×10^{10} cfu/head daily of propionibacteria strain P169 (Low-dose P169); and the high-dose group ($n=6$ primiparous, $n=5$ multiparous) received the control TMR plus 6×10^{11} cfu/head daily of P169 (High-dose P169) for 30 weeks *post partum*. This particular propionibacteria 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, USA) as a viable freeze-dried cell preparation containing strain P169 fermentation product and maltodextran as a carrier. Energy concentration of the diet was formulated to support a daily milk production of at least 45 kg. TMR was sampled throughout the study for analysis and averaged, on a dry matter basis, 17·1% crude protein, 7·0 MJ/kg net energy for lactation (NE_l), 68·7% total digestible nutrients (TDN), 25·1% acid detergent fibre (ADF), 38·7% neutral detergent fibre (NDF) and 0·97% Ca (Stein et al. 2006).

Cows calved between 26 August and 25 October 2002 and were stratified across treatments based on calving dates. Treatments were initiated 2 weeks prior to parturition, and the day of initiation did not differ ($P>0·28$) among control, low-dose, and high-dose treatment groups (13±2 d, 14±2 d and 18±2 d, respectively; Stein et al. 2006). Cows in each treatment group were housed in the same open-air free-stall barn divided into three separate free-stall and feeding areas to prevent contact between the dietary groups. Cows had free access to water and were milked twice daily at 4.00 and 16.00. Cows were provided with feed *ad libitum* in two allocations daily at 9.00 and 18.00. From weeks 1 to 30 *post partum*, blood samples were collected weekly after an a.m. milking (between 5·30 and 7.30) via coccygeal venipuncture in tubes (7 ml) containing EDTA. Blood samples were centrifuged at 1200 ***g*** at 4 °C for 15 min and plasma was decanted and stored frozen at −20 °C until assayed for glucose, insulin, IGF-I, leptin, NEFA and cholesterol. Average (±SEM) days on feed at the first week *post partum* blood collection for the control, high-dose and low-dose P169 groups were 3·4±0·60 d, 3·3±0·66 d, and 4·2±0·58 d, respectively, and did not differ ($P>0·10$) between groups. Cows were fed the P169 via top-dressing on a small amount of TMR (4·5 kg) once a day at 17·00 in the free stalls. To ensure complete consumption of the control and treatment TMR, the cows were confined to the free stalls after the p.m. milking until the small amount of top-dressed 'treatment TMR' was consumed.

To assess the effects of feeding P169 during concomitant bST administration, bST (POSILAC®, sterile sometribove zinc suspension; 500 mg; Monsanto, St Louis MO, USA) was administered every 2 weeks to all cows from weeks 25 to 30 of lactation; a total of three 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 prior to bST from each animal served as control values for bST effects.

Laboratory analyses

Plasma concentrations of glucose were determined using a spectrophotometer and a glucose kit (Thermo Electron Corporation, Louisville CO, USA). This procedure was based on the hexokinase coupled with

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glucose-6-phosphate dehydrogenase enzymic reaction. Standard curves were constructed between 6·25 and 100 mg/dl, and sensitivity of the assay was 6.25 mg/dl. Intrassay and interassay CV averaged 5% and 9%, respectively.

Plasma concentrations of insulin were determined using a solid-phase insulin radioimmunoassay (RIA) Kit (Micromedic Insulin Kit, ICN Biomedicals Inc., Costa Mesa CA, USA) using purified bovine insulin (28 i.u./mg) as the standard (Maciel et al. 2001). Standard curves were constructed between 0·0112 and 2·74 ng/tube, and sensitivity of the assay was 0·11 ng/ml. Intrassay and interassay CV averaged 11% and 8%, respectively.

Concentrations of IGF-I in plasma were determined by RIA after acid-ethanol extraction using recombinant human IGF-I as the standard (Echternkamp et al. 1990). Briefly, aliquots of blood plasma were diluted 1 : 4 with 87·5% acidic ethanol (final concentration, 0·25 M-HCl) and incubated at 4 °C for 16 h. Samples were then centrifuged at 1200 ***g*** at 4 °C for 30 min, the supernatant neutralized with 0·855 M-Tris, and diluted in assay buffer prior to RIA. Standard curves were constructed between 0·488 and 250 pg/tube, and sensitivity of the assay was 3·4 ng/ml. Intraassay and interassay CV averaged 8% and 9%, respectively.

