

EFFECT OF A DIRECT-FED MICROBIAL ON  
PLASMA CONCENTRATIONS OF HORMONES AND  
METABOLITES IN PRIMIPAROUS AND  
MULTIPAROUS HOLSTEIN COWS

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## TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
II. REVIEW OF LITERATURE.....	4
Genus <i>Propionibacterium</i> .....	4
Bacterial Fermentation in the Rumen.....	10
Plasma Hormones and Metabolites.....	15
Effect of Propionate on Plasma Hormones and Metabolites.....	15
Changes in Plasma Hormones and Metabolites during Lactation.....	25
LITERATURE CITED .....	30
III. EFFECT OF A DIRECT-FED MICROBIAL ON PLASMA CONCENTRATIONS OF HORMONES AND METABOLITES IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS.....	49
Abstract .....	49
Introduction .....	50
Methods and Materials .....	53
Experimental Design and Sample Collection.....	53
Statistical Analysis.....	56
IV. Results .....	58
Plasma Glucose.....	58
Plasma Insulin .....	59
Plasma Insulin/Glucose Ratio.....	60
Plasma Insulin-like Growth Factor-I.....	60

Chapter	Page
Plasma Leptin.....	61
Plasma Non-esterified Fatty Acids.....	62
Plasma Cholesterol.....	63
V. Discussion and Conclusion.....	109
Discussion.....	109
Conclusion.....	115
LITERATURE CITED .....	116

## LIST OF FIGURES

Figure	Page
Figure 4.1	69
Changes in plasma glucose concentration from wk 1 to 25 of lactation; values are averaged across dietary treatment groups. * First mean that differs from wk 1 ( $P < 0.01$ ); ** First mean that differ from wk 2 ( $P < 0.003$ ).....	
Figure 4.2.	71
Plasma glucose concentration (averaged of wk 1 to 25) as affected by dietary treatment x parity interaction. Across parity and treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	
Figure 4.3.	73
Plasma glucose concentration (average of wk 1 to 12) as influenced by dietary treatment x parity interaction. Across treatment and parity groups, Means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	
Figure 4.4.	75
Plasma glucose concentration (averaged of wk 13 to 25) affected by dietary treatment x parity. Across treatment and parity groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	
Figure 4.5.	77
Plasma insulin concentration (averaged of wk 1 to 25) influenced by dietary treatment. Across treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	
Figure 4.6	79
Plasma insulin concentration (averaged of wk 1 to 12) affected by treatment. Across treatment groups, Means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	
Figure 4.7	81
Weekly changes in plasma insulin concentrations from wk 1 to 12 of lactation; values are averaged across diet and parity treatment groups. Asterisk (*) indicates means differ from wk 2 and 3 means ( $P < 0.05$ ).....	
Figure 4.8	83
Plasma insulin concentration (averaged of wk 13 to 25) influenced by treatment. Across treatment groups, Means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	

Figure	Page
4.9 Weekly changes on plasma insulin during bST administration. * First mean that differ from wk 25 ( $P < 0.02$ ). Arrows indicate time of bST injection.....	85
4.10 Plasma insulin:glucose ratio (averaged of wk 1 to 25) by dietary treatment x parity interaction. Across parity and treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	87
4.11 Weekly changes of IGF-I concentration from wk 1 to 25 of lactation; values are averaged across parity and dietary treatment groups. * First mean that differ from wk 2 ( $P < 0.01$ ); ** First mean that differ from wk 4 ( $P < 0.03$ ).....	89
4.12 Weekly changes in plasma IGF-I on primi- and multiparous cows during bST administration. * First means that differ from wk 25 ( $P < 0.0002$ ). Arrows indicate time of bST injection.....	91
4.13 Plasma IGF-I concentration influenced by dietary treatment x parity during bST administration. Across treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	93
4.14 Changes in plasma leptin concentration during wk 1 to 25 of lactation. Asterisk (*) indicates first mean that differs from wk 1 values ( $P < 0.0002$ ).....	95
4.15 Plasma leptin concentration during bST administrations. Across treatment groups, means without a common letter differ ( $P < 0.05$ ). Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	97
4.16 Weekly changes in plasma NEFA concentrations during the first 25 wk of lactation. * First mean that differs from wk 1; ** First mean that differ from wk 3 ( $P < 0.05$ ).....	99
4.17 Weekly changes in plasma NEFA concentration during bST administration. * First means that differ from wk 25 ( $P < 0.0002$ ). Arrows indicate time of bST injection.....	101
4.18 Changing in plasma cholesterol concentration during wk 1 to 25 of lactation; values are averaged across diet and parity treatment groups. * First mean that differs from wk 1 ( $P < 0.0001$ ); ** First mean that differ from wk 3 ( $P < 0.0002$ ); *** First mean that differ from wk 13 ( $P < 0.03$ ).....	103

Figure	Page
4.19 Plasma cholesterol concentration (average of wk 1 to 25) as affected by dietary treatment x parity interaction. Across parity and diet groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.....	105
4.20 Weekly changes in plasma cholesterol during bST administration. Values are averaged across diet and parity treatment group. * First means that differ from wk 25 ( $P < 0.05$ ). Arrows indicate time of bST injection.....	107



## LIST OF TABLES

Table		Page
4.1	Ingredient and Nutrient Composition of the Control Diet (DM basis). Diet was fed from parturition to d 265 of lactation.....	65
4.2	Ingredient and Nutrient Composition of the Control Transition Diet (DM basis). Diet was fed from d -30 to parturition.....	67

## **Chapter I**

### **Introduction**

The rumen is considered a self-contained ecosystem in which feed consumed by the animal is fermented to volatile fatty acids (VFA) and microbial biomass (Church, 1993; Weimer, 1998). Microbial populations in the rumen evolved over millions of years consisting of anaerobic bacteria, ciliated protozoa, and lower numbers of fungi that interact in a very complex manner to provide the best example of microbial symbioses (Weimer, 1998). In production animals, the microbial population is comprised of  $10^{10}$  -  $10^{11}$  bacteria,  $10^5$  –  $10^6$  protozoa, and  $10^3$  –  $10^5$  fungi per ml of ruminal content (Church, 1993). Alteration of factors such as buffering capacity, osmotic pressure, dry matter content, and oxidation-reduction potential lead to a negative response on the activity and growth of the microbial population in the rumen (Church, 1993; Van Soest, 1994; Dehority, 2003). These changes result in a disruption of the ruminal homeostasis such that performance of the animal is affected by decreasing DMI, fiber digestibility, milk yield, increased health problems, and the need of feed additives (Allen, 1997; Cheng et al., 1998; Nagaraja and Chengappa, 1998; Owens et al., 1998; Russell and Rychlik, 2001).

Lactating dairy cows experience dramatic metabolic changes during the transition period commencing 3 wk before calving to 3 wk after calving in which the rumen microbial population as well as the liver must adapt quickly to meet the cow's increasing

nutrient consumption and metabolic demand (Grummer, 1995). The rumen microbial production of VFA are converted via gluconeogenesis to glucose by the liver for subsequent tissue use. The mammary gland requires approximately 80% of the glucose supply to support the high production of milk (Drackley et al., 2001). Glucose concentrations sharply increase 1 d prior to calving reflecting hormonal changes that promotes gluconeogenesis and glycogenolysis (Vazquez-Añon et al., 1994). Mobilization of adipose tissue increased 5 d before parturition reflected by the increase in nonesterified fatty acids prior to depression of DMI (Vazquez-Añon et al., 1994). Dramatic endocrine changes also occur pre- and post-partum. For example, plasma concentrations of insulin and insulin-like growth factor-I were greater before than immediately after parturition and increased gradually during lactation (Spicer et al., 1990; Spicer et al., 1993; Francisco et al., 2002; Pushpakumara et al., 2003). Likewise, leptin concentrations were greater before than after calving in lactating Holstein cows (Block et al., 2001; Ehrhardt et al., 2001; Liefers et al., 2003).

Various strategies have been used to ameliorate the dramatic changes in plasma hormones and metabolites that occur during the transition period to improve performance in lactating cows. These strategies include increasing energy density of the diet (Grum et al., 1996), infusion of either propionate or glucose (Subiyatno et al., 1996; Gabai et al., 2002; Lee and Hossner, 2002), and supplementation of fat (Spicer et al., 1993; Beam and Butler, 1998) or propionibacteria (Francisco et al., 2002).

Rumen microbial manipulation has been of interest to ruminant nutritionists in order to achieve improvement in the profitability and health of lactating cows. Therefore, propionibacteria have been used as a direct-fed microbial to prevent the risk of acidosis

(Swinney-Floyd, 1997; Davidson, 1998; Ghorbani et al., 2002; Yang et al., 2004) in feedlot cattle. Also, propionibacteria have been demonstrated to reduce nitrate toxicity in cattle (Swartzlander, 1994; Strickland et al., 2004). In the dairy industry, propionibacteria use has focused on increasing the production of propionate and to modulate changes in plasma hormones and metabolites that lead to improved metabolism in dairy cows (Francisco et al., 2002). Further studies are needed to more deeply evaluate the propionibacteria effects on metabolic indicators during the transition period in dairy cows.

Therefore, supplementation with propionibacteria to dairy cows could enhance metabolism by a positive alteration on plasma hormones and metabolites such that cows produce more milk yield, reduce ketoacidosis syndrome, and improve reproductive performance. The purpose of this literature review is to summarize our current understanding of the use of propionibacteria as well as summarize research concerning manipulation of rumenal bacteria and fermentation by the addition of dietary glucogenic precursors and ionophores to dairy cows. Because this manipulation may alter plasma hormones and metabolites, changes in plasma hormones and metabolites during lactation in Holstein dairy cows will also be reviewed.

## **Chapter II**

### **Review of Literature**

#### ***Genus Propionibacterium***

Propionibacteria are classified as gram-positive, non-spore forming, non-motile, and pleomorphic bacteria in nature. They are anaerobic to aerotolerant organisms, which range from 0.3 to 1.3  $\mu\text{m}$  in diameter and 1 to 10  $\mu\text{m}$  in length. Cells are arranged in various patterns such as single, pairs or V and Y configuration, and short chains or clumps with “Chinese letters” arrangement. Fermentation products from glucose include a mixture of propionic acid and acetic acid and lesser amounts of isovaleric, formic, succinic or lactic acid, and carbon dioxide (Moore and Holdeman, 1974).

*Propionibacterium ssp.* encompass a variety of co-factors involved in the transfer and rearrangement of C-1 compounds (Hugenholtz et al., 2002). They are also slow growing, acid intolerant bacteria (Kung, 2001) with maximal growth occurring at 30 - 37°C with a pH near 7 (Moore and Holdeman, 1974).

Other properties of the genus *Propionibacterium* include the ability to synthesize no- or low-calorie sugars such as trehalose (Hugenholtz et al., 2002) and produce antimicrobial agents such as propionic acid, propionins and bacteriocins (Grinstead and Barefoot, 1992; Hugenholtz et al., 2002). They are also used as inoculants for wheat,

sorghum, and maize silage and also in bread manufacturing (Merry and Davies, 1999; Suomalainen and Mayra-Makinen, 1999; Filya et al., 2004). Moreover, all *Propionibacterium* strains synthesize relatively large amounts of Vitamin B<sub>12</sub> (deoxy adenosyl cobalamin) under very low oxygen concentrations and, thus, have a huge advantage as a food-grade organism (Hugenholtz et al., 2002; Martens et al., 2002).

Cutaneous and classical (dairy) represent the two groups of the *Propionibacterium* *ssp.* found in humans, animals, and dairy products as saprophytes. Cutaneous species are present in the human skin and intestinal tract microbial population (Zárate et al., 2002), which include: *P. avidum*, *P. acnes*, *P. lymphophilum*, and *P. granulosum* (Moore and Holdeman, 1974). Dairy or classical propionibacteria include four different species: *P. freudenreichii*, *P. thoeni*, *P. acidopropionici*, and *P. jensenii* (Moore and Holdeman, 1974), which are extensively used in the dairy industry as starter cultures, and as a main agent in lipolysis in the production of typical Emmental type cheeses (Chamba and Perreard, 2002; Hugenholtz et al., 2002). These species are also responsible for the characteristic “eyes” or “holes”, and contribute to the development of the typical flavor of the Swiss-type cheeses (Rossi et al., 1999; Frohlich-Wyder et al., 2002).

In humans with maldigestion diseases, dietary supplements containing *Propionibacterium* *ssp.* improve their tolerance to lactose compounds (Zárate et al., 2000). Other research shows that adhesion of propionibacteria to intestinal epithelial cells may play an important role in gastric protection of the stomach (Jan et al., 2002; Zárate et al., 2002). Perez-Chaia et al. (1995) also found that *Propionibacterium* fed for 7 d to mice in a suspension with skim milk increased the levels of this strain in the gut during treatment, and they remained high after 1 wk of cessation of the treatment. In

studies with mice, the presence of the genus *Propionibacterium* alter intestinal metabolism (Perez-Chaia et al., 1995; 1999).

In ruminant animals such as sheep, 4.3% of the total bacterial population consisted of *Propionibacterium ssp.* isolated from the ruminal epithelium tissue when high roughage diets were fed (Mead and Jones, 1981). Oshio et al. (1987) showed that *Propionibacterium* comprise 1.4% of the overall microbial population in ruminants fed high concentrate diets. Davidson (1998) reported that the normal propionibacteria habitat population ranges from  $10^3$  to  $10^4$  cfu/mL in the bovine rumen.

In the cattle industry, propionibacteria have been used as a direct-fed microbial (DFM) to prevent the risk of acidosis in feedlot cattle receiving high concentrate diets due to the fact that propionibacteria utilize lactate and convert it to propionate, acetate and CO<sub>2</sub> (Ghorbani et al., 2002; Yang et al., 2004). Ghorbani et al. (2002) conducted an experiment to evaluate whether or not *Propionibacterium ssp.* as a DFM could reduce the risk of acidosis in steers fed high concentrate diets. Treatments were control, *Propionibacterium* P15 (P15), and P15 + *Enterococcus faecium* EF212 (PE). The bacteria were top-dressed once daily at the time of feeding and blended with whey powder to supply  $1 \times 10^9$  cfu/g of carrier. The experiment had a 2-wk adaptation and 1-wk measurement period. There was no effect on ruminal pH, blood pH, blood glucose, propionate, isobutyrate and isovalerate, or acetate/propionate ratio after DFM supplementation; however, acetate concentrations were greater in steers fed PE compared with steers fed P15 alone or control. Although DFM supplementation did not alter pH, some rumen and blood variables indicated a reduction in acidosis (Ghorbani et al., 2002). In support of these results, Yang et al. (2004) found no effect of DFM supplementation

on the mean fermenter pH, acetate, propionate, butyrate or total VFA concentrations, or acetate/propionate ratio when feedlot cattle were fed diets containing *Propionibacterium* P15 (PB), *Enterococcus faecium* EF212 (EF) and *Enterococcus faecium* with a yeast, *Saccharomyces cerevisiae* (EFY). The total bacteria count in the fermenter fluid tended to be higher with control or PB than with EF or EFY. Likewise, the number of lactate-utilizing bacteria was higher for control or EF than for PB or EFY treatments. Bacterial population, digestibility, microbial protein synthesis or fermentation in continuous cultures was not affected when DFM was supplemented. In contrast, Kim et al. (2001a) showed that propionate increased as the dosage levels increased (0,  $10^7$ ,  $10^8$ ,  $10^9$ , and  $10^{10}$  cfu). Acetate/propionate ratio decreased in the DH42 treated group at most dosages, and the acetate/propionate ratio did not return to the pre-test levels during the post-test period. Acetate/propionate ratio in a treatment with DH42 + LAB (*Lactobacillus plantarum* MC2 a lactate producing bacteria) was greater in the post-test period than the pre-test period, suggesting *P. acidipropionici* alters metabolism toward less acetate and more propionate production. In support of this latter study, Kim et al. (2001b) reported that the acetate/propionate ratio decreased after in-vitro fermentation for 6, 12 and 24 h when *P. acidipropionici* was used at a concentration of  $4.7 \times 10^{10}$  cfu. Total VFA, acetate, and propionate concentrations increased 9, 5, and 18%, respectively, when *Propionibacterium* strain P5 (PB) was prepared at  $1 \times 10^3$ ,  $1 \times 10^6$ , and  $1 \times 10^9$  cfu/g; however, when PB was autoclaved a significant decrease was observed in total VFA, acetate, and propionate concentrations compared with PB suggesting that the changes in VFA concentrations were due to PB (Akay and Dado, 2001).



*Propionibacterium jensenii* strain 169 (P169) fed to early lactating multiparous Holstein dairy cows from -2 to 12 wk postpartum increased NEFA concentrations at wk 1 of lactation, but had no effect on milk production (Francisco et al., 2002). Leptin concentrations were greater in treated cows through the course of the study, whereas plasma glucose, insulin, cholesterol, and IGF-I concentrations were not affected by feeding P169. Francisco et al. (2002) was the first published study using P169 in dairy cattle and indicated that P169 may alter some metabolic changes during lactation. An in-vitro ruminal model suggested that *P. jensenii* strains have the greatest potential to utilize lactic acid in the rumen of beef cattle fed high concentrate diets due to increasing pH and the suppression of lactic acid accumulation (Parrott, 1997). Swinney-Floyd (1997) reported that during the first 9 d of a 21 d experiment for the evaluation of adaptation to high concentrate diets, acidosis never occurred and lactic acid concentrations did not accumulate in ruminally-cannulated heifers inoculated with a mixed culture of *P. acidipropionici* and *P. freudenreichii*. Acidosis occurred after 12 d, but lactic acid concentration did not accumulate in the rumen. These results suggest that the dose of *Propionibacterium* strain ( $2 \times 10^{11}$  cfu/mL) was too low to become established in the rumen. Similar results were observed when a mixed culture of *P. acidipropionici*, *P. freudenreichii*, and *P. jensenii* was inoculated daily ( $6.3 \times 10^{11}$  cfu/mL) to heifers during adaptation to large amounts of grain (Davidson, 1998). Furthermore, Swinney-Floyd (1997) evaluated the effect of inoculation of *P. jensenii* strain P63 into feedlot cattle. Results indicated that acidosis was reduced in treated animals compared with control animals. Ten heifers were used in this experiment in which 5 animals received  $3 \times 10^{11}$  cfu of P63 intraruminally 14 d before the introduction of concentrate diets. Heifers were

fasted for 1 d and the following 2 d were switched to a 90% concentrate diet containing high concentrations of ground wheat. Lactic acid concentration was lower in inoculated compared with non-inoculated heifers. Swinney-Floyd (1997) concluded that P63 could reduce acidosis problems in animals fed high concentrate diets due to the anti-acidotic activity.