Concentrations of leptin were measured in plasma samples collected during even weeks throughout the study and determined by RIA using a modification of Linco's Multi-Species Leptin Kit (Linco Research Inc., St Charles MO, USA) as previously described (Maciel et al. 2001). Bovine plasma (40, 60, 80, and 100 µl) produced curves parallel to standard curves which were constructed between 1 and 20 ng/ml. Sensitivity of the assay was 1·2 ng/ml, and intraassay and interassay CV averaged 9% and 5%, respectively.

Concentrations of NEFA were determined in plasma samples collected during even weeks throughout the study by an enzymic colorimetric method using NEFA-C Kits (WaKo Chemicals Inc., Richmond VA, USA) as previously described (Francisco et al. 2002). Standard curves were constructed between 0·25 and 2·0 mmol/l, and sensitivity of the assay was 0·099 mmol/l. Intraassay and interassay CV was 5% and 14%, respectively.

Concentrations of total plasma cholesterol were determined by an enzymic colorimetric method using a Cholesterol E Kit (Wako Chemicals Inc., Richmond VA, USA) as previously described (Francisco et al. 2002). Standard curves were constructed between 50 and 400 mg/dl, and sensitivity of the assay was 53 mg/dl. Intrassay and interassay CV averaged 7% and 15%, respectively.

Statistical analyses

Plasma concentrations of glucose, insulin, IGF-I, leptin, NEFA and cholesterol were analysed as a completely randomized design ANOVA for repeated measures, using the MIXED procedure of SAS and the model: $Y_{ijkl} = U + D_i + P_j + (D \times P)_{ij} + C(D \times P)_{ijk} + W_l + (D \times W)_{il} + (P \times W)_{jl} + (D \times P \times W)_{ijl} + e_{ijkl}$, where U = overall mean, D = diet, P = parity, C(D × P) = cow within group and parity, W = week *post partum*, (D × P) = diet by parity interaction, (D × W) = diet by week *post partum* interaction, (P × W) = parity by week *post partum* interaction, (D × P × W) = diet by parity by week *post partum* interaction, and e = residual error. For the variables that were measured weekly, two separate analyses were conducted for weeks 1–25 and weeks 25–30 to better evaluate the effect of treatment on the variables during the pre-bST portion of the study, and during bST treatment, respectively. The model of the covariate structure for repeated measurements was an autoregressive with lag equal to one (Littell et al. 1996). If the week × diet interaction was significant, simple effects of diet were analysed using the slice option for the LSMEANS statement. Conversely, main effects were analysed using LSMEANS with the PDIFF option if one or more main effects were significant but the interactions were not significant.

Results

Plasma glucose

For weeks 1–25, there were week ($P<0·01$), parity ($P<0·001$) and diet × parity ($P<0·0002$) effects on plasma glucose concentrations; all other main effects ($P>0·32$) and interactions ($P>0·74$) were not significant. Plasma glucose concentrations decreased by 5% from weeks 1 to 2 and then increased by 8% until the end of week 5 after which it did not change (results not shown). Averaged over weeks 1–25, primiparous cows had a 5% greater ($P<0·001$) plasma glucose concentrations compared with multiparous cows. High-dose P169 multiparous cows (63·3 ± 1·1 mg/dl) had 6% greater ($P<0·05$) plasma glucose compared with the low-dose P169 multiparous cows, whereas primiparous cows fed the low-dose P169 (67·9 ± 1·1 mg/dl) had 9% and 6% greater ($P<0·05$) plasma glucose concentrations than primiparous control and high-dose P169 groups, respectively (Fig. 1).

Plasma glucose was not affected by diet ($P>0·37$) or diet × parity ($P>0·16$) during bST administration. However, plasma glucose concentrations were influenced by parity ($P<0·02$) during bST administration such that primiparous cows (67·3 ± 1·1 mg/dl) had concentrations of plasma glucose 6% greater than multiparous cows (63·6 ± 1·1 mg/dl).

Plasma insulin

Plasma concentrations of insulin during weeks 1–25 of lactation were influenced by dietary treatment ($P<0·001$) but not other main effects ($P>0·20$) or their interactions ($P>0·60$). Insulin concentrations were 11–20% lower ($P<0·05$) in low-dose P169 cows (0·66 ± 0·03 ng/ml)

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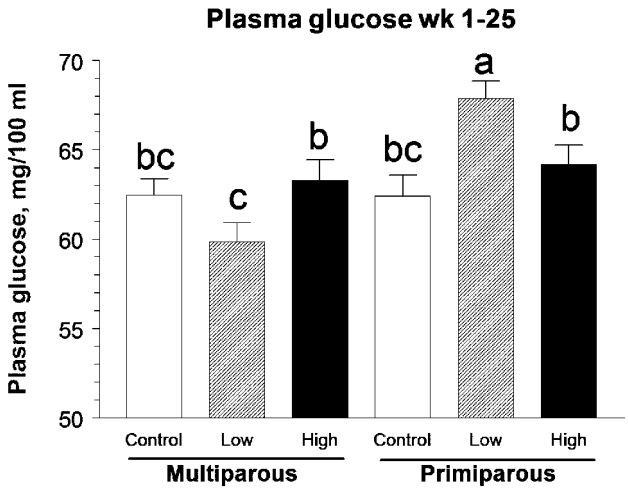

Fig. 1. Changes in plasma glucose concentrations during lactation (averaged over weeks 1–25) as affected by dietary treatment and parity in Holstein cows. Across parity and diet groups, means without a common letter differ ($P<0·05$). Low = low-dose P169 group; High = high-dose P169 group.

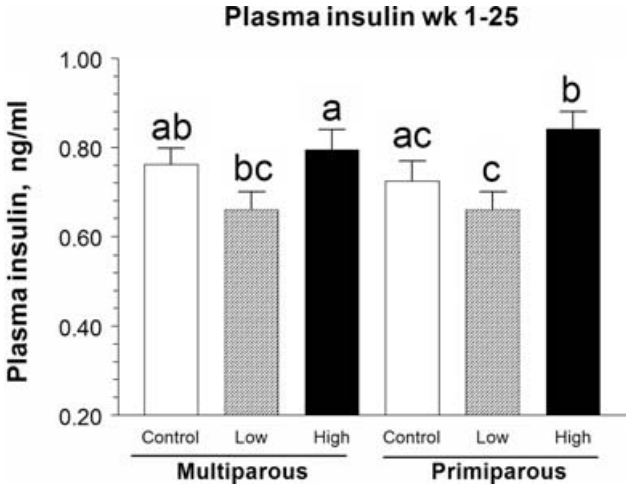

Fig. 2. Changes in plasma insulin concentrations during lactation (averaged over weeks 1–25) as affected by dietary treatment and parity in Holstein cows. Across parity and diet groups, means without a common letter differ ($P<0·05$). Low = low-dose P169 group; High = high-dose P169 group.

than high-dose P169 (0·82 ± 0·03 ng/ml) and control (0·74 ± 0·03 ng/ml) cows during the 25-week study (Fig. 2).

During treatment with bST, plasma insulin concentrations were affected by week ($P<0·05$) but not parity ($P>0·14$), dietary treatment ($P>0·29$) or their interactions ($P>0·79$). Plasma insulin appeared to increase in response to the second and third bST injection such that the concentration of insulin at week 29 (1·02 ± 0·07 ng/ml) was 32% greater ($P<0·02$) than at week 25 (0·77 ± 0·07 ng/ml).