Other research has suggested that *Propionibacterium ssp.* may play an important role in reducing nitrate toxicity in beef cattle. For example, Swartzlander (1994), in an in-vitro study, suggested that of the 154 propionibacteria strains tested, 11% had the ability to denitrify of which *P. acidipropionici*, *P. freudenreichii*, and *P. jensenii* accounted for 69, 29, and 6%, respectively of the denitrification. Due to the greater response of the *P. acidipropionici* (P5) on denitrification, Swartzlander (1994) evaluated the effect of this strain in vivo in beef cattle. Heifers received a daily dose of P5 ( $10^7$  cfu/mL) via the ruminal cannula. Ruminal populations of P5 were monitored by differential-selective media and plasmid DNA profiling. In one experiment, P5-inoculated heifers had 43% less ruminal nitrite and rapidly decreased blood nitrite levels. In a second experiment, P5 did not affect ruminal nitrate levels; however, blood nitrite was reduced by 38% within 8 h and methemoglobin was reduced by 35%. Swartzlander (1994) concluded that pre-inoculation of cattle with *P. acidipropionici* reduces nitrate toxicity. Similarly, Strickland et al. (2004) reported that propionibacteria have the capacity to reduce ruminal nitrite and blood methemoglobin by 40 to 50% in cattle.

Further research is needed to evaluate all the different properties of *Propionibacterium ssp.* as a DFM to improve metabolic and hormonal changes in beef cattle, feedlot cattle, and high-producing dairy cattle.

## **Bacterial Fermentation in the Rumen**

The rumen is an open, oxygen-free, self-contained ecosystem representing an ideal environment to maintain a stable microbial population in which feed consumed by the ruminant animal is fermented to VFA and microbial biomass. These byproducts then serve as sources of energy and protein (Church, 1993; Weimer, 1998). The rumen is also a combustion chamber that maintains a temperature between 38 - 42°C and a pH ranging from 6 to 7. Alterations of these factors and other factors such as the buffering capacity, osmotic pressure, dry matter content, and oxidation-reduction potential results in a negative response on the activity and growth of the microbial population in the rumen (Church, 1993; Van Soest, 1994; Dehority, 2003). Low pH causes a disruption of the homeostasis in the rumen, affecting the performance of the animal by decreasing DMI, fiber digestibility, and microbial yield. These responses decrease milk production, increase health problems, and increase the need for feed additives (ionophores, buffers, and antibiotics) which increase feed costs (Allen, 1997; Cheng et al., 1998; Nagaraja and Chengappa, 1998; Owens et al., 1998; Russell and Rychlik, 2001).

Microbial species have evolved over millions of years in the rumen where they interact in a very complex manner to provide the best example of microbial symbioses (Weimer, 1998). Microbial populations of the rumen consist of anaerobic bacteria, ciliated protozoa, and lower numbers of fungi responsible for the digestion and fermentation of large amounts of fiber fed to the ruminant animal (Church, 1993; Russell and Rychlik, 2001; Dehority, 2003). Bacterial populations in the rumen account for  $10^{10}$  to  $10^{11}$  cells/mL of ruminal content, comprising a relatively large proportion of the total population. Protozoa can reach numbers of  $10^5$  to  $10^6$  cells/mL of ruminal content,

representing 40% of total microbial N and 60% of microbial fermentation products in the rumen. Ruminal fungi, found in a lower numbers ( $10^3$  to  $10^5$  cells/ml), have been shown to have a role in fiber degradation and contribute about 8% of the microbial mass (Church, 1993). Therefore, because of the diverse morphology and physiological characteristics of the ruminal microorganisms, techniques, such as 16S rRNA sequence and DNA:DNA hybridization, have been developed to characterize and quantify the microbial populations; however, these techniques need more refinement and improvement due to the low predicted population densities (Krause and Russell, 1996a, 1996b; Weimer et al., 1999)

Studies in vitro and in vivo have been conducted to evaluate the parameters involved in the ruminal fermentation process when different diets are consumed by ruminant animals. For example, Bernard et al. (2001) conducted an in vitro mixed ruminal microorganism fermentation study to evaluate the effect of coating whole cottonseed (WCS) with gelatinized cornstarch (0.0, 2.5, and 5.0%) and feed grade urea (0.0, 0.25, 0.5, and 1.0%). Total VFA,  $H_2$ ,  $CH_4$ , molar proportions of acetate, and acetate/propionate ratio decreased linearly as starch concentrations increased. Lactate concentrations were greater for 2.5% starch compared with 0 or 5.0%. However, urea treatment did not affect acetate, propionate, acetate/propionate ratio, total VFA or Lactate (Bernard et al., 2001). This experiment was also conducted in vivo (Bernard et al., 2003) using the same substrates in four ruminally- and duodenally-cannulated Jersey cows to evaluate the effect of WCS on ruminal fermentation, fiber digestion, and bacterial protein synthesis. WCS compromised 15% of the ration dry matter fed as a TMR once daily. The coating provided two concentrations of gelatinized cornstarch (2.5 [2S] or 5.0%

[5S]) and feed urea (0.25 [2U] or 0.50% [5U]). Ruminant pH increased and propionate concentrations tended to increase, whereas valerate decreased with 2S compared with 5S (Bernard et al., 2003). Acetate tended to decrease and butyrate increased more for 5U than 2U. Neither ruminant nor total tract digestibility was affected nor the flow of total N or bacterial N to the duodenum. WCS is primarily cellulose and coating WCS with starch potentially increased the density of amylolytic microorganisms. These results suggest that using a combination of starch and urea in coating WCS slightly altered ruminant fermentation.

Other studies have evaluated the effect on ruminant fermentation and N metabolism by replacing cornstarch with sucrose in a dual-effluent continuous culture in Holstein cows. Vallimont et al. (2004) reported that ruminant pH, total VFA or acetate/propionate ratio were not affected by the replacement of cornstarch (0 to 7.5% of DM) with sucrose (0 to 7.5% of DM); however, branched chain VFA were greater for the control treatment compared with the 7.5% sucrose. Sucrose treatments altered the molar proportions of all VFAs, acetate/propionate ratio, and gluconeogenic to lipogenic ratios after 5 h postfeeding. Digestibility of DM and N were not affected by treatment. Therefore, inclusion of sucrose to the diet when rumen-degradable protein (RDP) is adequate does not influence ruminant fermentation. However, to alter microbial populations in the rumen, sucrose needs to be at the highest level resulting in less ammonia N capture efficiency (Vallimont et al., 2004).

Microbial populations can be changed also by the addition of anaerobic fungus (Lee et al., 2004). Other research has reported that rumen microbial activity could be improved by various processing of the corn grain when fed to early lactating dairy cows resulting in

an increase in digestion, metabolism, and milk production (Allen, 1997; Knowlton et al., 1998). Moreover, ruminal microbial populations differ within species such that swamp buffaloes raised on traditional systems (native grasses, rice stubble and rice straw) over the Northeast of Thailand have greater bacterial populations, lower protozoa, and more fungal zoospores in ruminal fluid compared with crossbred cattle (Brahman x Native); (Wanapat, 2001). However, using oscillating dietary protein in wethers showed that microbial efficiency was not affected and also did not cause a negative effect on ruminal or postruminal digestion (Ludden et al., 2002). In contrast, microbial populations in beef cattle could be altered by the addition of compounds such as ionophores and sarsaponin. These compounds stimulated mixed ruminal microorganism fermentation by decreasing methane, hydrogen, and ammonia concentrations (Lila et al., 2003).

Weimer et al. (1999) compared the effects of four contrasting diets comprised of alfalfa silage or corn silage on the population of three cellulolytic species of ruminal bacteria (*Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*). Weimer et al. (1999) found that the major difference in the total number of these three species is mostly accounted for by individual cow variance than to the contrasting effect of diets, and concluded that cows maintained a unique assemblage of ruminal microbial strains that determine the cause of failure of the cellulolytic bacteria to respond to differences in source or concentration of fiber. These results support the fact that the ruminal microbial population is a result of a complex interaction with the host (Church, 1993; Van Soest, 1994; Weimer et al., 1999).

Collectively, the rumen is an ideal environment to maintain a diverse microbial population. The disruption of the rumen homeostasis results in increasing ruminant

disorders such as acidosis and health problems. Addition of certain compounds has been used to ameliorate this problem by the stimulation of the mixed ruminal microorganism to favor fermentation in the ruminant animal.

Drackley (1999) recommended the implementation of proper management practices to improve the transition period in order to ensure the profitability of the lactation period.

This author suggested that research is warranted in specific critical areas including:

1. periparturient control of DMI;
2. quantification of nutrient supply during the transition period;
3. interaction among the immune system, nutrition, and metabolism;
4. metabolic regulation and interaction among liver, adipose tissue, muscle and the digestive tract; and
5. effects of body condition on transition and metabolic responses due to different transition management practices.

## Plasma Hormones and Metabolites

### Effects of propionate on plasma hormones and metabolites

**Glucose.** Glucose uptake by the mammary gland is essential for milk synthesis (Rigout et al., 2003; Lemosquet et al., 2004). Nervous tissue, muscle, adipose, and fetal tissue also require glucose primarily via gluconeogenic pathways (Church, 1993). In addition, glucose impacts ovarian cell response to trophic stimulation (Stewart et al., 1995).

Research has been conducted to evaluate the effect of propionate, a glucogenic precursor, on glucose metabolism in cattle and sheep. For example, Peters et al. (1983) found that glucose concentrations in ewes were increased two fold after 15 min of intramesenterical infusion of propionate and levels remained increased for about 45 min after infusion. Moreover, intravenous infusion of propionate in sheep doubled the concentration of plasma glucose after 5 min of beginning propionate infusion and glucose levels remained elevated until 30 min postinfusion of propionate (Lee and Hossner, 2002). Primiparous cows fed Cr and infused with propionate over 5 min at a dosage of 2.5 mmol/kg of BW peaked glucose levels at 20 min after infusion, after which concentrations decreased slowly (Subiyatno et al., 1996). Similarly, Sano et al. (1993a) found a dose-dependent increase in glucose, glucagon, and insulin in sheep when propionate was infused intravenously at a dose of 1 to 64  $\mu\text{mol}\cdot\text{kg BW}^{-1}\cdot\text{min}^{-1}$  for 30



min. In contrast, Casse et al. (1994) infused propionate intrasenterically (2.5 M solution) to primiparous Holstein cows which caused a decrease in splanchnic glucose release, while increasing the release of acetate and alanine. However in sheep, intrasenteric infusion of propionate at dosages of 1, 2, 4, 8 and 32  $\mu\text{mol}\cdot\text{kg BW}^{-1}\cdot\text{min}^{-1}$  for 30 min did not alter glucose concentrations (Sano et al., 1995). Likewise, glucose metabolism was unchanged when propionate was infused intraruminally to lambs (Majdoub et al., 2003) or when propionibacteria P169, a glucogenic precursor, was fed top-dressed to multiparous Holstein cows (Francisco et al., 2002). Therefore, infusion of propionate increase plasma glucose concentrations; however, the response may depend on the dose and route of infusion as well as the basal diet the animals are fed.

Other research has focused on the effect of propylene glycol, also a glucogenic precursor (Pickett et al., 2003), on glucose metabolism. Drenching of propylene glycol (500 mL) from 7 to 42 d of lactation to Holstein cows caused a rapid increase in plasma glucose by 30 min, and a gradual increase through 90 min after drenching (Miyoshi et al., 2001). Furthermore, Grummer et al. (1994) suggested that 296 mL of propylene glycol also provoked a linear increase in glucose concentrations. Although conflicting reports exist, theoretically increasing the availability of glucogenic precursors such as propionate may increase liver metabolism of glucose (Danfaer et al., 1995), suggesting an increase in uptake of glucose and utilization by the mammary gland.

**Insulin.** Insulin regulates the storage of compounds such as protein, lipid, glycogen, and minerals, with major functions on secretory rate and receptor localization (M<sup>c</sup>Cusker, 1998). In particular, insulin stimulates glucose uptake in most tissues including the

mammary gland, adipose, and muscle (Peters et al., 1983; Danfaer et al., 1995; McGuire et al., 1995). Insulin also plays an important role in ovarian function, follicle growth, and cell proliferation (Spicer et al., 1993; Spicer and Echtenkamp, 1995; Miyoshi et al., 2001; Francisco et al., 2003)

The utilization of propionate appears not to be affected by insulin concentrations; however, incorporation of other glucose precursors into glucose is decreased by 30 – 50% in sheep (Brockman, 1990). Istasse et al. (1987) reported that propionate treatments increased insulin secretion. Johnson et al. (1982) showed that propionate-fed calves had a greater increase in insulin concentration (264.6  $\mu$ U/mL) at 0.5 h postfeeding compared with calves receiving glucose. Insulin rise when glucose declined suggests that the greater response of insulin is due to an inherent ability of propionate in preruminant calves. Either propionate or glucose infusion independent of the site of infusion has elicited insulin secretion in lactating dairy cows, beef cows, and sheep (Peters and Elliot, 1984; Istasse et al., 1987; Quigley and Heitmann, 1991; Sano et al., 1993a, 1995; Cole and Hallford, 1994; Lemosquet et al., 1997; DiCostanzo et al., 1999; Gabai et al., 2002; Jan et al., 2002; Lee and Hossner, 2002; Patton et al., 2004). Similarly, drenching with a glucogenic precursor such as propylene glycol produced a linear increase in insulin concentrations (Grummer et al., 1994; Miyoshi et al., 2001). In contrast, Pickett et al. (2003) found that when propylene glycol was administered for 3 d postpartum to lactating Holstein cows insulin concentrations were unchanged. These results could be attributed to the infrequent sampling frequency and thus not being able to detect an increase in insulin after a propylene glycol drenching. Likewise, feeding

propionibacteria P169 to multiparous Holstein cows during early lactation failed to alter plasma insulin concentrations (Francisco et al., 2002).

Collectively, these results suggest that plasma insulin concentrations are increased by propionate infusion, and that this increase may improve metabolic performance of cattle and sheep.

**Insulin-like growth factor-I.** Circulating levels of insulin-like growth factor-I (IGF-I) are regulated by nutritional status, energy intake, negative energy balance (NEB), and insulin treatment (Spicer et al., 1990; Straus, 1994; McGuire et al., 1995; Zulu et al., 2002). Ovarian cell mitogenesis and steroidogenesis is stimulated by IGF-I (Spicer and Echternkamp, 1995). Also, IGF-I plays an important role in mammary gland function stimulating mammary epithelial mitosis (Weber et al., 1999; Akers et al., 2000; Kleinberg et al., 2000) and synthesis of milk proteins (Keys et al., 1997).

Lemosquet et al. (2004) reported that infusion of propionate via the rumen increased IGF-I concentrations in lactating dairy cows during a 14 d study. Moreover, intravenous infusion of propionate at a dosage of 2.5 mmol/kg of BW over 5 min increased IGF-I levels when Cr was supplemented in early lactating primiparous cows (Subiyatno et al., 1996); however, glucose infusion during early lactation did not affect IGF-I concentrations (Chelikani et al., 2003). Lee and Hossner et al. (2002) found similar results in sheep such that infusion of propionate at a rate of  $64 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg BW}^{-1}$  for 30 min did not affect IGF-I concentration. Furthermore, supplementation of propionibacteria P169 from -2 to 12 wk postpartum to multiparous Holstein cows did not affect IGF-I levels in plasma; however, concentrations of IGF-I increased with weeks

postpartum (Francisco et al., 2002). These results suggests that further studies are warranted to better understand the effects of different sources of glucogenic precursors on IGF-I concentrations.

**Leptin.** Leptin, discovered in 1994, is a 16 kDa protein that contains 146 amino acids and is synthesized by adipose cells (Zhang et al., 1994). Leptin secretion by the adipose cell has been implicated in the regulation of feed intake, as well as the neuroendocrine-reproductive axis of several species (Spicer, 2001). It is also involved in energy metabolism and has an important role in the regulation of homeostatic and homeorhetic control (Reist et al., 2003).

Supplemental feeding of propionibacteria P169 increased leptin levels ( $8.10 \pm 1.0$  ng/mL) above control ( $5.25 \pm 1.0$  ng/mL) in Holstein cows during early lactation (Francisco et al., 2002). Feeding high concentrate diets (50%) from wk 1 to 10 postpartum to multiparous Holstein cows also increased leptin levels and this increase could be crucial for high milk yield due to its association with metabolic, enzymatic, and endocrine traits (Reist et al., 2003). Likewise, infusion of short-term propionate into sheep stimulates leptin mRNA. This response is related to the excess of energy due to propionate infusion (Lee and Hossner, 2002). In contrast, glucose infusion to Italian Simmental cows did not affect leptin concentrations (Gabai et al., 2002). Increasing leptin levels, via an increase in propionate concentration in the rumen, may improve reproductive function via an indirect effect; however, further studies are needed to elucidate the function of propionate on leptin concentrations.

**Nonesterified fatty acids.** Nonesterified fatty acids (NEFA) are transporters of fatty acids in blood. The release of NEFA from adipose tissue is a result of triglyceride lipolysis by action of hormone sensitive lipase (Murray et al., 2003). A dramatic increase in concentrations of NEFA occur prior to parturition due to the increase in nutrient demand for milk production (Drackley, 1999). Concentrations of plasma NEFA in lactating cows are negatively correlated with energy balance (EB); ( $r = -0.40$ ) such that as EB increased NEFA concentrations decreased (Canfield and Butler, 1991).

Many experiments have been conducted to evaluate changes in this metabolite with the addition of different compounds with glucogenic activity. For example, primi- and multiparous cows were used to examine the effect of propylene glycol on NEFA concentration and found that propylene glycol decreased plasma NEFA levels independent of the dose (Grummer et al., 1994; Miyoshi et al., 2001; Pickett et al., 2003). Similarly, infusion of propionate to either dairy cows (Casse et al., 1994; Subiyatno et al., 1996; Rigout et al., 2003; Patton et al., 2004) or sheep (Lee and Hossner, 2002) results in about a 70% decrease in plasma NEFA concentrations. Supplementation of propionibacteria P169 also decreased NEFA concentrations during early lactation in multiparous Holstein cows (Francisco et al., 2002). Collectively, experimental evidence indicates that glucogenic precursors decrease NEFA concentrations. Further evidence suggests that increasing insulin levels by glucose precursors lead to improved lipid deposition rather than mobilization and hence decreased NEFA concentrations (Picard et al., 1999; Lee and Hossner, 2002).

**Cholesterol.** The major site for cholesterol synthesis is the liver (Emmanuel and Robblee, 1984). Cholesterol production is regulated by various factors such as dietary feedback inhibition, circadian rhythms, feeding/fasting fluctuations, and enterohepatic bile acid circulation (Ott and Lachance, 1981). Moreover, cholesterol levels are positively correlated to DMI (Francisco et al., 2003) such that cholesterol concentrations increased two fold during wk 1 to 7 in Holstein cows as DMI increased (Francisco et al., 2002). Cholesterol is also required for ovarian steroidogenesis (Carroll et al., 1990; Spicer and Echternkamp, 1995; Spicer et al., 1996) which can be obtained from the blood as HDL/LDL (Veldhuis et al., 1984), or via de novo cholesterol synthesis within ovarian cells (Spicer et al., 1996).

Although no study has evaluated effects of acute propionate infusion on plasma cholesterol levels, research indicates that propionate may stimulate cholesterol synthesis by two mechanisms: 1.)  $\alpha$ - oxidation of propionate to yield acetyl-CoA, and 2.)  $\beta$ -oxidation of propionate to form malonyl-CoA (Emmanuel and Robblee, 1984). However, this mechanism also is dependent on the balance between incorporation of propionate into cholesterol and its inhibition of HMG-CoA synthase (Emmanuel and Robblee, 1984). Plasma cholesterol was not affected by supplemental feeding of propionibacteria P169 postpartum (Francisco et al., 2002).