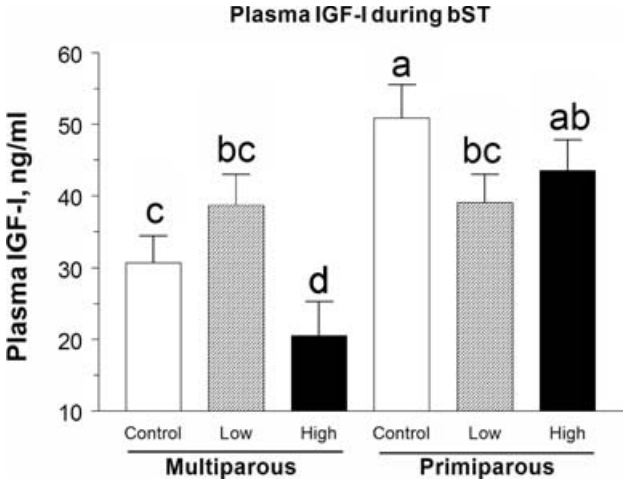

Fig. 3. Plasma IGF-I concentrations (averaged over weeks 25–30) as affected by dietary treatment × parity interaction during bST treatment in Holstein cows. Across parity and dietary treatment groups, means without a common letter differ ($P<0·05$). Low = low-dose P169 group; High = high-dose P169 group.

Plasma insulin : glucose ratio

During weeks 1–25, plasma insulin : glucose ratio was affected by dietary treatment ($P<0·005$) but not other main effects ($P>0·33$) or their interactions ($P>0·27$). Plasma insulin : glucose ratios were 13–18% lower in low-dose P169 cows (37·2 ± 1·8) than in control (42·7 ± 1·8) or high-dose P169 cows (45·6 ± 1·8). During bST treatment, parity ($P<0·06$) and week ($P<0·09$) tended to affect insulin:glucose ratios whereas other main effects and interactions did not ($P>0·30$). Multiparous cows (52·2 ± 3·2) tended to have 21% greater ($P<0·06$) insulin:glucose ratios than primiparous cows (43·3 ± 3·2). Also, insulin: glucose ratios tended to increase ($P<0·09$) by an average of 18% 2 weeks after each bST injection (results not shown).

Plasma IGF-I

During weeks 1–25, there was a week ($P<0·004$) and parity ($P<0·02$) effect on IGF-I concentrations, but other main effects ($P>0·10$) or interactions ($P>0·19$) were not significant. Primiparous cows (20·1 ± 1·1 ng/ml) had 22% greater ($P<0·02$) plasma IGF-I concentrations than multiparous cows (16·5 ± 1·1 ng/ml). Furthermore, IGF-I concentrations increased ($P<0·004$) more than 2-fold between week 1 and week 25 of lactation (results not shown).

There was a week ($P<0·001$), parity ($P<0·001$), week × parity ($P<0·0001$) as well as diet × parity ($P<0·01$) effect on plasma IGF-I concentrations during bST administration (Fig. 3). Primiparous cows had greater ($P<0·01$) increases in plasma IGF-I than multiparous cows following each of the bST administrations at weeks 25, 27 and 29 (results not shown). In addition, high-dose P169 multiparous cows had

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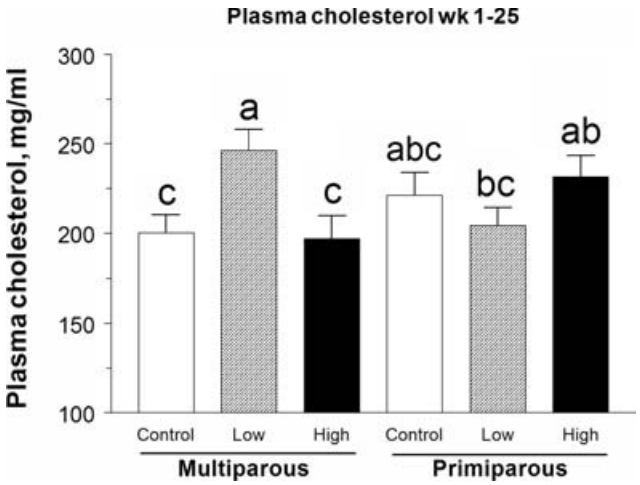

Fig. 4. Plasma cholesterol concentrations during lactation (averaged over weeks 1–25) as affected by dietary treatment and parity in Holstein cows. Across parity and diet groups, means without a common letter differ ($P<0·05$). Low = low-dose P169 group; High = high-dose P169 group.

33% lower concentrations of plasma IGF-I than their respective control group, whereas low-dose P169 primiparous cows had 31% lower plasma IGF-I concentrations than their respective control group (Fig. 3).

Plasma leptin

During weeks 1–25, only week affected ($P<0·001$) plasma leptin concentrations such that leptin levels were increased ($P<0·05$) by 24% between week 1 (3·89 ± 0·3 ng/ml) and week 3 (4·89 ± 0·3 ng/ml) after which plasma leptin concentrations remained constant. Other main effects ($P>0·50$) and interactions ($P>0·15$) did not affect leptin concentrations during weeks 1–25. Plasma leptin concentrations averaged 4·4 ± 0·4 ng/ml and 4·7 ± 0·4 ng/ml in primiparous and multiparous cows, respectively.

Plasma leptin concentrations were not affected by week ($P>0·98$), diet ($P>0·11$), parity ($P>0·15$) or their interaction ($P>0·44$) during bST administration.

Plasma NEFA

Week ($P<0·001$) and parity ($P<0·005$) but not diet ($P>0·56$) affected plasma NEFA concentrations during weeks 1–25. Plasma NEFA concentrations decreased ($P<0·001$) 64% between week 1 (0·62 ± 0·02 mmol/l) and week 15 (0·22 ± 0·02 mmol/l) after which NEFA concentrations did not significantly change (results not shown). In addition, multiparous cows (0·35 ± 0·01 mmol/l) had 18% greater ($P<0·005$) plasma NEFA concentrations than primiparous cows (0·29 ± 0·01 mmol/l).

During bST administration, diet ($P<0·01$), week ($P<0·001$) and parity ($P<0·05$) affected plasma NEFA concentrations. All interactions were not significant ($P>0·13$). Plasma NEFA concentrations in high-dose P169-treated cows (0·31 ± 0·01 mmol/l) were 24% and 19% greater ($P<0·01$) than control (0·25 ± 0·01 mmol/l) and low-dose P169-treated (0·26 ± 0·01 mmol/l) cows, respectively. In contrast to weeks 1–25 analysis, primiparous cows (0·30 ± 0·01 mmol/l) had 12% greater ($P<0·05$) plasma NEFA levels than multiparous cows (0·27 ± 0·01 mmol/l). Moreover, concentrations of NEFA were increased by 27–39% ($P<0·001$) 2 weeks after each bST injection (results not shown).

Plasma cholesterol

Plasma cholesterol concentrations were affected by week ($P<0·001$) and diet × parity ($P<0·005$), but not other main effects ($P>0·40$) or interactions ($P>0·12$) during weeks 1–25. Plasma cholesterol concentrations increased ($P<0·001$) throughout lactation (week 1 = 89, week 5 = 157, week 10 = 206, week 15 = 244 ± 8 mg/ml), and multiparous low-dose P169 cows had 25% greater ($P<0·05$) levels of plasma cholesterol than their respective high-dose P169 and control groups (Fig. 4). In contrast, plasma cholesterol concentrations in primiparous cows did not differ among treatment groups during the 25-week study (Fig. 4).

During bST administration, plasma cholesterol concentrations were affected by week ($P<0·03$) but not by diet ($P>0·22$), parity ($P>0·21$) or their interactions ($P>0·12$). Plasma cholesterol concentrations increased ($P<0·05$) between week 25 (287 ± 10 mg/ml) and week 29 (317 ± 10 mg/ml).

Discussion

As previously reported for the same cows of the present study, milk production (4% FCM) increased by 8·5% in low-dose P169-fed cows and by 7·1% in high-dose P169-fed cows, and P169 also changed milk fat and lactose levels (Stein et al. 2006), indicating that P169 feeding altered metabolism. Results of this study should be interpreted with caution owing to the small number of animals evaluated in each subgroup (n = 5–8). Further studies with larger numbers of animals should be conducted to confirm the findings of this study.