Addition of fat to diets increases plasma cholesterol concentrations. For example, Carrol et al. (1990) found that feeding 5% prilled long-chain fatty acids in combination with three different forage ratios ( 45:55, 64:36, 84:16) to Holstein cows starting at 5 d postpartum increased plasma cholesterol concentrations throughout 100 d of milking regardless of the forage ratio. Similarly, Spicer et al. (1993) found that when inert fat

was supplemented to Holstein cows from wk 0 to 12 postpartum, cholesterol concentrations increased 69% between wk 5 to 12, whereas in cows receiving the control diet cholesterol increased by 23%. Furthermore, when high concentrate diets were offered to multiparous Holstein cows during the first 20 wk postpartum, plasma cholesterol concentrations responded positively (Reist et al., 2003). Collectively, plasma cholesterol concentrations responded positively to either propionate or fat supplementation. Moreover, it is also positively correlated to DMI, suggesting that plasma cholesterol could be used as a predictor of metabolic status in Holstein cows.

## Monensin

Monensin, an ionophore that increases the ratio of propionate to acetate produced within the rumen (Nevel and Demeyer, 1977), has been used in the beef industry for several years to increase feed efficiency and has recently been approved (Nov 3, 2004) for use in lactating dairy cows. It has the potential to reduce metabolic disorders (Duffield et al., 2002; 2003a), increase milk production (Phipps et al., 2000; Duffield et al., 2003b), and improve reproductive function (Heuer et al., 2001; Tallam et al., 2003). Monensin also increases the molar proportion of propionate in the rumen and alters energy metabolism in dairy cows (Stephenson et al., 1997; Arieli et al., 2001). However, discrepancies exist relative to the effects of monensin on metabolic indicators in dairy cows. For example, monensin treatment increases glucose metabolism in multiparous Holstein cows (Arieli et al., 2001), whereas other research found that monensin treatment had no positive effect on plasma glucose (Stephenson et al., 1997; Juchem et al., 2004; Gallardo et al., 2005). Plasma concentrations of insulin were increased in ruminally-cannulated beef heifers treated with 0 or 200 mg per day of monensin (Reed and Whisnant, 2001). These results suggest that the decrease in plasma concentrations of NEFA (Gallardo et al., 2005) was due to the antilipolytic effect of insulin (Picard et al., 1999). In contrast, other research was unable to elicit an effect of monensin on either insulin or NEFA concentrations (Stephenson et al., 1997; Juchem et al., 2004). Cholesterol concentrations were greater when monensin treatment was offered to Holstein cows 3 wk precalving (Duffield et al., 2003a). Further studies are warranted to



clarify the effects of monensin on metabolic indicators such as glucose, insulin, NEFA, and cholesterol in lactating Holstein cows.

Monensin supplementation to lactating cows also increases milk production and reduces milk fat. For example, Phipps et al. (2000) conducted two experiments using lactating cows. Experiment 1 consisted of 0, 150, 300, or 450 mg of monensin during wk 7 to 26 of lactation. Experiment 2 consisted of 0 or 300 mg/d of monensin for two consecutive lactations. Milk production increases in Experiment 1 were 2.8, 2.5, and 1.5 kg/d in response to 150, 300, and 450 mg of monensin/d, respectively. In Experiment 2, milk production increases in response to monensin were 0.8 and 1.1 kg/d. Therefore, monensin increased milk production in both experiments; however, in Experiment 1, milk production efficiency was greater by 5% compared to Experiment 2, suggesting that monensin favors ruminal fermentation metabolism by increasing the availability of glucogenic precursors. Moreover, a decrease in the percentage of milk fat is associated with monensin supplementation and results depend on dose and dietary characteristics (Phipps et al., 2000; Duffield et al., 2003b). Whether monensin shifts ruminal fermentation and increases the availability of glucogenic precursors in lactating dairy cows will require additional studies.

## **Plasma Hormones and Metabolites**

### **Changes in plasma hormones and metabolites during lactation**

The transition period is defined as commencing 3 wk before calving to 3 wk after calving in which lactating cows overcome dramatic metabolic changes (Grummer, 1995). These metabolic changes are accounted for by the state of pregnancy, diminished feed intake during late pregnancy, lactogenesis, and parturition (Grummer, 1995; Drackley, 1999). Plasma NEFA concentrations increase during the last 10 d of expected calving and are followed by a decrease in feed intake (Grummer, 1995). Glucose concentrations decrease during the transition period with a sharp increase at parturition. This acute increase in glucose at calving is associated with an 80% increase in glucose required by the mammary gland for milk synthesis (Bell and Bauman, 1997). Insulin and IGF-I concentrations were greater before parturition; however, just prior to parturition those concentrations decreased and recovered slowly during lactation (Spicer et al., 1990; Francisco et al., 2002; Pushpakumara et al., 2003; Radcliff et al., 2003). Leptin concentrations decreased over 50% between 1 mo before calving and 1 wk postpartum and then increased between 1 and 4 wk postpartum (Kadokawa et al., 2000; Block et al., 2001; Liefers et al., 2003).

Much research has been conducted to evaluate the fluctuations in plasma NEFA and glucose during lactation. For example, Vasquez-Añon et al. (1994) reported that NEFA concentrations increased 5 d before calving, peaked at d 1 after calving and decreased during the first 3 wk of lactation. These authors suggested that the acute increase in

NEFA concentration could be due to the decrease in DMI and the increase in lipolytic hormones at calving. Similar changes in plasma NEFA have been reported by others (Canfield and Butler, 1991; Grummer, 1995; Block et al., 2001; Pushpakumara et al., 2003; Jorritsma et al., 2004).

Glucose concentrations increased prior to calving, decreased during the first wk of lactation and then gradually increased (Canfield and Butler, 1991; Vazquez-Añon et al., 1994; Grummer, 1995; Grum et al., 1996; Doepel et al., 2002; Francisco et al., 2002; Radcliff et al., 2003). Changes in glucose concentrations around the time of calving are due to increases in DMI, nutrient demands for milk production and changes in hormones such as cortisol that promote gluconeogenesis and glycogenolysis (Maciel et al., 2001; Francisco et al., 2002; Nikolic et al., 2003).

Fluctuations in insulin concentration during the periparturient period are not well defined, but a consistent increase between wk 1 and 12 has been observed (Spicer et al., 1993; Francisco et al., 2002). For instance, Radcliff et al. (2003) reported that insulin concentration before parturition was increased and then declined for approximately 5 d after parturition, after which it increased gradually. Pushpakumara et al. (2003) suggested that prior to calving insulin concentrations were greater and dramatically decreased at wk 1 of calving, after which insulin concentrations gradually increase during lactation. Doepel et al. (2002) reported that insulin concentration declined from wk -3 to calving and remained low through wk 3 postpartum in multiparous Holstein cows. Moreover, Francisco et al. (2002) reported that insulin concentrations at wk 4 were 50% greater than wk 1 and that plasma insulin concentrations increased gradually between wk 1 and 12 in lactating Holstein cows. These increases in insulin were positively correlated

with DMI (Francisco et al., 2002). Overall, changes in insulin concentrations could be due to change in the ability of the pancreas to secrete insulin and (or) a change in peripheral insulin sensitivity during lactation (Lomax et al., 1979).

Concentrations of IGF-I decline at calving, remain low for several weeks, and gradually increase postpartum (Aribat et al., 1990; Spicer et al., 1990; Kerr et al., 1991; Pushpakumara et al., 2003; Radcliff et al., 2003). Concentrations of IGF-I are also correlated with EB such that cows experiencing positive EB had greater IGF-I concentrations compared to cows in negative energy balance (NEB) (Spicer et al., 1990; Beam and Butler, 1998; Lucy, 2000; Walters et al., 2002). Moreover, primiparous cows had greater IGF-I concentrations compared to multiparous cows and these differences were due to milk yield (Taylor et al., 2004). Also, lower IGF-I concentrations and BCS after calving were associated with a failure to conceive (Pushpakumara et al., 2003; Taylor et al., 2004). Increasing energy density of the diet or addition of supplemental inert fat did not affect IGF-I concentrations (Spicer et al., 1993; Grum et al., 1996), and systemic IGF-I concentrations were not significantly correlated with DMI in two (Spicer et al., 1990; 1993) of three studies (Francisco et al., 2002)

Leptin concentrations during the transition period have been investigated in order to evaluate the relationship with energy balance, milk yield, DMI, and live weights (Liefers et al., 2003). In all species, leptin concentrations were greater before parturition and declined about 50% after parturition (Kadokawa et al., 2000; Block et al., 2001; Ehrhardt et al., 2001; Liefers et al., 2003). These reductions in leptin concentrations could be associated with reduction of leptin mRNA expression (Block et al., 2001; Ehrhardt et al., 2001). Leptin concentration recovery after parturition depends on energy status and

adipose accumulation (Liefers et al., 2003). Cows in NEB had lower leptin concentrations, produced more milk, had less feed intake, and lighter live weight compared to cows in positive EB (Liefers et al., 2003). Moreover, plasma leptin concentration is positively correlated with glucose and insulin concentrations and negatively correlated with plasma NEFA concentrations (Block et al., 2001).

In summary, the transition period is characterized for a sharp increase in glucose concentration at parturition, after which decreased following calving. Insulin, IGF-I, leptin were greater before than after parturition and increase during the postpartum period, whereas NEFA concentrations increase toward calving and remains high during the early postpartum associated with NEB, diminished of DMI and feed intake. The physiological significance of these changes in hormones and metabolites are not completely known, but in vitro evidence clearly indicates that insulin (Halmiton et al., 1999; Amstrong et al., 2001), IGF-I (Halmiton et al., 1999; Guitierrez et al., 2000; Amstrong et al., 2001), leptin (Gregoraszczuk et al., 2003; Swaim et al., 2004), and glucose (Boland et al., 1994; Stewart et al., 1995) can directly impact ovarian function.

In order to meet the increasing nutrient demands for milk production in the lactating dairy cow, marked hormonal and metabolic changes occur during the transition period. Supplemental feeding of glucogenic precursors (e.g. propionibacteria) have been used to ameliorate the dramatic metabolic changes that occur in the lactating cow. Therefore, propionibacteria supplementation could influence the metabolism of the lactating cow, such that changes in plasma hormones and metabolites are not as drastic when stress stimuli, such as parturition and lactation, are present. Our goal was to evaluate the effect

of supplemental feeding of propionibacteria P169 on plasma hormones and metabolites in  
primi- and multiparous Holstein cows from 14 d prepartum to 175 d postpartum.

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## Chapter III

### EFFECT OF A DIRECT-FED MICROBIAL ON PLASMA CONCENTRATIONS OF HORMONES AND METABOLITES IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

#### Abstract

To determine the effect of feeding propionibacteria on metabolic indicators during lactation, multi- and primiparous Holstein cows were fed one of three dietary treatments in a 2x3 factorial design from 14 d prepartum to 175 d postpartum: 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  $6 \times 10^{11}$ /head/d (high-dose P169) of Propionibacterium Strain P169; or 3) low-dose group (primiparous n=8, multiparous n=6), fed TMR plus  $6 \times 10^{10}$ /head/d (low-dose P169) of P169. Blood samples were collected weekly for 30 wk and analyzed for plasma concentrations of glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, nonesterified fatty acids (NEFA) and cholesterol (CHOL). Between wk 25 and 30, bovine somatotropin (bST) was given to all groups every 2 wk. Plasma glucose was affected by diet x parity ( $P < 0.001$ ) such that glucose levels in low-dose P169 multiparous cows ( $59.8 \pm 1.1$  mg/dL) were 5.5% lower than in high-dose P169 multiparous cows; low-dose P169 primiparous cows ( $67.9 \pm 0.9$  mg/dL) had 6% to 9% greater plasma glucose concentrations than high-dose P169 and Control primiparous cows. Plasma insulin was affected by diet ( $P < 0.001$ ) such that

low-dose P169 had less plasma insulin than high-dose P169 and Control cows (during wk 13-25), and high-dose P169 cows had greater insulin than Controls (during wk 1-12). Plasma IGF-I, NEFA, and leptin concentrations did not differ ( $P > 0.15$ ) among diet groups between wk 1 and 25, but primiparous cows had greater ( $P < 0.02$ ) IGF-I and lower ( $P < 0.01$ ) NEFA concentrations than multiparous cows. Plasma CHOL was affected by diet x parity ( $P < 0.01$ ) such that low-dose P169 multiparous cows ( $246 \pm 11$  mg/dL) had 25% greater concentrations than high-dose P169 and Control multiparous cows; CHOL concentrations in primiparous cows did not differ among diet groups. During bST, high-dose P169 multiparous cows and low-dose P169 primiparous cows had lower ( $P < 0.01$ ) IGF-I levels than their respective Controls. Regardless of parity, low-dose P169 cows had greater ( $P < 0.10$ ) leptin levels than Control cows, and high-dose P169 cows had greater ( $P < 0.01$ ) NEFA than Control cows. We conclude that P169 may hold potential as a direct-fed microbial to enhance metabolic efficiency during early and mid-lactation.

## **Introduction**

Propionibacteria are natural inhabitants of the rumen microflora such that in ruminant animals fed either high concentrate or high roughage diets, propionibacteria comprise 1.4% to 4.3% of the total microbial population (Mead and Jones, 1981; Oshio et al., 1987). In dairy cows, the population of probionibacteria ranged from  $10^3$  to  $10^4$  cfu/mL in the rumen (Davidson, 1998). One end product of propionibacteria is the production of propionic acid (Grinstead and Barefoot, 1992; Hugenholtz et al., 2002).

Propionate is the only major volatile fatty acid (VFA) that is glucogenic and the major site for metabolism is the liver (Van Soest, 1994). Gluconeogenesis is an important process for the ruminant animal in which glucose synthesis occurs and results in a source of energy for the animal (Church, 1993). The volatile fatty acids can supply up to 70 to 80% of the ruminant's energy requirement (McDonald et al., 2002). The efficiency of propionate as a source of energy for ATP is about 108% compared to glucose (McDonald et al., 2002). The efficiency of propionic acid for utilization for maintenance is 0.86 compared to acetic acid (0.59) or butyric acid (0.76), and the efficiency of these VFA for growing ruminants animals is 0.56, 0.33-0.60, and 0.62, respectively (McDonald et al., 2002).

During the periparturient period in dairy cows, the requirements for nutrients increase due to the fact that lactation requirements are even greater than that for fetal development (Church, 1993; Drackley, 1999). Consequently, maintaining energy balance in high producing dairy cows is a difficult process to achieve and involves metabolic and hormonal changes. Leptin and insulin concentrations decrease during negative energy balance, and thus insulin has been suggested to be a modulator of leptin secretion in lactating dairy cows (Block et al., 2003; Liefers et al., 2003). Nonesterified fatty acids (NEFA) concentrations increase during parturition as a result of fat mobilization and provides fuel to lactating cows for milk production and maintenance (Grummer, 1993).

Venous propionate infusion in sheep for 30 min at 1.2 M increased plasma glucose and insulin concentrations (Sano et al., 1993a). Patton et al. (2004) showed that when fat and calcium propionate were added to diets of multiparous Holstein cows, decreased blood levels of NEFA and increase glucose and insulin. Infusion of 50% dextrose

(glucose) to Holstein cows increased glucose and insulin without affecting leptin and IGF-I concentrations (Chelikani et al., 2003). Similarly, 33% glucose solution did not alter leptin concentrations in Italian Simmental cows (Gabai et al., 2002). However, Lee and Hossner (2002) found that propionate infusion increased leptin mRNA in adipose tissue of sheep. Therefore, infusion or feeding glucose precursors such as propionate have been used as a management strategy to improve metabolic status in the lactating dairy cow (Drackley, 1999; Oba and Allen, 2003a, 2003b).

Of such strategies, the use of propionibacteria as a glucogenic precursor has been investigated. Propionibacteria fed to lactating multiparous dairy cows during a 12 wk period did not alter plasma glucose, insulin, cholesterol, or IGF-I concentrations; however, leptin concentration tended to be greater in cows fed propionibacteria (Francisco et al., 2002). Therefore, we hypothesized that long-term (>25 wk) feeding of propionibacteria P169 could promote metabolic and hormonal changes that enhance overall metabolism in lactating Holstein cows. Thus, our objectives were to determine the effect of supplemental feeding of propionibacteria P169 on key metabolic indicators such as plasma glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, nonesterified fatty acids (NEFA), and cholesterol concentrations.

## Materials and Methods

### Experimental Design and Sample Collection

Two weeks prior to parturition thirty-eight multi- and primiparous Holstein cows were randomly allotted to one of three dietary treatments groups: 1) control group (primiparous n=5, multiparous n=8) received a total mixed ration (TMR) throughout day 175 postpartum; 2) high-dose group (primiparous n=6, multiparous n=5) received the control diet plus  $6 \times 10^{11}$  cfu/head/d Propionibacterium Strain 169 (high-dose P169); and 3) low-dose group (primiparous n=8, multiparous n=6) received the control diet plus  $6 \times 10^{10}$  cfu/head/d Propionibacterium Strain 169 (low-dose P169); (Agtech Inc., Waukesha, WI). Multiparous and primiparous cows were stratified across treatments based on the previous year's milk production, and predicted transmitting ability (PTA), respectively. The 305-d mature equivalent (ME) milk production for multiparous cows averaged  $10,265 \pm 716$ ,  $10,439 \pm 905$ , and  $10,174 \pm 905$  kg in Control, low-dose, and high-dose treatment groups, respectively, and did not differ among dietary groups. The PTA for primiparous averaged  $120 \pm 104$ ,  $236 \pm 78$  and  $187 \pm 95$  kg for Control, Low-dose, and High-dose treatment groups, respectively and did not differ among dietary groups. The TMR was composed of sorghum/sudan silage, alfalfa hay, bermuda hay, whole cottonseed, corn gluten, yeast (Diamond VXP Yeast culture), and mineral concentrate (Table 1). Energy concentration of the diet was formulated to support daily milk production of at least 45 kg. The TMR was sampled weekly and composited monthly

throughout the study for analysis. The forage analysis was completed by Dairy One Inc., Forage Laboratory, Ithaca, NY.

Cows calved between August 26 and October 25, 2002 and were stratified across treatments based on calving dates. On average, cows in high-dose and low-dose P169 groups had treatments initiated at  $13.1 \pm 2.1$ ,  $14.5 \pm 2.0$ , and  $18.0 \pm 2.3$  d prior to parturition, respectively. Thirty days prepartum, body weights averaged  $581 \pm 21$ ,  $574 \pm 15$  and  $588 \pm 27$  kg for the Control, low-dose and high-dose P169 primiparous cows, respectively, and  $771 \pm 32$ ,  $811 \pm 26$  and  $767 \pm 37$  kg for the Control, low-dose and high-dose P169 multiparous cows, respectively. Cows had free access to water and were provided feed ad libitum. 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 were milked twice daily at 0400 and 1600, and fed twice daily at 0900 and 1600. Blood samples were collected after the a.m. milking, at 0430, via coccygeal venipuncture in tubes (7 mL) containing EDTA. Samples were collected from wk 1 through wk 30 postpartum. After collection of the blood, samples were centrifuged at  $1,200 \times g$  for 15 min ( $4^{\circ}\text{C}$ ) and plasma was decanted and stored frozen at  $-20^{\circ}\text{C}$  for glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, nonesterified fatty acids (NEFA), and cholesterol analyses. The average days on feed at the first week postpartum blood collection for the Control, high-dose, and low-dose dietary groups were  $3.4 \pm 0.60$ ,  $3.3 \pm 0.66$ , and  $4.2 \pm 0.58$  d, respectively, and did not differ between dietary groups. Cows were fed the Propionibacterium Strain P169 via top-dress on a small amount of TMR (4.5 kg) once per day (p.m.) in the free stalls. To assure a 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. One low-dose primiparous cow was taken off the study at wk 23 due to health problems.

The original 25-wk (175 d) study was extended for an additional 5-wk (35 d) period to assess the effects of the Propionibacterium treatment during concomitant bST treatment (Posilac, Monsanto Company, St Louis, MO). Following the final measurements for the 25-wk study, bST was administered at a dose of 500 mg for three consecutive 2-wk (14 d) periods by subcutaneous injection in the *ischiorectal fossa* immediately following weekly blood collection; this was in addition to the continuation of the once per day dosage of Propionibacterium Strain P169 to the applicable treatment groups.

### **Laboratory Analyses**

Plasma concentrations of glucose were determined using a spectrophotometer and a glucose kit (Thermo Electron Corporation, Louisville, CO). This procedure was based on the hexokinase coupled with glucose-6-phosphate dehydrogenase enzymatic reaction. Intrassay and interassay coefficients of variations averaged 5.2 and 9.4%, respectively.

Plasma concentrations of insulin were determined using a solid-phase insulin radioimmunoassay (RIA) Kit (Micromedic Insulin Kit, ICN Biomedicals Inc, Costa Mesa, CA) as previously described using purified bovine insulin (28 Iu/mg) as the standard (Simpson et al., 1994). Intrassay and interassay coefficients of variations averaged 11.4 and 7.6%, respectively.

Concentration of IGF-I in plasma was determined by RIA after acid-ethanol extraction as previously described 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 N HCl) and incubated for 16 h at 4°C. The samples were then centrifuged for 30 min at 1,200 x g at 4° C and then neutralized with 0.855 M tris.

Intraassay and interassay coefficients of variation averaged 8.3 and 7.9%, respectively.

Plasma concentrations of leptin was measured in samples collected during even weeks throughout the 25 wk study and determined by RIA using a Linco's Multi-Species Leptin Kit (Linco Rsearch, Inc., St. Charles, MO) as previously described (Maciel et al., 2001). Intraassay and interassay variations averaged 8.8 and 4.7%, respectively.

Concentrations of NEFA were determined in plasma samples collected during even weeks throughout the study by an enzymatic colorimetric method using NEFA-C Kits (WaKo Chemicals USA, Inc., Richmond, VA). The intraassay and interassay coefficient of variation was 4.7% and 13.5%, respectively.

Concentrations of total plasma cholesterol were determined by an enzymatic colorimetric method using a Cholesterol E Kit (Wako Chemicals USA, Inc., Richmond, VA). Standard curves were constructed between 0 and 400 mg/100mL. Intrassay and interassay coefficients of variations averaged 6.9 and 15%, respectively.

### **Statistical Analyses**

Plasma concentrations of glucose, insulin, IGF-I, leptin, NEFA and cholesterol were analyzed as a completely randomized design for repeated measures, utilizing the Mixed Model of SAS:  $Y_{ijkl} = U + D_i + P_j + (D \times P)_{ij} + C(D \times P)_{ijk} + W_l + (D \times W)_{il} + (P \times W)_{jl} + (D \times P \times W)_{ijl} + e_{ijkl}$ , where U = overall mean, D = diet, P = parity, C(D x P) = cow within group and parity, W = week postpartum, (D x W) = diet by week postpartum

interactions, (P x W) = parity by week postpartum interactions, (D x P x W) = diet by week by parity postpartum interactions, and e = residual error. For the variables that were measured weekly, four separate analyses were conducted for wk 1-25, wk 1-12, wk 13-25 and wk 25-30 in order to better evaluate the effect of treatment on the variables during: the complete study, early lactation, mid-lactation, and during bST treatment, respectively. The model of the covariate structure for repeated measurements was an autoregressive with lag equal to one. If the week x 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 PDIFF option if one or more main effects were significant but the interactions were not significant.

## Chapter IV

### Results

#### Plasma Glucose

There were week ( $P < 0.01$ ), parity ( $P < 0.001$ ), and diet x parity ( $P < 0.0002$ ) effects on plasma glucose concentration; all other main effects ( $P > 0.32$ ) and interactions ( $P > 0.74$ ) were not significant. Plasma glucose concentrations were influenced by week, such that glucose concentration decreased 5% from wk 1 to 2 and then increased 8% through wk 5 after which it did not change (Figure 4.1). Primiparous cows had 5% greater plasma glucose concentrations compared with multiparous cows ( $P < 0.001$ ). Multiparous cows receiving low-dose P169 had a 5% decrease in plasma glucose compared with the high-dose P169 treated cows, whereas primiparous cows fed the low-dose P169 had 8% increase in plasma glucose concentrations compared with their respective control and high-dose groups (Figure 4.2).

Plasma glucose concentration, during wk 1 to 12 of lactation, was influenced by diet x parity interaction ( $P < 0.02$ ), such that primiparous cows fed the low-dose P169 had a 9% increase in glucose concentrations compared with control cows, whereas glucose concentrations in multiparous cows did not differ among treatment groups ( $P > 0.10$ ); (Figure 4.3).

Similar to wk 1 to 12 analysis, during wk 13 to 25 of lactation plasma glucose concentration was influenced ( $P < 0.001$ ) by a diet x parity interaction such that primiparous cows treated with low-dose P169 had 10% greater plasma glucose concentrations, compared with primiparous controls, whereas, plasma glucose concentrations in multiparous cows did not differ among treatment groups (Figure 4.4).

Plasma glucose was not affected by diet ( $P > 0.37$ ) or diet x parity ( $P > 0.16$ ) during bST administration. However, plasma glucose concentration was influenced by parity ( $P < 0.02$ ) during bST administration such that primiparous cows ( $67.3 \pm 1.1$  mg/dL) had 6% greater concentrations of plasma glucose compared with multiparous cows ( $63.6 \pm 1.1$  mg/dL).

### **Plasma Insulin**

Plasma concentrations of insulin were influenced by dietary treatment ( $P < 0.001$ ), but not other main effects ( $P > 0.20$ ) or their interactions ( $P > 0.60$ ). Plasma insulin concentrations decreased 11% in low-dose P169-treated cows compared with high-dose P169 and control cows during the 25 wk study (Figure 4.5).

For the period of wk 1 to 12 of lactation, plasma insulin concentrations were influenced by diet ( $P < 0.01$ ) and diet x week ( $P < 0.01$ ). Compared to the control, plasma insulin concentration increased 16% in high-dose P169-treated cows and numerically ( $P = 0.33$ ) decreased 7% in low-dose P169 cows (Figure 4.6). Average plasma insulin concentrations differed ( $P < 0.01$ ) among weeks, such that plasma insulin concentrations were greater in wk 9 and 11 versus wk 2 and 3 of lactation (Figure 4.7).

Somewhat similar to wk 1 to 12 analysis, plasma insulin concentration was influenced by dietary ( $P < 0.02$ ) during wk 13 to 25 of lactation such that control and high-dose P169-treated cows had greater ( $P < 0.02$ ) insulin concentrations than the low-dose P169 cows (Figure 4.8).

During treatment with bST, plasma insulin concentrations were affected ( $P < 0.05$ ) by week but not parity ( $P > 0.14$ ) or dietary treatment ( $P > 0.29$ ). Plasma insulin appeared to increase in response to second and third bST injection such that the concentration of insulin at wk 29 was greater ( $P < 0.02$ ) than at wk 25 (Figure 4.9).

### **Plasma Insulin:Glucose Ratio**

During the period of wk 1 to 25, plasma insulin/glucose ratio was affected by dietary treatment ( $P < 0.002$ ), but not other main effects ( $P > 0.33$ ) or their interactions ( $P > 0.27$ ). Compared to control, plasma insulin:glucose ratio decreased 15% in low-dose P169 dietary group whereas insulin:glucose ratio was decreased 18% in low-dose P169 compared with high-dose P169 treatment group (Figure 4.10).

### **Plasma Insulin-like Growth Factor-I**

There was a week ( $P < 0.004$ ) and parity ( $P < 0.02$ ) effect on IGF-I concentration, but other main effects ( $P > 0.10$ ) or interactions ( $P > 0.19$ ) were not significant. Primiparous cows ( $20.1 \pm 1.1$  ng/mL) had 22% greater plasma IGF-I concentrations than multiparous cows ( $16.5 \pm 1.1$  ng/mL). Furthermore, IGF-I concentrations increased ( $P < 0.004$ ) over twofold between wk 1 and 25 of lactation (Figure 4.11).

During wk 1 to 12, diet ( $P > 0.07$ ) or parity ( $P > 0.13$ ) did not affect IGF-I concentrations. Week affected ( $P < 0.01$ ) IGF-I concentration such that there was a significant increase in IGF-I concentration between wk 1 to 4 and remained constant thereafter.

Parity influenced ( $P < 0.04$ ) IGF-I concentration between wk 13 and 25, all other main effects ( $P > 0.52$ ) and interactions ( $P > 0.18$ ) were not significant, such that primiparous cows ( $23.3 \pm 1.6$  ng/mL) had 26% greater IGF-I than multiparous cows ( $18.5 \pm 1.6$  ng/mL).

There was a week x parity ( $P < 0.0001$ ) as well as diet x parity ( $P < 0.01$ ) effect on plasma IGF-I concentration during bST administration. Plasma IGF-I concentration in primiparous cows were greater than in multiparous cows throughout the 5 wk bST administration (Figure 4.12). Primiparous cows had greater ( $P < 0.01$ ) increases in plasma IGF-I than multiparous cows following each of the bST administrations at wk 25, 27, and 29 (Figure 4.12). In addition, high-dose P169 multiparous cows exhibited a 33% decrease in plasma IGF-I compared with their respective control group, whereas low-dose P169 primiparous cows had 31% lower plasma IGF-I concentrations than their respective control groups (Figure 4.13).

### **Plasma Leptin**

Plasma leptin concentration was increased ( $P < 0.0001$ ) between wk 1 and 3 after which plasma leptin concentrations remained constant (Figure 4.14). However, the other main effects ( $P > 0.50$ ) or interactions ( $P > 0.15$ ) did not affect leptin concentrations

between wk 1 and 25. Because samples collected every other week were analyzed, wk 1 to 12 and wk 13 to 25 analysis were not conducted.

Plasma leptin concentration was not affected by week ( $P > 0.98$ ), parity ( $P > 0.15$ ) or their interaction ( $P > 0.44$ ) during bST administrations. However, diet tended to influence ( $P < 0.11$ ) plasma leptin concentration such that plasma leptin was greater ( $P < 0.11$ ) in low-dose P169 than controls during bST administration (Figure 4.15).

### **Nonesterified fatty acid (NEFA)**

There was an effect of week ( $P < 0.001$ ) and parity ( $P < 0.005$ ) but not diet ( $P > 0.56$ ) on plasma NEFA concentrations. Plasma NEFA concentrations decreased ( $P < 0.0001$ ) between wk 1 and 15 after which NEFA concentrations did not change (Figure 4.16).

Also, multiparous cows ( $0.35 \pm 0.01$  mmol/L) had 18% greater ( $P < 0.005$ ) plasma NEFA concentration than primiparous cows ( $0.29 \pm 0.01$  mmol/L). Because samples collected every other week were analyzed, wk 1 to 12 and wk 13 to 25 analyses were not conducted.

During bST administration there was a diet ( $P < 0.01$ ) and week ( $P < 0.0001$ ) effect but not parity effect ( $P > 0.09$ ). Plasma NEFA concentration was 24% greater ( $P < 0.01$ ) in high-dose P169-treated cows ( $0.31 \pm 0.01$  mmol/L) versus control ( $0.25 \pm 0.01$  mmol/L) and low-dose P169-treated cows ( $0.26 \pm 0.01$  mmol/L). NEFA concentration was affected by week ( $P < 0.0001$ ) during bST treatment such that NEFA concentrations were increased by 39% ( $P < 0.0001$ ) at wk 27, then declined by 15% ( $P < 0.02$ ) at wk 28, and rose again by 26% ( $P < 0.001$ ) at wk 29 and then declined by 12% ( $P < 0.01$ ) at wk 30 (Figure 4.17).

## Plasma Cholesterol

Plasma cholesterol was affected by week ( $P < 0.001$ ) and diet x parity ( $P < 0.002$ ), but not other main effects ( $P > 0.40$ ) or interactions ( $P > 0.12$ ). Plasma cholesterol concentration was influenced ( $P < 0.0001$ ) by week (Figure 4.18) such that cholesterol levels increased throughout the course of the experiment. Plasma cholesterol concentration was influenced by diet x parity ( $P < 0.002$ ), such that multiparous cows in low-dose P169 group had 25% greater levels of plasma cholesterol than their respective high-dose P169 and control groups (Figure 4.19). In contrast, plasma cholesterol in primiparous cows did not differ among treatment groups during the 25 wk study (Figure 4.19).

During wk 1 to 12, week ( $P < 0.0001$ ) and diet x week x parity ( $P < 0.01$ ) influenced cholesterol concentrations, but not diet ( $P > 0.72$ ) or parity ( $P > 0.52$ ) interactions. Plasma cholesterol was influenced by week, such that cholesterol concentrations increased 1.3-fold from wk 1 to wk 3 after which cholesterol concentrations remained high. Also, multiparous cows fed low-dose P169 had greater plasma cholesterol concentrations during wk 1 to 12.

During wk 13 to 25, plasma cholesterol concentration was influenced by week ( $P < 0.005$ ) and diet x parity ( $P < 0.002$ ), but not other main effects ( $P > 0.26$ ) or interactions ( $P > 0.44$ ). Plasma cholesterol concentration was influenced by week ( $P < 0.005$ ) such that cholesterol concentrations increased over 22% from wk 13 to wk 25. Multiparous cows fed low-dose P169 had a 1.3 fold greater of cholesterol concentration compared with their respective high-dose and control groups, whereas, low-dose P169 primiparous



cows ( $250.33 \pm 13.8$  mg/dL) had 14% and 9% lower concentrations of cholesterol than high-dose and control primiparous cows.

During bST administration, plasma cholesterol concentration was affected by week, ( $P < 0.03$ ) but not diet ( $P > 0.22$ ), parity ( $P > 0.21$ ) or their interactions ( $P > 0.12$ ). Plasma cholesterol concentrations increased between wk 25 and 26 ( $P < 0.05$ ); (Figure 4.20).

**Table 4.1:** Ingredient and Nutrient Composition of the Control Lactation Diet (DM basis). Diet was fed from parturition to d 265 of lactation.

Table 4.1

## Ingredient and Nutrient Composition of the Control Lactation Diet (DM basis)

Ingredient	%	Nutrient	
Sorghum Silage	16.44	DM, % (as fed)	55.9
Alfalfa (RFV 180)	16.94	CP, %	17.1
Bermuda Grass Hay	6.53	ADF, %	25.1
Whole Cottonseed	4.07	NDF, %	38.7
Corn Gluten Feed	6.60	NE <sub>l</sub> , Mcal/kg	1.67
MEGALAC <sup>®</sup> -R*	0.91	Ca, %	0.97
Lactation Cow Grain Mix		P, %	0.41
Ground Corn	27.84	Mg, %	0.36
Wheat Midds	8.16		
Soybean Meal	6.79	K, %	1.44
Extruded/Expeller SB Meal	2.59		
Calcium Carbonate	0.94	Na, %	0.25
Sodium Bicarbonate	0.54		
Diamond V XP Yeast **	0.55	S, %	0.20
Magnesium Oxide	0.27		
Salt – White	0.27		
Zinpro 4-plex ***	0.07	Zn, ppm	58.00
Vit/Trace Mineral pak	0.27	Fe, ppm	281.25
		Cu, ppm	16.83
		Mn, ppm	75.42
		Mo, ppm	1.20

\* Arm & Hammer<sup>®</sup> Animal Nutrition Group, Princeton, NJ; MEGALAC<sup>®</sup>-R contains:

Fat (as fatty acids) - 82.5%, Calcium - 8.5%, IOD (moisture) – 3 to 4%

\*\* Diamond V-XP Yeast Culture; Diamond V Mills Inc., Cedar Rapids, IA

\*\*\* Zinpro Corp., Eden Prairie, MN contains:

Zinc – 2.58%, Mn – 1.43, Cu – 0.90%, Co – 0.18%, Methionine – 8.21%, Lysine – 3.8%

**Table 4.2:** Ingredient and Nutrient Composition of the Control Transition Diet (DM basis). Diet was fed from d -14 to parturition.