Feeding the high-dose P169 increased ruminal propionate in the same cows as the present study (Stein et al. 2006), the majority of which is presumed to be removed from the portal venous system via hepatic uptake and subsequently increases hepatic glucose output (Bergman, 1990; Britton & Krehbiel, 1993; Kristensen & Harmon, 2004). Plasma glucose concentrations during early and mid lactation were 9% greater in low-dose P169 primiparous cows than in controls, and were 6% greater in high-dose P169 multiparous cows than in low-dose P169 multiparous cows. Francisco et al. (2002) found no effect of feeding P169 (low-dose) on glucose concentrations,

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measured weekly, in early (weeks 1–12) lactating multiparous dairy cows and further indicates that the level of P169 fed to cows may affect plasma glucose concentrations. Nevertheless, feeding sources of propionate or infusion of it at high enough levels can transiently increase both glucose and insulin concentrations in sheep (Sano et al. 1993a) and cattle (Sartin et al. 1985; Oba & Allen, 2003). In these latter studies, frequent (e.g. hourly) blood samples were collected post-infusion or feeding. The duration and magnitude of these increases were dependent on dose of propionate infused (Sano et al. 1993a). Thus, once weekly blood collection as conducted in the present study may not have been a frequent enough regime to detect transient increases in plasma glucose concentrations that may have occurred in response to P169 feeding, and increased ruminal propionate levels in high-dose P169-fed cows supports this supposition (Stein et al. 2006). Because the entire amount of P169 was fed over a short period of time prior to the afternoon feeding, a transient increase in ruminal propionate in low-dose as well as high-dose cows would be expected. Reasons why blood glucose levels were elevated in low-dose P169 primiparous cows and not in either group of P169-fed multiparous cows may be linked to the fact that the low-dose P169 primiparous group was the only group with no increase in milk lactose levels (Stein et al. 2006).

Because glucose uptake by mammary epithelial cells is not dependent on acute changes in insulin (Laarveld et al. 1985; Bauman & Griinari, 2003), why the same cows in the high-dose P169 group of the present study had increased milk lactose (Stein et al. 2006) in spite of greater plasma insulin concentrations will require further study. Francisco et al. (2002) observed that neither plasma insulin levels nor milk lactose levels differed between P169 (low-dose) fed and control multiparous cows during early lactation, supporting the concept that changes (or lack of) in plasma insulin and milk lactose may be linked. Hayirli et al. (2001) reported that the molar ratio of insulin to glucose is lower in cows fed supplemental chromium-methionine, indicating that feed supplements do have potential for improving tissue sensitivity to insulin. Although not studied extensively, the molar ratio of insulin to glucose observed in the present study was within the range reported for cattle (Hayirli et al. 2001; Liu et al. 2004). Insulin responses to propionate infusion also depend on the level of nutrients fed to non-lactating, non-pregnant mature ewes (Quigley & Heitmann, 1991). Thus, lower feed intake or energy balance may have reduced the insulin response to propionibacteria feeding in the previous study (Francisco et al. 2002). Other researchers found that stage of lactation influences the insulin response to glucose and propionate owing to differences in hepatic clearance rates and levels of energy balance (Sartin et al. 1985; Sano et al. 1993b). As discussed earlier for plasma glucose, because samples were collected only once a day each week, it is also likely that acute changes in plasma insulin in response to feeding either low-dose P169 or high-dose P169 may have gone undetected in the present study.