Table 4.2

## Ingredient and Nutrient Composition of the Control Transition Diet (DM basis)

Ingredient	%	Nutrient	
Sorghum Silage	28.53	DM, % (as fed)	60.8
Alfalfa (RFV 180)	0.00	CP, %	12.8
Bermuda Grass Hay	13.95	ADF, %	33.8
Whole Cottonseed	0.00	NDF, %	53.5
Corn Gluten Feed	21.87	NE <sub>t</sub> , Mcal/kg	1.43
MEGALAC <sup>®</sup> -R*	0.00	Ca, %	0.80
Dry Cow Grain Mix		P, %	0.31
Ground Corn	21.26	Mg, %	0.39
Wheat Midds	3.92		
Soybean Meal	3.94	K, %	1.08
Extruded/Expeller SB Meal	3.43		
Calcium Carbonate	1.28	Na, %	0.23
Sodium Bicarbonate	0.00		
Diamond V XP Yeast **	0.45	S, %	0.15
Magnesium Oxide	0.29		
Salt – White	0.20		
Zinpro 4-plex ***	0.20	Zn, ppm	72.75
Vit/Trace Mineral pak	0.38		
Calcium Chloride	0.20	Fe, ppm	247.75
		Cu, ppm	18.75
		Mn, ppm	103.5
		Mo, ppm	< 1.0

\* Arm & Hammer<sup>®</sup> Animal Nutrition Group, Princeton, NJ; MEGALAC<sup>®</sup>-R contains:

Fat (as fatty acids) - 82.5%, Calcium - 8.5%, IOD (moisture) – 3 to 4%

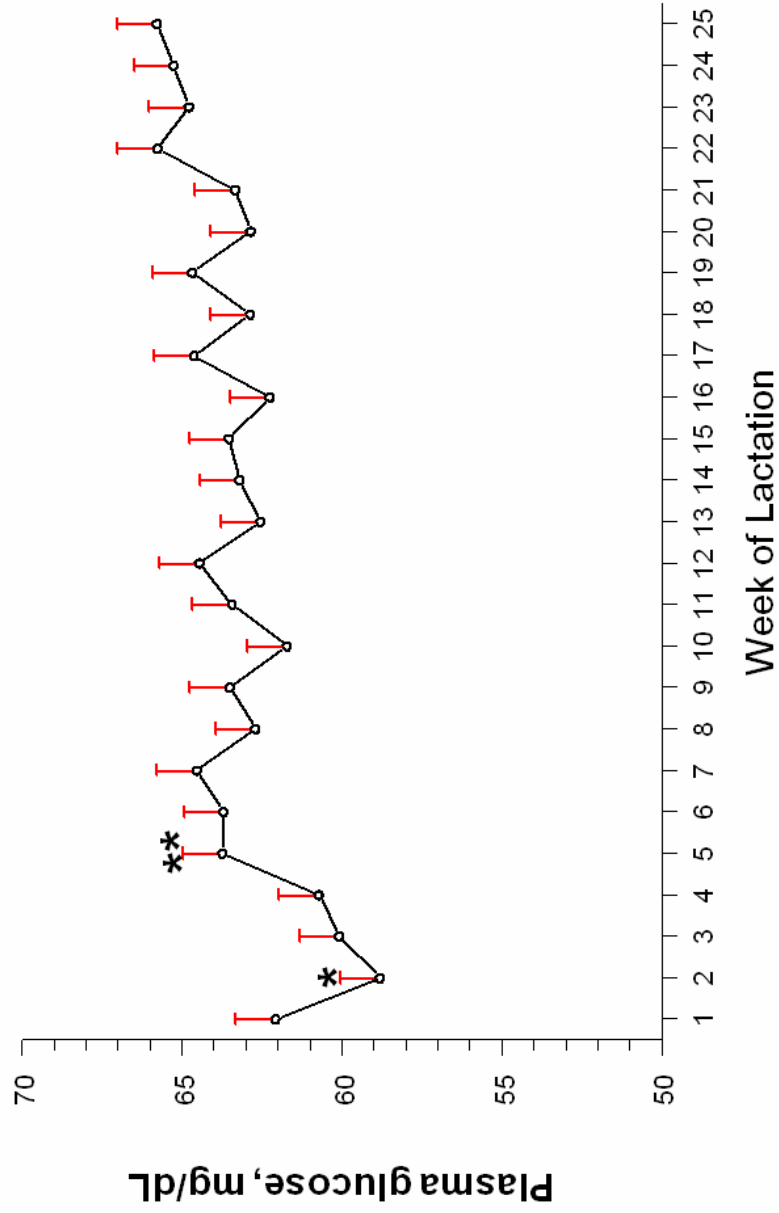
\*\* Diamond V-XP Yeast Culture; Diamond V Mills Inc., Cedar Rapids, IA

\*\*\* Zinpro Corp., Eden Prairie, MN contains:

Zinc – 2.58%, Mn – 1.43, Cu – 0.90%, Co – 0.18%, Methionine – 8.21%, Lysine – 3.8%

**Figure 4.1.** Changes in plasma glucose concentration from wk 1 to 25 of lactation; values are averaged across dietary treatment groups. \* First mean that differs from wk 1 ( $P < 0.01$ ); \*\* First mean that differ from wk 2 ( $P < 0.003$ ).

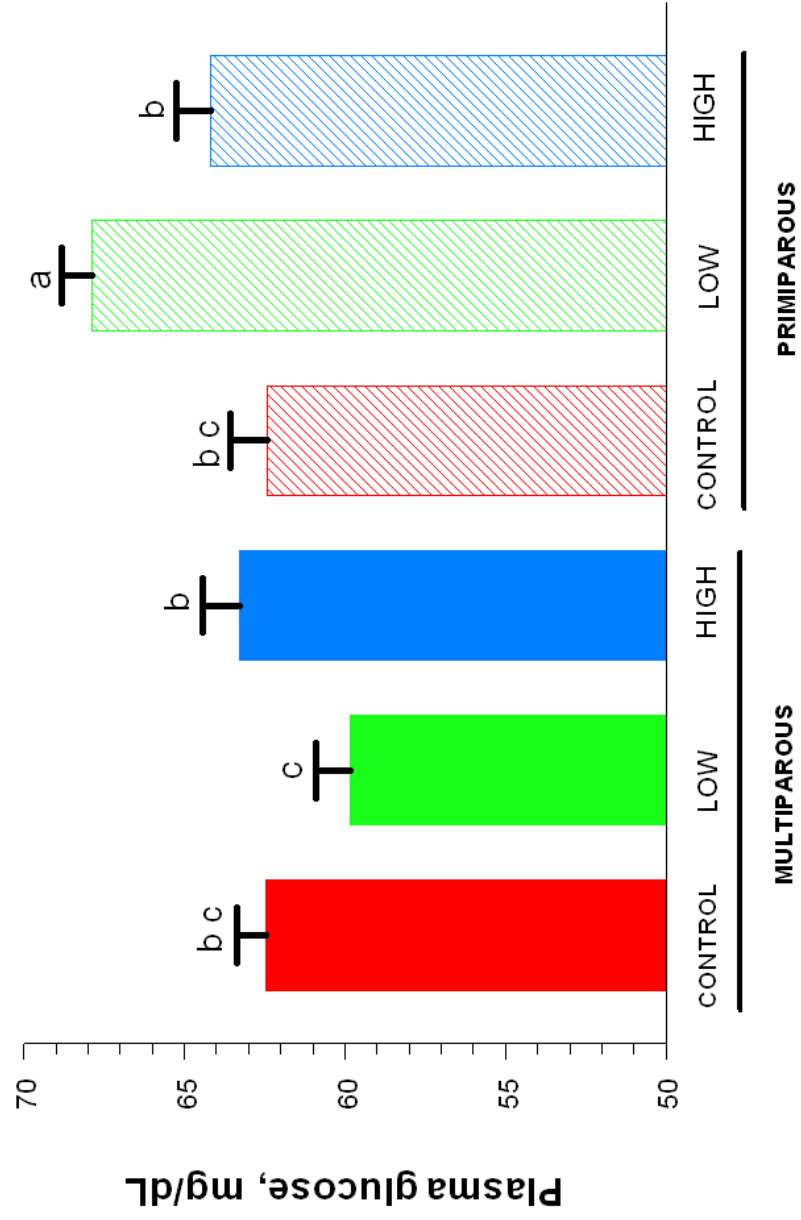
# Plasma glucose wk 1-25



**Figure 4.2.** Plasma glucose concentration (averaged of wk 1 to 25) as affected by dietary treatment x parity interaction. Across parity and treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

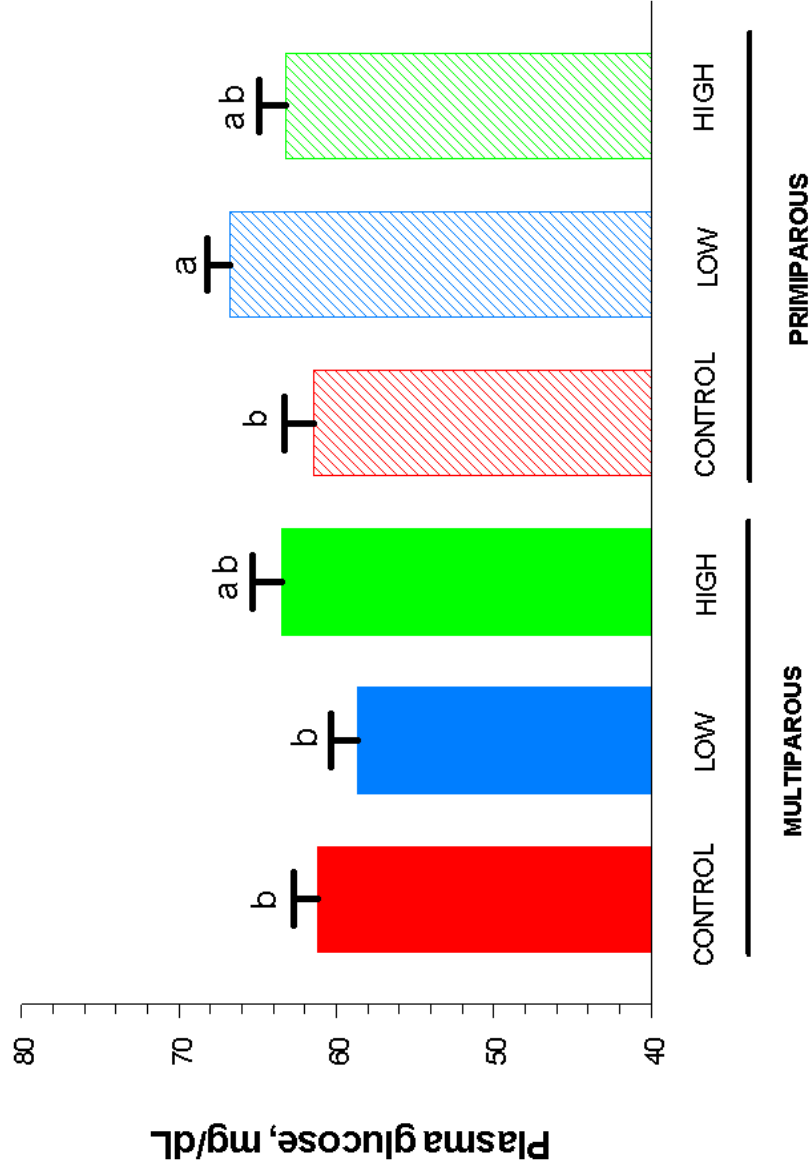


# Plasma glucose wk 1-25



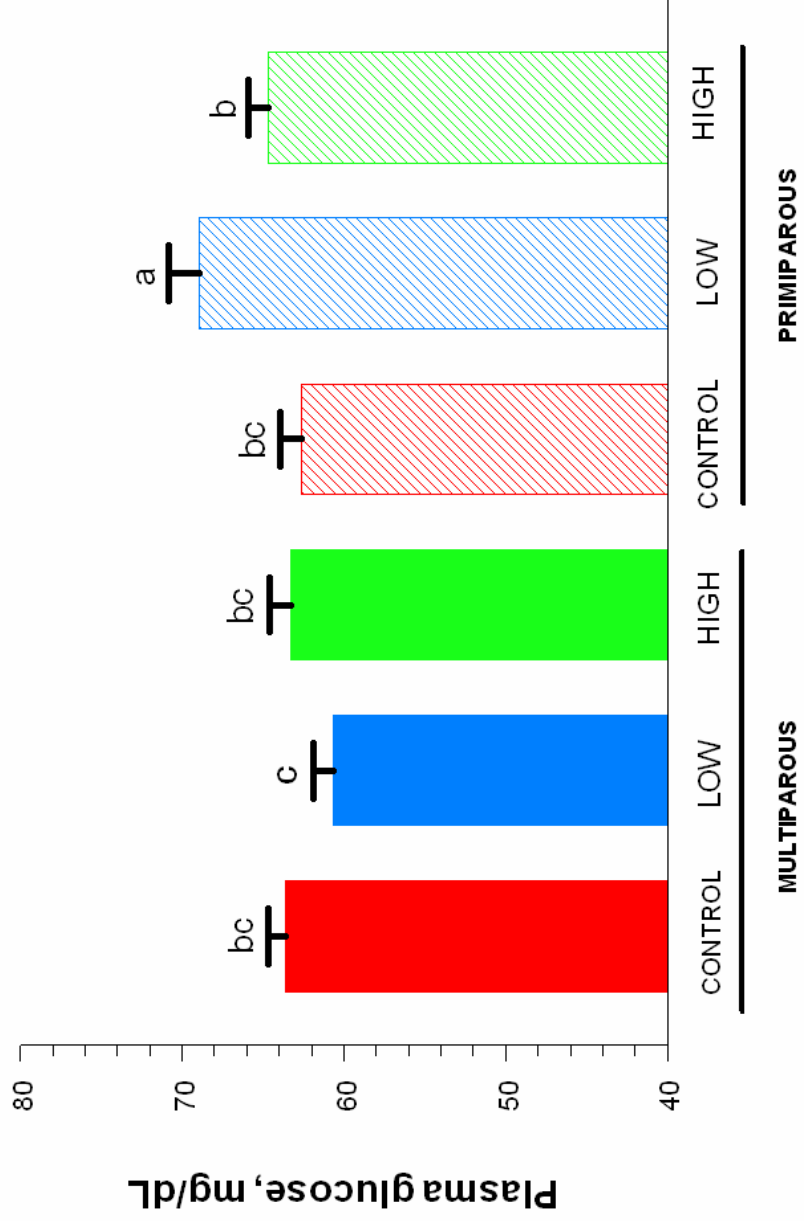
**Figure4.3.** Plasma glucose concentration (average of wk 1 to 12) as influenced by dietary treatment x parity interaction. Across treatment and parity groups, Means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

# Plasma glucose wk 1-12



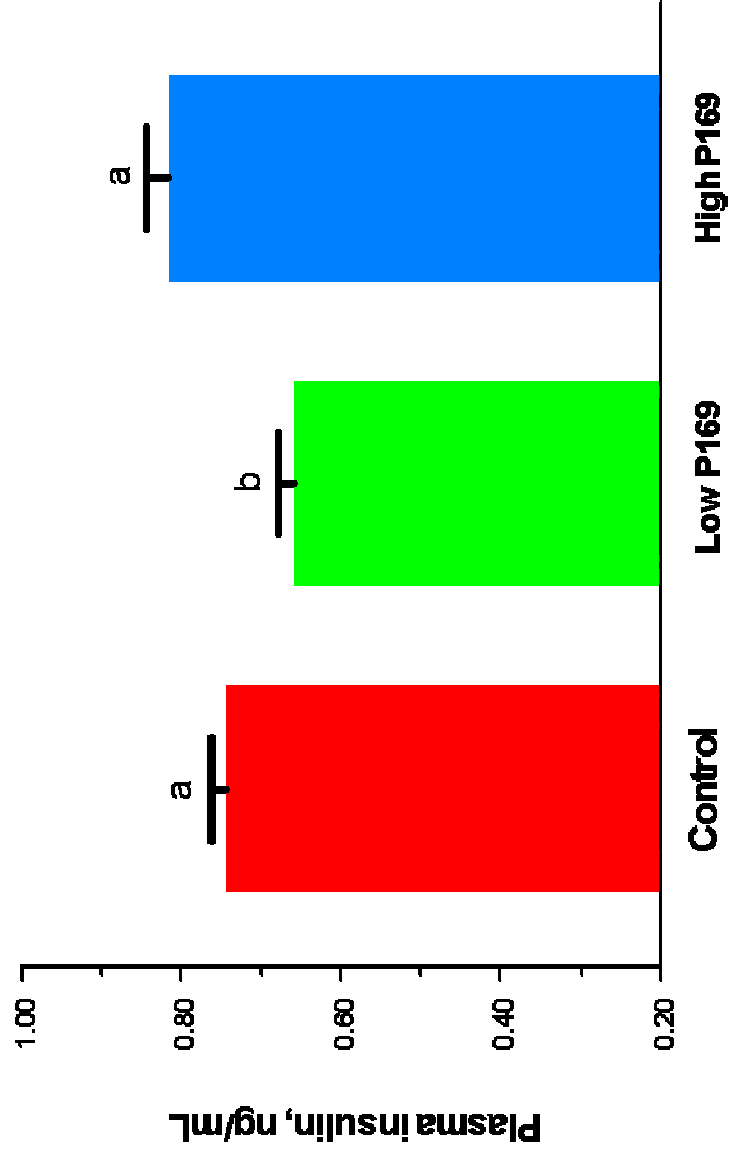
**Figure 4.4.** Plasma glucose concentration (averaged of wk 13 to 25) affected by dietary treatment x parity. Across treatment and parity groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

# Plasma glucose wk 13-25



**Figure 4.5.** Plasma insulin concentration (averaged of wk 1 to 25) influenced by dietary treatment. Across treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

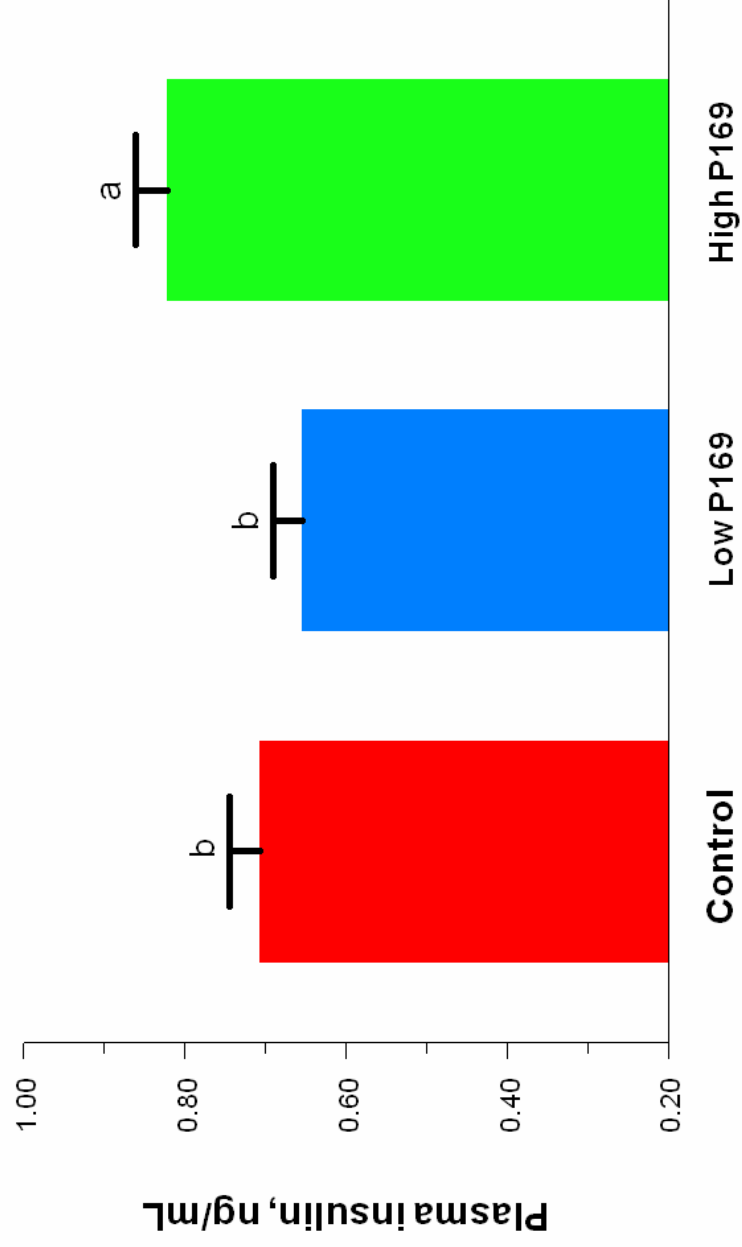
## Plasma insulin wk 1-25



**Figure 4.6.** Plasma insulin concentration (averaged of wk 1 to 12) affected by treatment. Across treatment groups, Means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