Similarly to previous studies, systemic IGF-I concentrations in the present study were unaltered by addition of P169 to the diet (Francisco et al. 2002) and increased during the first 7–16 weeks of lactation (Spicer et al. 1990, 1993). Because cows in positive energy balance have greater concentrations of IGF-I (Spicer et al. 1990), we speculate that the primiparous cows with greater IGF-I levels were in greater positive energy balance than multiparous cows. In support of this, multiparous cows had 18% greater NEFA concentrations than primiparous cows between weeks 1 and 25 of lactation, suggesting that multiparous cows had greater lipid mobilization probably because of their greater milk production (Stein et al. 2006). Moreover, primiparous cows had greater glucose and IGF-I concentrations than multiparous cows following bST administration and further supports the idea that primiparous cows were in greater positive energy balance than multiparous cows. Consistent with a more rapid return to positive energy balance in primiparous *v.* multiparous cows, Stein et al. (2006) reported that these same primiparous cows recovered their body weight quicker between week 1 and week 25 than the multiparous cows of the present study. Other studies (Chelikani et al. 2003; Lemosquet et al. 2004) indicate that energy balance status of the cows may influence IGF-I response to increased glucose or bST. Increased IGF-I and NEFA after bST in the present and previous (Gulay et al. 2003) studies support this suggestion. Other research suggests that bST treatment plays an important role in metabolism owing to increases in rates of gluconeogenesis and NEFA irreversible loss and oxidation, resulting in an extra energy source for milk production (Bauman, 1999). During bST treatment in the present study, high-dose P169 cows had greater NEFA concentrations, but not during weeks 1–25. Previously, Francisco et al. (2002) found that multiparous cows fed P169 (low-dose) had greater plasma NEFA levels than control cows only during week 1 *post partum* and greater plasma leptin levels during weeks 1–12 than control cows. In lactating animals, treatment with propionate or glucose precursors decreases NEFA concentrations (Lemosquet et al. 1997; Gabai et al. 2002) and this response is thought to be due to an increase in systemic insulin which acts as an antilipolytic hormone to increase lipid deposition rather than mobilization (Picard et al. 1999). Because insulin concentrations did not differ among treatment groups during bST treatment, differences in insulin secretion cannot explain the greater NEFA levels in high-dose P169 cows during bST treatment but decreased insulin sensitivity (i.e. increased insulin : glucose ratio) might be an explanation. Perhaps P169 treatment over time provides precursors for lipid deposition within adipocytes (Bergman, 1990) and when subsequently challenged with increased endogenous (e.g. at parturition) or exogenous bST, more lipids can be mobilized (Bauman, 1999). The 25–32 kg greater body weights in the high-dose P169

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group *v.* the control and low-dose P169 groups support this notion (Stein et al. 2006). Greater body weights existed in low-dose P169 *v.* control cows in the study of Francisco et al. (2002) but not in the present study (Stein et al. 2006), and may explain the discrepancy in the leptin data between these two studies. Although the role of propionate as an antiketogenic compound has been suggested, the mechanism by which P169 increases NEFA concentrations during bST treatment will require further study.

In contrast to the present study, feeding P169 (low-dose) for 12 weeks had no effect on plasma cholesterol levels of multiparous cows (Francisco et al. 2002). The reason for this discrepancy is unclear but may involve body weight differences, differences in dry matter intake and (or) the composition of rations used in the two studies. For example, Francisco et al. (2002) fed 0·86% (of dry matter) of Rumofat (contains free fatty acids) and no yeast whereas the present study fed 0·91% (of dry matter) of Megalac-R (contains Ca-salts of long-chain fatty acids) and supplemented with yeast. Feeding inert fat (e.g. Megalac) to multiparous cows in early lactation has been shown to increase cholesterol concentrations (Spicer et al. 1993). Because multiparous cows fed low-dose P169 had greater cholesterol levels than primiparous cows fed the low-dose P169, and multiparous cows fed high-dose P169 had less plasma cholesterol than primiparous cows fed the high-dose P169, the present results indicate that dose of P169 can interact with parity to affect metabolism.

In summary, feeding P169 from 2 weeks prior to parturition until the end of week 30 *post partum* to Holstein cows resulted in complex hormone and metabolite changes (present study), and increased milk production by 7·1–8·5% as reported for these same cows (Stein et al. 2006). These hormone and metabolite changes were influenced by dose of P169 and parity, and included increased plasma insulin and decreased glucose in high-dose P169 primiparous cows (*v.* low-dose P169), increased plasma insulin and increased glucose in high-dose P169 multiparous cows (*v.* low-dose P169), and increased plasma cholesterol in low-dose P169 multiparous cows (*v.* controls and high-dose P169). During bST treatment, plasma concentrations of IGF-I decreased and NEFA increased in high-dose P169 multiparous cows (*v.* control and low-dose P169). However, these results are based on a small number of animals and should be interpreted with caution. Further studies with a greater number of animals are warranted to evaluate in more detail the interaction between parity and dose of supplemental feeding of P169 on metabolism in lactating Holstein cows.

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