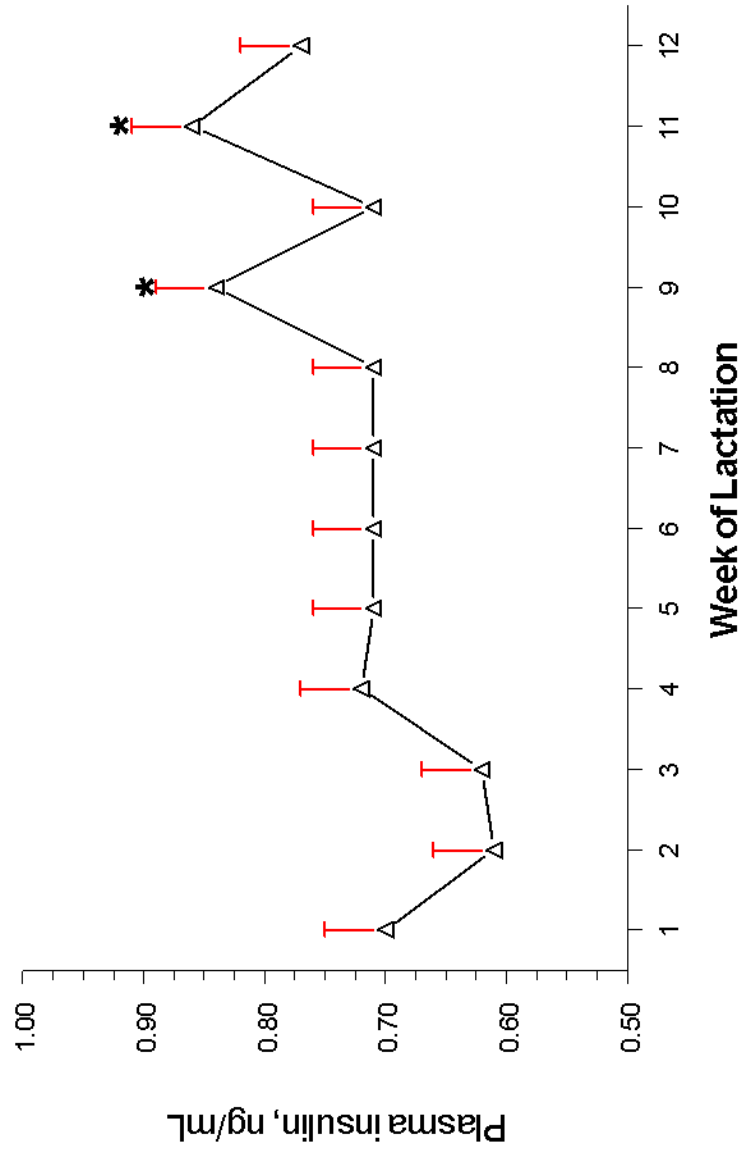


# Plasma insulin wk 1-12



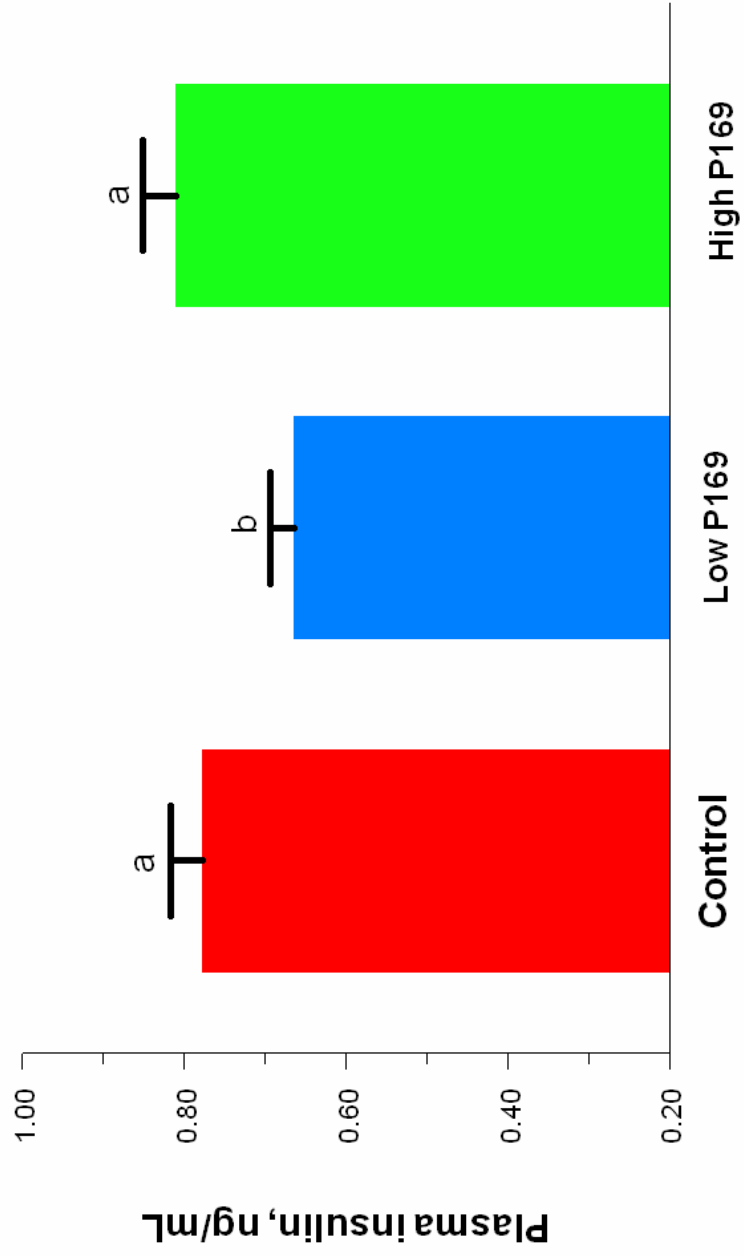
**Figure 4.7.** Weekly changes in plasma insulin concentrations from wk 1 to 12 of lactation; values are averaged across diet and parity treatment groups. Asterisk (\*) indicates means differ from wk 2 and 3 means ( $P < 0.05$ ).

# Plasma insulin wk 1-12



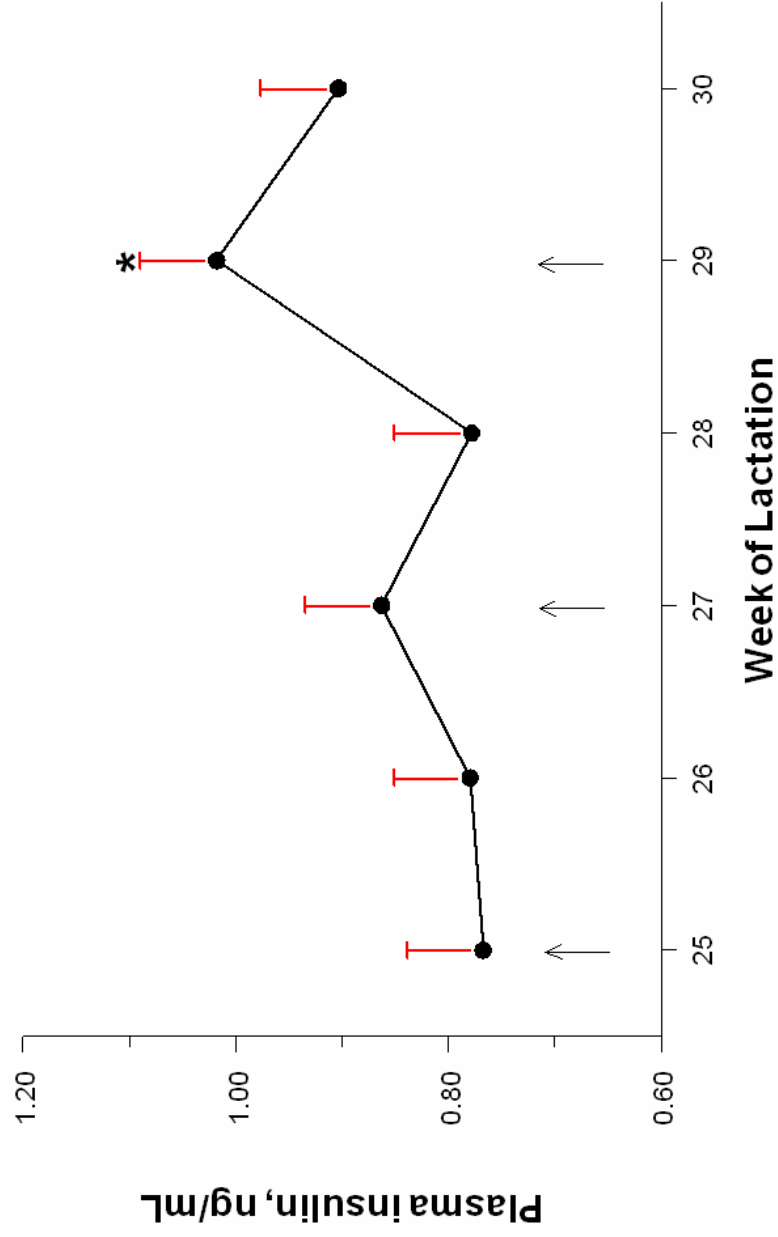
**Figure 4.8.** Plasma insulin concentration (averaged of wk 13 to 25) influenced by treatment. Across treatment groups, Means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

## Plasma insulin wk 13-25



**Figure 4.9.** Weekly changes on plasma insulin during bST administration. \* First mean that differ from wk 25 ( $P < 0.02$ ). Arrows indicate time of bST injection.

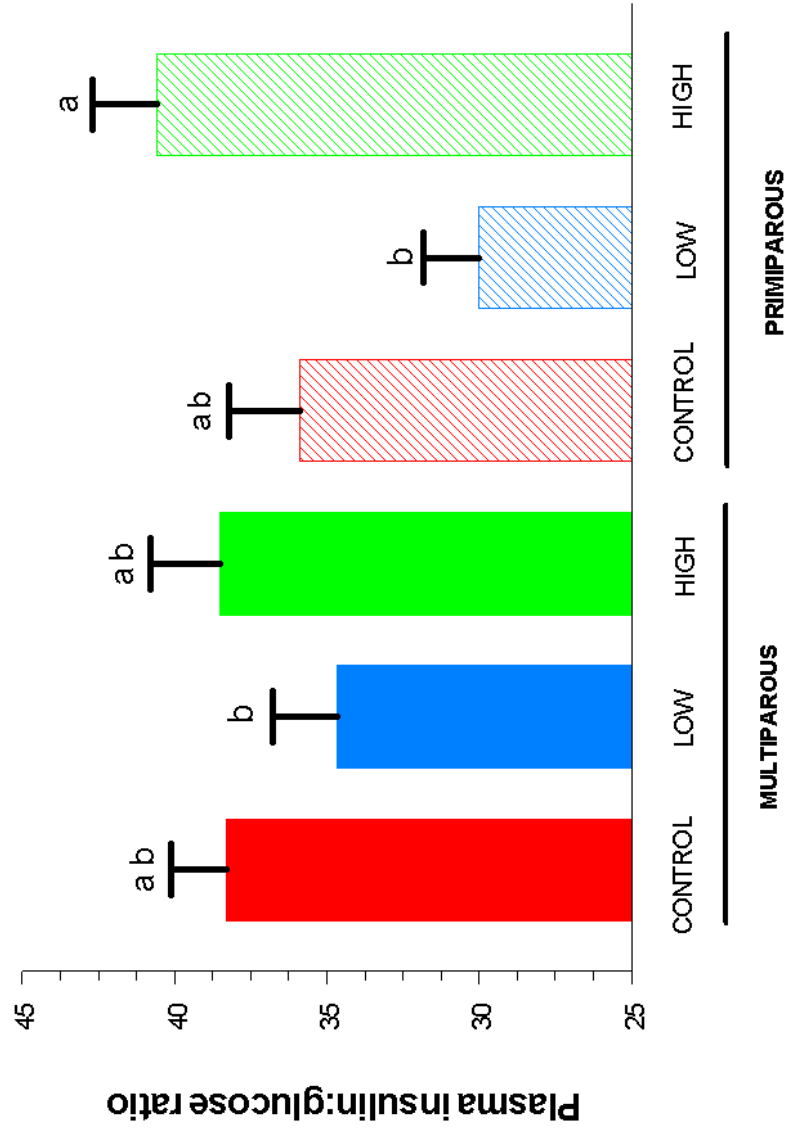
## Plasma insulin during bST



**Figure 4.10.** Plasma insulin:glucose ratio (averaged of wk 1 to 25) by dietary treatment x parity interaction. Across parity and treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

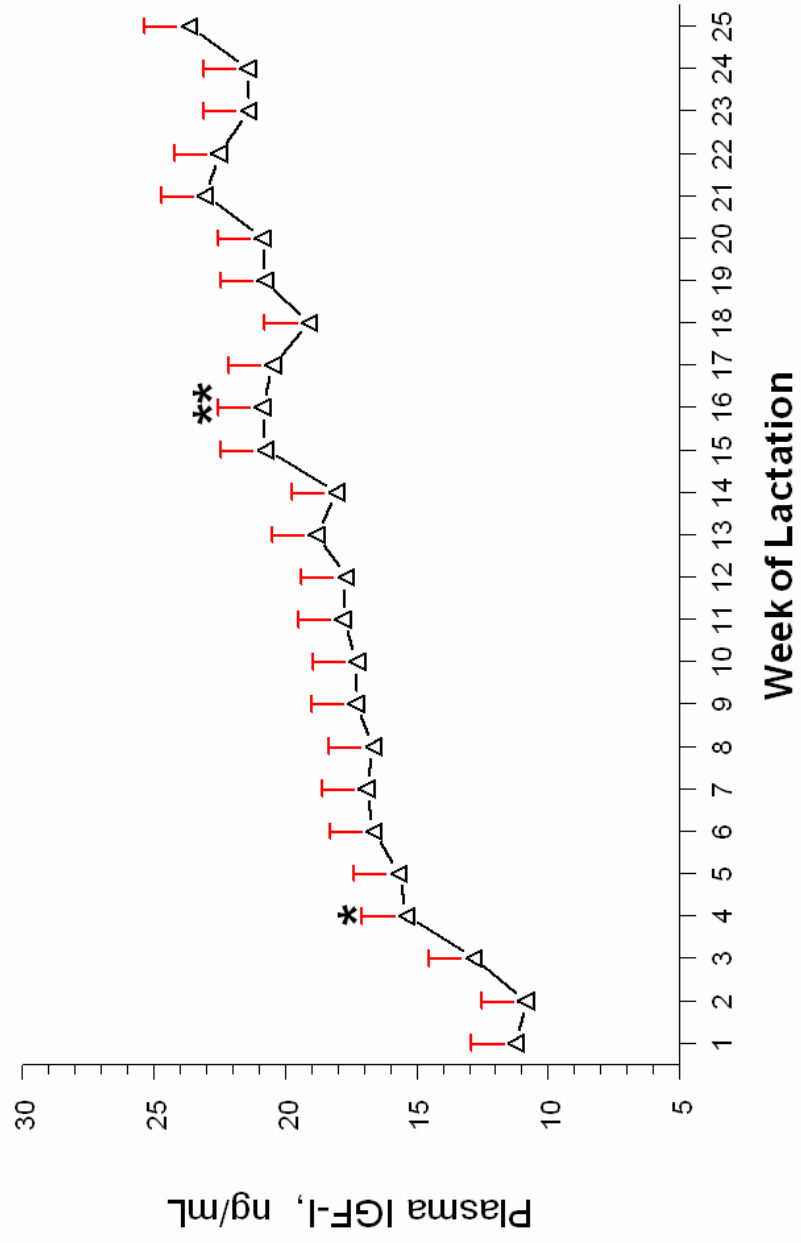


# Plasma insulin:glucose ratio wk 1-25



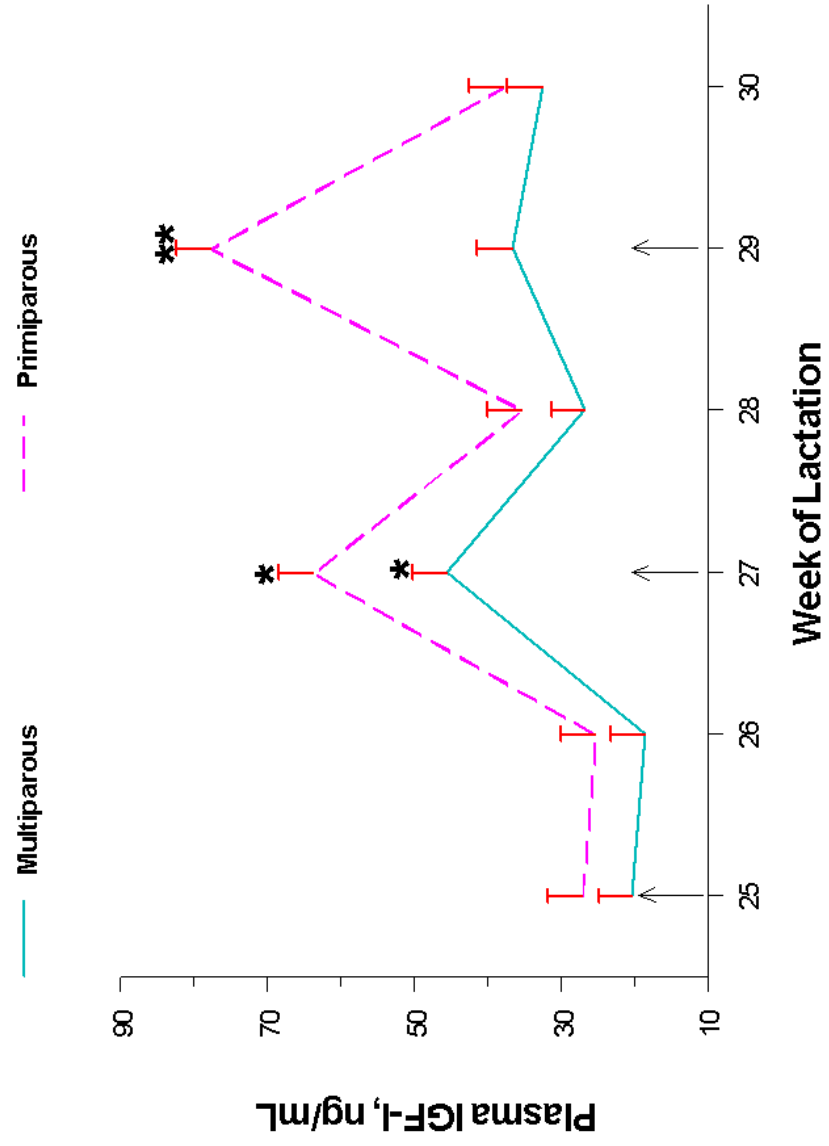
**Figure 4.11.** Weekly changes of IGF-I concentration from wk 1 to 25 of lactation; values are averaged across parity and dietary treatment groups. \* First mean that differ from wk 2 ( $P < 0.01$ ); \*\* First mean that differ from wk 4 ( $P < 0.03$ ).

## Plasma IGF-I by wk 1-25



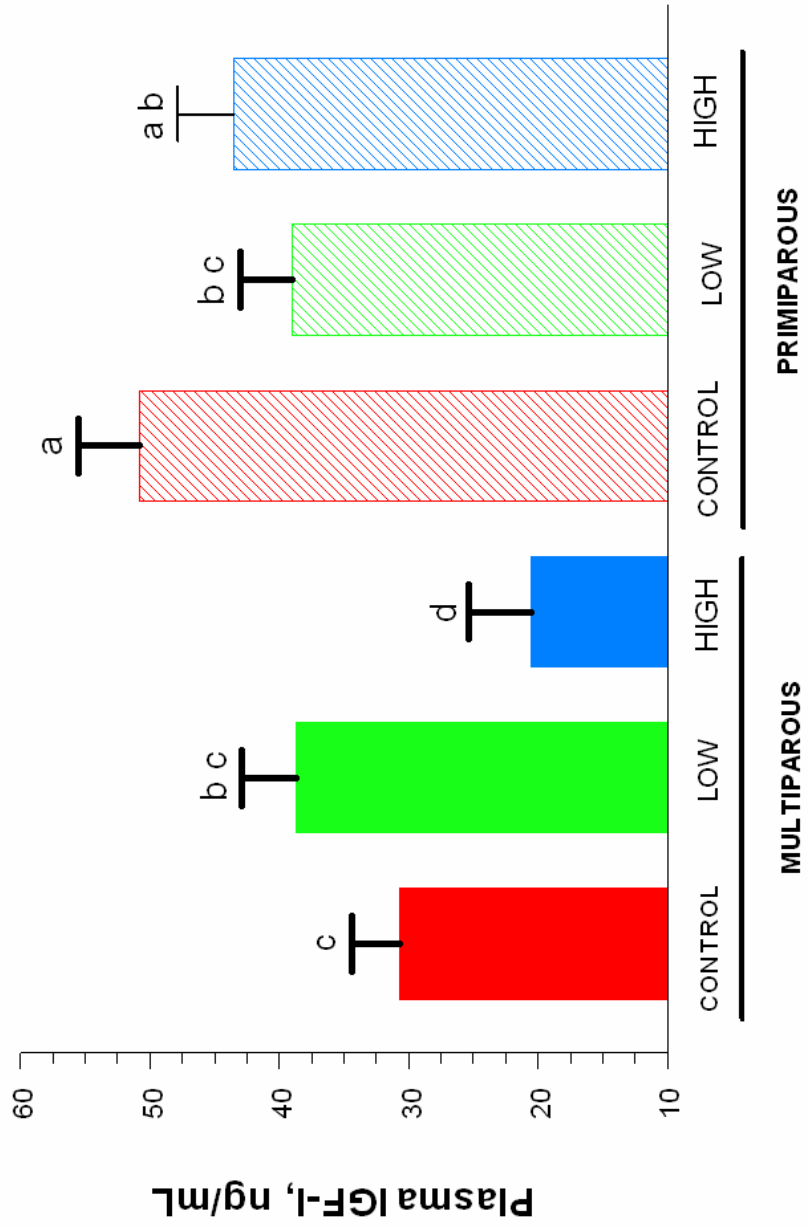
**Figure 4.12.** Weekly changes in plasma IGF-I on primi- and multiparous cows during bST administration. \* First means that differ from wk 25 ( $P < 0.0002$ ). Arrows indicate time of bST injection. \*\* Mean differ from respective multiparous mean ( $P < 0.05$ ).

# Plasma IGF-I during bST



**Figure 4.13.** Plasma IGF-I concentration influenced by dietary treatment x parity during bST administration. Across treatment groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

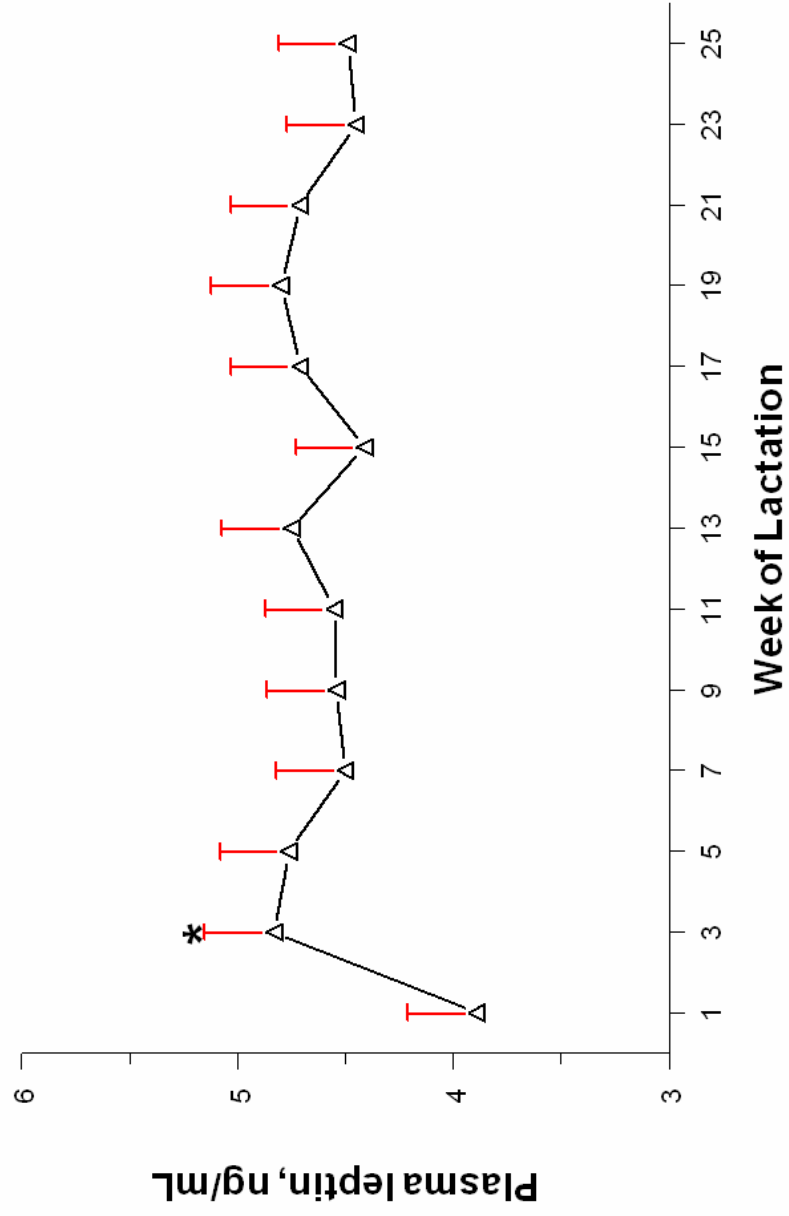
## Plasma IGF-I during bST



**Figure 4.14.** Changes in plasma leptin concentration during wk 1 to 25 of lactation. Asterisk (\*) indicates first mean that differs from wk 1 values ( $P < 0.0002$ ).

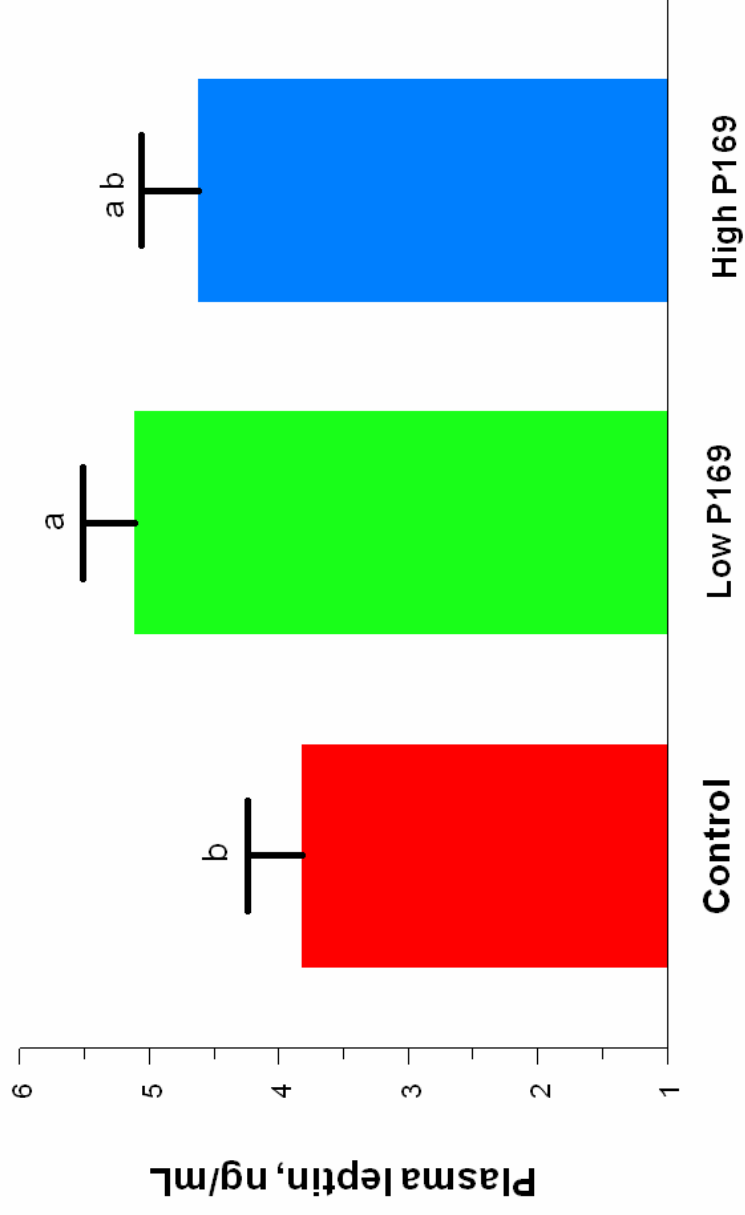


## Plasma leptin wk 1-25



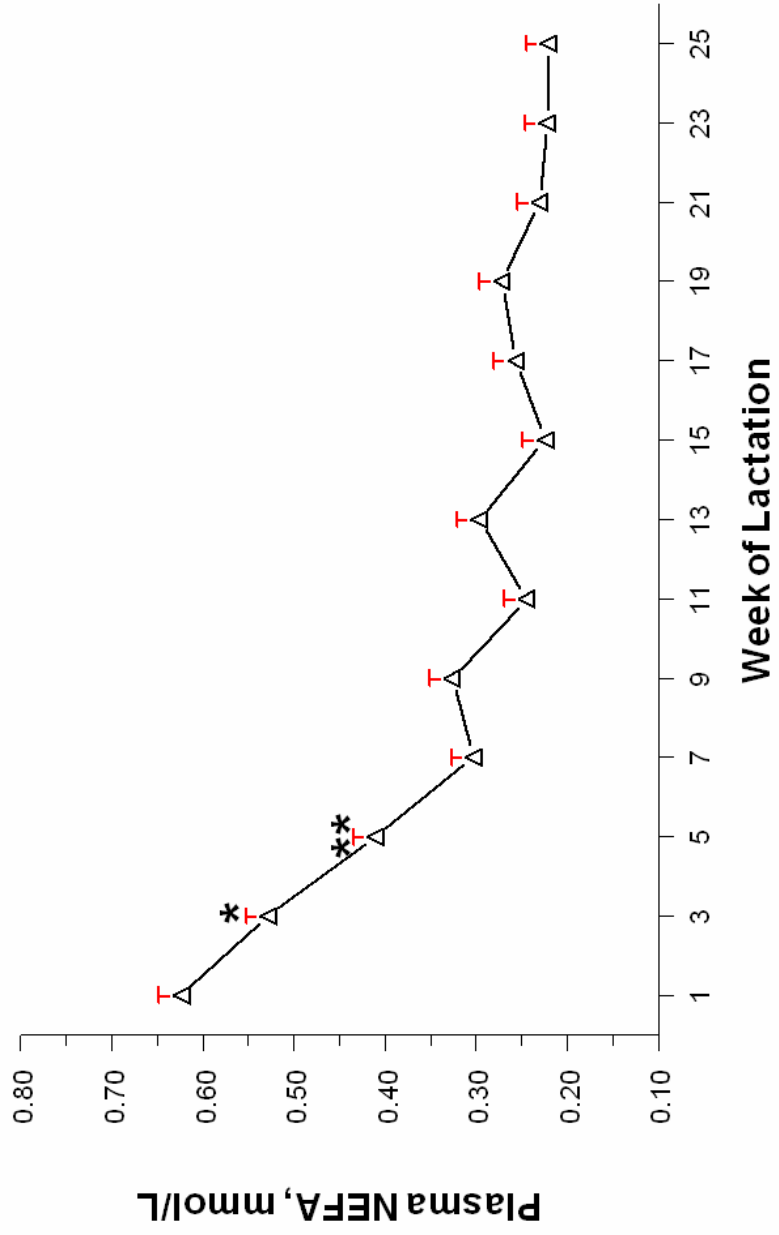
**Figure 4.15.** Plasma leptin concentration during bST administrations. Across treatment groups, means without a common letter differ ( $P < 0.05$ ). Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

## Plasma leptin during bST



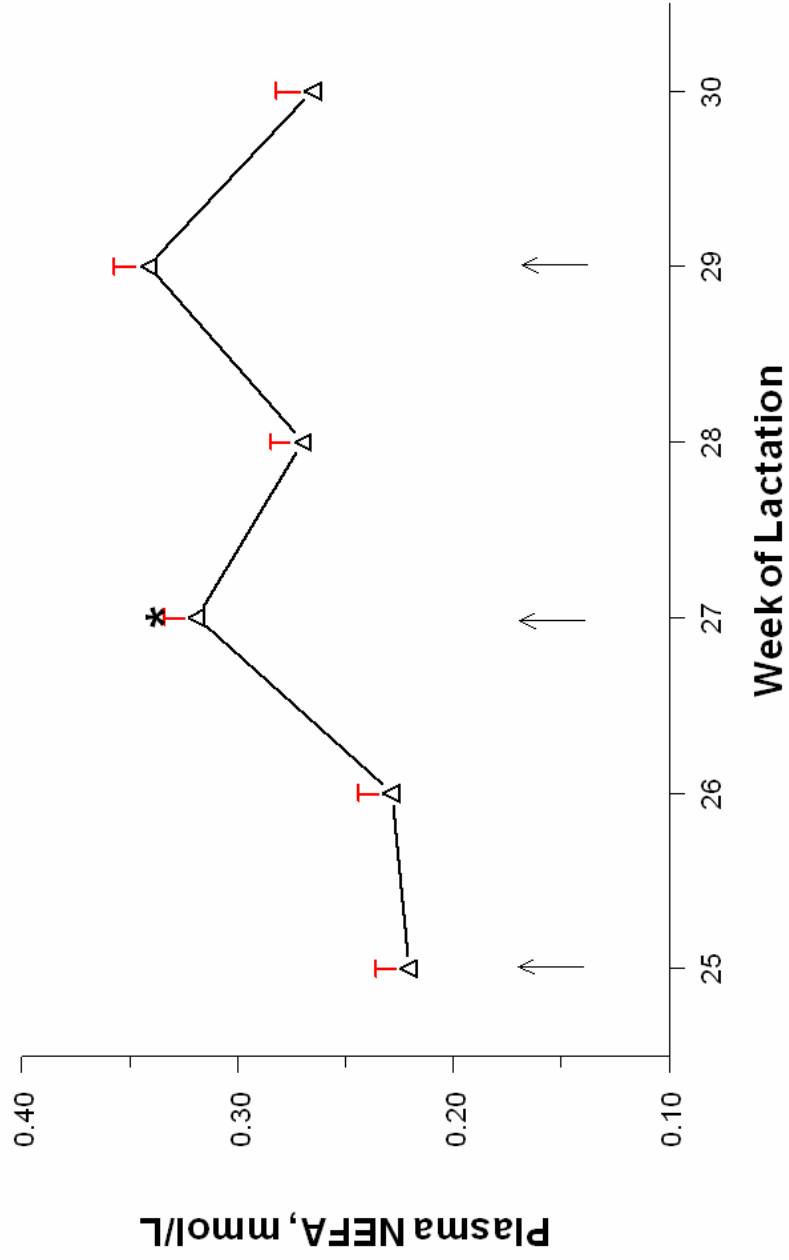
**Figure 4.16.** Weekly changes in plasma NEFA concentrations during the first 25 wk of lactation. \* First mean that differs from wk 1; \*\* First mean that differ from wk 3 (P < 0.05).

## Plasma NEFA wk 1-25



**Figure 4.17.** Weekly changes in plasma NEFA concentration during bST administration.  
\* First means that differ from wk 25 ( $P < 0.0002$ ). Arrows indicate time of bST injection.

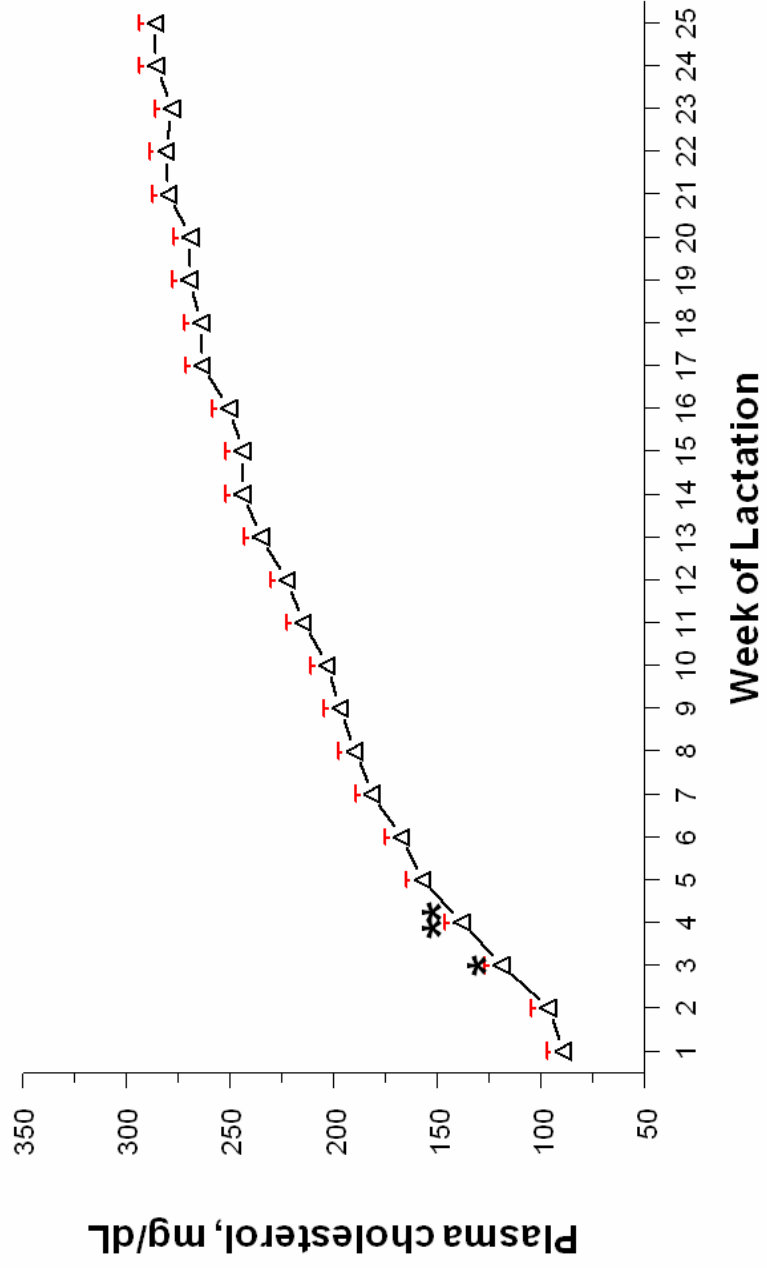
## Plasma NEFA during bST



**Figure 4.18.** Changing in plasma cholesterol concentration during wk 1 to 25 of lactation; values are averaged across diet and parity treatment groups. \* First mean that differs from wk 1 ( $P < 0.0001$ ); \*\* First mean that differ from wk 3 ( $P < 0.0002$ ); \*\*\* First mean that differ from wk 13 ( $P < 0.03$ ).

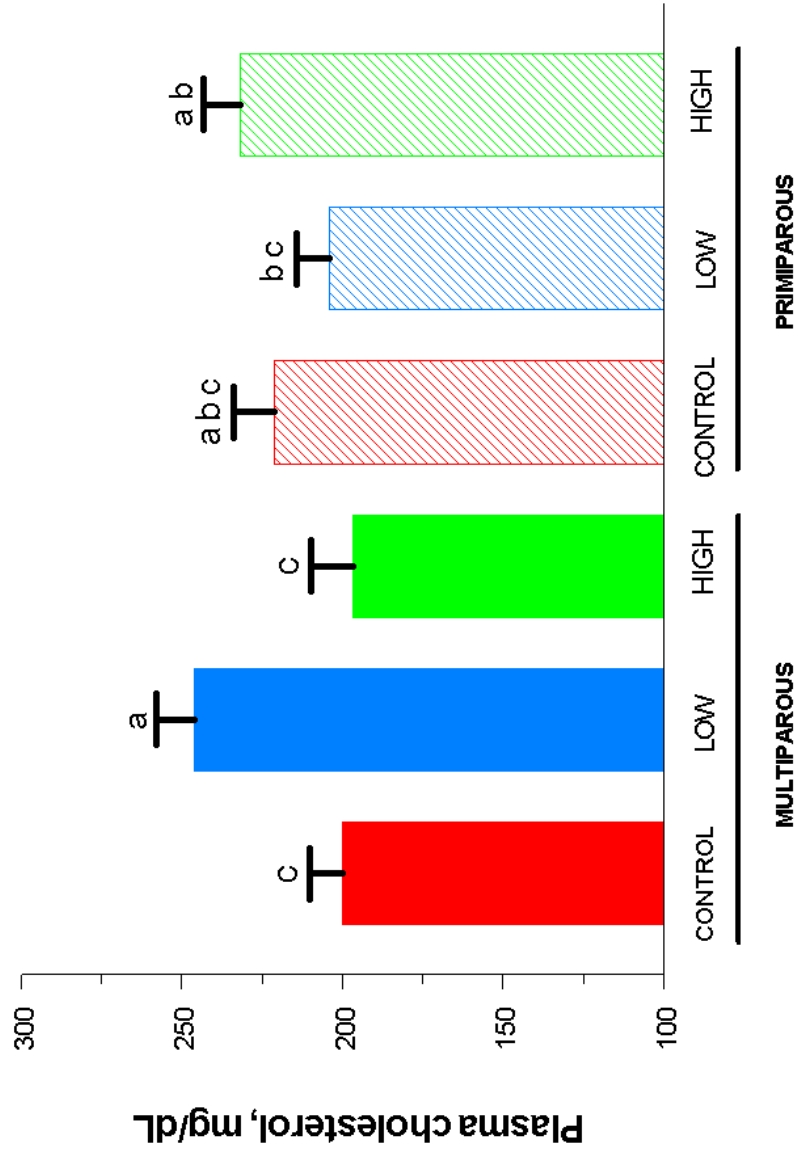


# Plasma Cholesterol wk 1-25



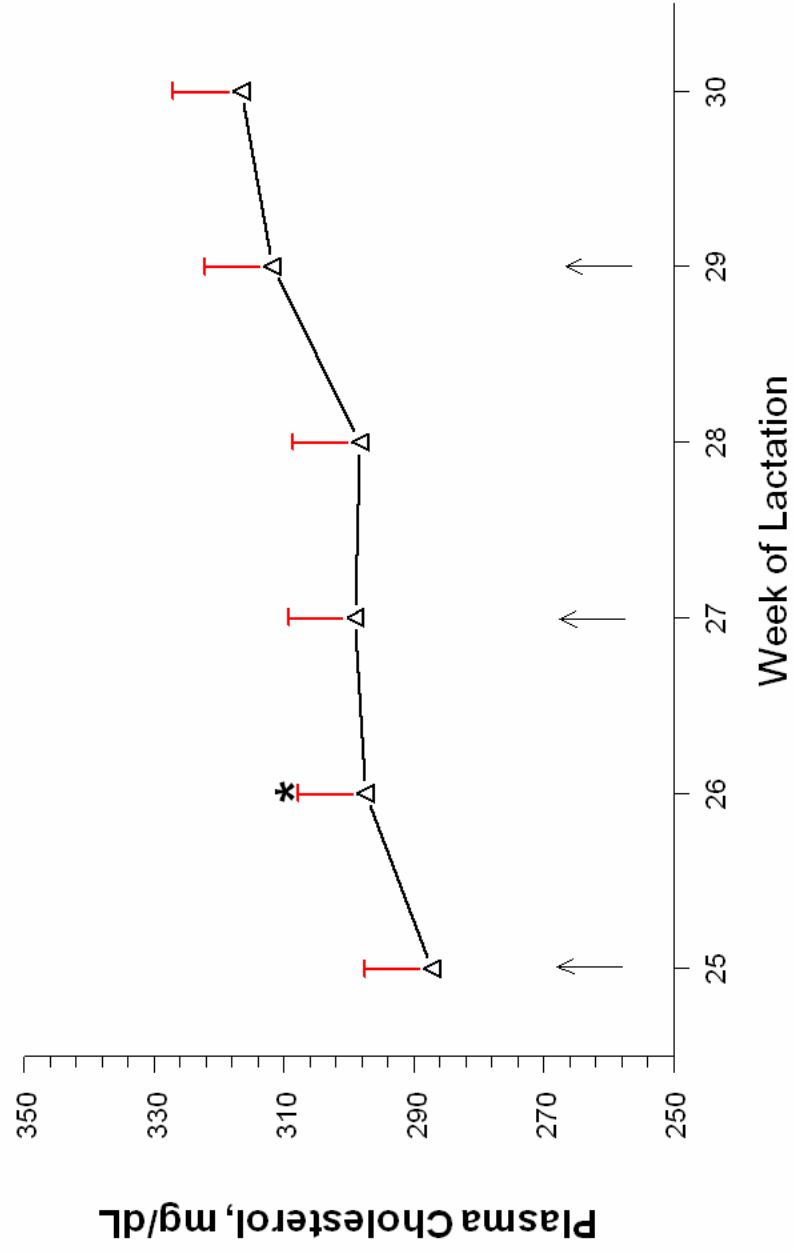
**Figure 4.19.** Plasma cholesterol concentration (average of wk 1 to 25) as affected by dietary treatment x parity interaction. Across parity and diet groups, means without a common letter differ ( $P < 0.05$ ); Low P169 = Low-dose P169 group, High P169 = High-dose P169 group.

# Plasma cholesterol wk 1-25



**Figure 4.20.** Weekly changes in plasma cholesterol during bST administration. Values are averaged across diet and parity treatment group. \* First means that differ from wk 25 ( $P < 0.05$ ). Arrows indicate time of bST injection.

## Plasma cholesterol during bST



## **Chapter V**

### **Discussion**

The aim of this experiment was to determine if supplemental feeding of Propionibacteria P169 to dairy cows during early and mid lactation could affect plasma hormones and metabolites. Previously reported, milk production (4% FCM) was increased 7.5 in low-dose P169-fed cows and 8.5% in high-dose P169-fed cows (Stein, 2004), indicating that P169 feeding altered metabolism. Plasma glucose concentration during early and mid lactation was 9% greater in low-dose P169 primiparous cows versus controls. However, glucose concentrations in multiparous cows did not differ among treatments in either lactation period. Similarly, Francisco et al. (2002) found no effect of feeding P169 on glucose concentrations, measured weekly, in early lactating multiparous dairy cows. 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, 1995; Leuvenink et al., 1997; Lee and Hossner, 2002) and cattle (DiCostanzo et al., 1999; Oba and Allen, 2003a, 2003b). The duration and magnitude of these increases were dependent on dose of propionate infused (Sano et al., 1993a). In these latter studies, frequent (e.g. hourly) blood samples were collected post-infusion or feeding. 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.

In the present study during early lactation (wk 1-12), insulin levels were 16% greater in cows fed high-dose P169 compared to controls and those fed low-dose P169. During mid-lactation (wk 13-25) and throughout the study (wk 1-25) plasma insulin concentrations were less in low-dose P169 fed cows than controls. This is in contrast to Francisco et al. (2002) who found that insulin did not differ between P169 (low-dose) fed cows and controls during early lactation. Insulin responses to propionate infusion also depends on the level of nutrients fed to non-lactating, non-pregnant mature ewe (Quigley and Heitmann, 1991). Thus, level of feed intake or energy balance may have influenced the insulin response to propionibacteria feeding in the present study. Other researchers found that the stage of lactation influences the insulin response to glucose and propionate due 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 per day, it is also likely that acute changes in plasma insulin in response to feeding either low-or high-dose P169 may have gone undetected in the present study. In agreement to previous studies (Spicer et al., 1993; Francisco et al., 2002), plasma insulin concentrations significantly increased between wk 1 to 10 of lactation.

Because glucose concentrations were increased and insulin concentrations were decreased in primiparous cows fed low-dose P169, this group had lower insulin:glucose molar ratios as compared to control and high-dose P169 cows. Decreased insulin:glucose ratios suggest that cows fed low-dose P169 had improved tissue sensitivity to insulin. Interestingly, insulin concentrations were increased while glucose concentrations were unchanged in high-dose P169 cows, yet their insulin:glucose ratios were unchanged.

This suggests that despite the elevated plasma insulin in these cows, tissue sensitivity to insulin was not compromised. In fact, glucose levels were significantly greater in high-dose P169 multiparous cows than low-dose P169 (but not control) multiparous cows. A previous study (Hayirli et al., 2001) has shown that the molar ratio of insulin to glucose was lower for cows fed supplemental chromium-methionine, indicating that feed supplements do have potential for improving tissue sensitivity to insulin. The molar ratio of insulin to glucose observed in the present study was within the range of ratios previously reported for cattle (Hayirli et al., 2001; Liu et al., 2004).

Similar to previous studies (Spicer et al., 1990, 1993; Francisco et al., 2002), systemic IGF-I concentrations were unaltered by addition of P169 to the diet, and increased during the first 7 to 16 wk of lactation. Moreover, primiparous cows had 22% greater IGF-I concentrations than multiparous cows during the first 25 wk of lactation and are consistent with observations of Taylor et al. (2004). Because cows in positive energy balance have greater concentrations of IGF-I (Spicer et al., 1990), we speculate that the primiparous cows were in greater positive energy balance than multiparous cows. In support of this, Stein (2004) found that primiparous cows recovered their body weight quicker from wk 1 through wk 25 compared with multiparous cows. In contrast to the present study, early lactating primiparous cows receiving a supplement of chromium (Cr) and infused with propionate had increased IGF-I concentrations (Subiyatno et al., 1996), and glucose infusion increased IGF-I concentrations during late lactation (Chelikani et al., 2003). However, IGF-I was not affected when glucose was infused to Holstein cows in early lactation (Chelikani et al., 2003) suggesting that energy balance status of the cows may influence IGF-I response to increased glucose.



Plasma cholesterol levels during wk 1 to 25 of lactation were greater in only multiparous cows fed low-dose P169 than controls cows of current experiment, and this increase in plasma cholesterol was evident during wk 1 to 12 and wk 13 to 25. A previous study reported no effect of feeding P169 for 12 wk on plasma cholesterol levels of multiparous cows (Francisco et al., 2002). Reasons for the discrepancy between the present and previous study are unclear but may involve differences in the composition of rations and the addition of yeast culture used in the two studies. For example, Francisco et al. (2002) fed 0.86% (of DM) of Rumofat whereas the present study fed 0.91% (of DM) of Megalac-R. Feeding inert fat to multiparous cows in early lactation has been shown to increase both cholesterol and IGF-I concentrations (Spicer et al., 1993). Alternatively, because plasma cholesterol is positively correlated to energy balance and dry matter intake (Francisco et al., 2002; Francisco et al., 2003), perhaps differences in either of these components between studies influenced the cholesterol response to feeding P169.

The present study found that low-dose P169 treatment increased leptin concentrations during bST treatment but not during wk 1 to 25. Francisco et al. (2002) found that feeding P169 increased plasma leptin concentration between wk 1 and 12 of lactation. Although, glucose infusion did not alter plasma leptin concentrations in Italian Simmental cows (Gabai et al., 2002), acute systemic propionate infusions increased leptin mRNA abundance in adipose tissue of sheep (Lee and Hossner, 2002). Thus, further research is needed to clarify the influence of propionate and glucose on leptin secretion in ruminants.

In the present study, NEFA concentrations between wk 1 and 25 were not affected by feeding P169. However, regardless of parity, cows treated with high-dose P169 had greater NEFA concentrations than control and low-dose cows during bST treatment. Previously, Francisco et al. (2002) found that multiparous cows fed P169 had greater NEFA concentrations than controls cows only during wk 1 postpartum. Infusing glucose duodenally in Holstein cows resulted in decreased NEFA concentrations (Lemosquet et al., 1997). Similarly, in lactating animals, infusion with propionate or glucose precursors decreased NEFA concentrations (Subiyatno et al., 1996; Lemosquet et al., 1997; Gabai et al., 2002; Oba and Allen, 2003b; Patton et al., 2004) and this response was 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; Lee and Hossner, 2002). Similar to the present study, Francisco et al. (2002) observed that NEFA concentrations decreased dramatically between wk 1 to 10 of lactation and remained unchanged thereafter. In addition, multiparous cows had 18% greater NEFA concentrations than primiparous cows, suggesting that multiparous cows had greater lipid mobilization likely due to their high milk production (Stein, 2004). Perhaps P169 treatment ultimately provides precursors for lipid deposition within adipocytes and when subsequently challenged with increased endogenous or exogenous bST, more lipids are able to be mobilized. Although the role of propionate as an antiketogenic compound has been suggested (Drackley, 1999), the mechanism by which P169 increases NEFA concentrations during bST treatment will require further study.

During bST treatment, multiparous high-dose P169 cows and primiparous low-dose P169 cows had lower plasma IGF-I concentrations than controls. Prepartum Holstein

cows given bST biweekly had increased insulin and NEFA concentrations and unaltered glucose and IGF-I levels, whereas, postpartum cows treated with bST had increased NEFA concentrations and unaltered glucose, insulin or IGF-I levels (Gulay et al., 2003). Gulay et al. (2003) suggested that the prepartum response to bST treatment is due to greater positive energy balance, whereas the postpartum response is accounted for by an increase in lipolysis. Somatotropin administration to mature Holstein cows fed either high or medium protein levels increased plasma IGF-I, insulin, glucose and free fatty acids (FFA) levels (Boer et al., 1991). In the present study, 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. Because no untreated controls were evaluated, definitive bST treatment effects cannot be determined. Other research suggests that bST treatment plays an important role in metabolism due to increases in both rates of gluconeogenesis and oxidation, and increased rates in NEFA irreversible loss and oxidation, resulting in an extra energy source for milk production when feed intake is diminished (Bauman et al., 1988; Knapp et al., 1992). During bST treatment in the present study, high-dose P169 cows had greater NEFA and low-dose P169 cows had greater leptin concentrations, indicating a complex interaction among hormones and metabolic status.

## Summary and Conclusions

Feeding P169 from 2 wk prior to parturition throughout 175 d postpartum to Holstein cows increased milk production 8.5% and resulted in complex metabolic changes such that primiparous cows receiving a low-dose P169 had an 8% increase in plasma glucose compared with control and other groups of cows. During bST treatment, plasma glucose levels in primiparous cows were 6% greater compared with multiparous cows. Therefore, greater milk production in multiparous cows likely drives lower plasma glucose levels. High-dose P169 increased plasma insulin concentrations whereas low-dose P169 decreased insulin concentrations. Plasma insulin concentrations increased gradually after bST treatment. Thus, differences in insulin concentrations due to diet may be accounted for by differences in feed efficiency. Concentrations of IGF-I were not altered by feeding P169 during wk 1 to 25. In contrast, during bST treatment both low- and high-dose P169 treatment decreased plasma IGF-I. Concentrations of NEFA were not affected by P169 between wk 1 to 25, but during bST treatment, plasma NEFA levels were increased in high-dose P169 cows. Increased NEFA and decreased IGF-I indicates that high-dose P169 cows had lower negative energy balance than the other groups. Cholesterol concentrations were increased by low-dose P169 feeding between wk 1 to 25 and may be due to increased feed intake. Therefore, supplemental feeding of P169 to primiparous and multiparous Holstein cows impacted their metabolism in such a way that cows could meet the greater needs of nutrients for milk production. Further studies are warranted to evaluate in more detail the metabolic effects of supplemental feeding of P169 to Holstein cows.

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## VITA

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Master of Science

Thesis : EFFECT OF A DIRECT-FED MICROBIAL ON PLASMA  
CONCENTRATIONS OF HORMONES AND METABOLITES  
IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

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Title of Study: EFFECT OF A DIRECT-FED MICROBIAL ON PLASMA  
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PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

Pages in Study: 134

Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope of Study: This study was conducted to evaluate the effect of supplemental feeding Propionibacterium P169 on key metabolic indicators in lactating cows. From 14 d prepartum to 175 d postpartum, multi- and primiparous Holstein cows were fed one of three dietary treatments: 1) Control (n = 13) fed a total mixed ration (TMR); 2) High-dose group (n = 11), fed TMR plus  $6 \times 10^{11}$  /head /d (high-dose P169) of Propionibacterium Strain P169; or 3) Low-dose group (n = 14), fed TMR plus  $6 \times 10^{10}$  /head/d (low-dose P169) of P169. Blood samples were collected weekly for 30 wk and analyzed for plasma concentrations of glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, nonesterified fatty acids (NEFA), and cholesterol (CHOL). Between wk 25 and 30, bovine somatotropin (bST) was given to all groups every 2 wk.

Findings and Conclusions: Plasma glucose concentrations were affected by diet x parity such that plasma glucose in low-dose P169 multiparous cows ( $59.8 \pm 1.1$  mg/dL) were 5.5% lower than in high-dose P169 multiparous cows; low-dose P169 primiparous cows ( $67.9 \pm 0.9$  mg/dL) had 6% to 9% greater plasma glucose concentrations than high-dose P169 and Control primiparous cows. Plasma insulin concentrations were affected by diet such that low-dose P169 had less plasma insulin than high-dose P169 and Control cows (during wk 13-25), and high-dose P169 cows had greater insulin than Controls (during wk 1-12). Plasma IGF-I, NEFA and leptin concentrations did not differ among diet groups between wk 1 and 25, but primiparous cows had greater IGF-I and lower NEFA levels than multiparous cows. Plasma CHOL was affected by diet x parity such that low-dose P169 multiparous cows ( $246 \pm 11$  mg/dL) had 25% greater concentrations than high-dose P169 and Control multiparous cows; CHOL levels in primiparous cows did not differ among diet groups. During bST, high-dose P169 multiparous cows and low-dose P169 primiparous cows had lower IGF-I concentrations than their respective Controls. Regardless of parity, low-dose P169 cows had greater leptin concentrations than Control cows, and high-dose P169 cows had greater plasma NEFA than Control cows. We conclude that P169 may hold potential as a direct-fed microbial to enhance metabolic efficiency during early and mid-lactation.

Advisor's Approval: Dr. Leon Spicer