DIRECTLY FED PROPIONIBACTERIA: EFFECTS ON ENERGY BALANCE, METABOLITES, HORMONES AND REPRODUCTION IN EARLY POSTPARTUM DAIRY COWS

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To Almighty, for giving me the strength and wisdom

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CHAPTER I

INTRODUCTION

Early in lactation, dairy cows undergo several physiological events simultaneously, including maximal lactation and return to ovarian function (Swanson, 1989). The energy requirements for these biological events are met through an increase in combination of feed intake and mobilization of body energy reserves (Coppock et al., 1974; Butler and Smith, 1989). Because lactation is a prioritized physiological state, cows change their pattern of tissue metabolism, "homeorhesis", to support their physiological state (Bauman and Curie, 1980). Insufficient dry matter intake at early lactation of many high-producing cows predisposes the animals to a state of negative energy balance (EB) that consequently affects the reproductive status of the cows (Wagner and Oxenreider, 1971; Folman et al., 1973; Butler and Smith, 1979; Spicer et al., 1990; Beam and Butler, 1998).

Return to normal ovarian function measured as number of days open has been associated with two main factors, dry matter intake and milk yield (Whitmore et al., 1974; Staples and Thatcher, 1990). Thus, the negative EB early in lactation is likely to delay the interval from calving to conception. The estimated cost per day of delayed conception rate is $2.00, a $60.00 savings per cow per lactation can be realized by reducing the calving interval from 13.5 to

12.5 months (Speicher, 1977; Senger, 1994). With 10 million dairy cows in the U.S., $600 million will be saved by the industry by shortening the calving interval by a month.

To achieve recovery from negative energy balance status, several studies have supplemented diets of early lactating cows with fat (Jerred et al., 1990; Spicer et al., 1993; Drackley and Elliot, 1993), protein (Ferguson and Chalupa, 1989; Butler, 1998), and volatile fatty acids (Sartin et al., 1985; Hurtaud et al., 1998). Responses in dry matter intake (DMI), milk yield and measures of reproductive function have been variable.

Supplementing direct-fed microbials can alter ruminal fermentation and thus may improve EB of early lactating cows. Recently, supplemental yeast cultures to the diet of lactating cows also produced variable results (Piva et al., 1993; Robinson, 1997; Dann et al., 2000). Propionibacteria culture, a direct-fed microbial, alters ruminal fermentation of propionate, which serves as a precursor of glucose in ruminants. Consequently, the present study was conducted to test the hypothesis that increasing the population of Propionibacteria in the rumen may increase EB and thereby increase production of metabolites and hormones necessary for rapid return to normal reproductive functions. Furthermore, models were developed using the variables measured above to predict plasma cholesterol and progesterone concentrations and days to first and second postpartum ovulations. Models included several metabolic and endocrine modulators (insulin like growth factor-I, insulin, glucose, progesterone and cholesterol) that have been identified previously as regulators of postpartum

return to reproductive functions. This study was designed to measure the effects of supplementing Propionibacteria culture in the diet of early lactating cows by measuring reproductive and metabolic hormones, production and reproductive variables.

CHAPTER II

REVIEW OF LITERATURE

Postpartum Changes in Nutrition of Early Lactating Dairy Cows

The interaction of nutrition and reproduction in dairy cattle is most pronounced during early (wk 1-12) postpartum. Dairy cows undergo several physiological events that are simultaneously occurring at early postpartum namely maximal lactation, uterine involution and return to cyclic ovarian functions (Smidt and Farris, 1982; Swanson, 1989). The energy requirements of the animals for these biological events are met through a combination of dietary intake and mobilization of body reserves (Coppock et al., 1974; Butler and Smith, 1989). The cows undergo the process of "homeorhesis", a term coined by Bauman and Curie (1980), to mean the orchestrated changes in the metabolism of body tissues necessary to support the present physiological state.

Early postpartum period (i.e., 1-12 wk) is the time that most high-producing dairy cows experience negative energy balance (EB) that consequently affects the reproductive status of the animals (Wagner and Oxenreider, 1971; Folman et al., 1973; Coppock et al., 1974; Butler et al., 1981; Villa-Godoy et al., 1988; Butler and Smith, 1989; Spicer et al., 1990; Staples et al., 1990; Beam and Butler, 1998). Moreover, lactation is a prioritized

physiological state usually allowed to proceed at the temporary expense of the next reproductive cycle (Staples et al., 1990). Recovery or improvement in energy balance (EB) from its most negative state towards a positive state at early lactation may provide an important signal for restoration of ovarian activity (Butler et al., 1981; Berghorn et al., 1988; Staples et al., 1988; Butler and Smith, 1989). The amount of nutrients available to the cow (dry matter intake (DMI) and body tissue mobilization) and quantity of milk produced are the two main factors that have been associated with the number of days for the cow to return to normal ovarian activity (Marion and Gier, 1968; Whitmore et al, 1974; Staples et al., 1990). To achieve recovery from negative EB status, the diet is often altered to increase its energy density, but the responses are usually variable (Coppock et al., 1974; Smith et al., 1978; Carroll et al., 1990; Jerred et al., 1990; Sklan et al., 1991; Lucy et al., 1993; Spicer et al., 1993b; Beam and Butler, 1998; Blum et al., 1999).

Dry Matter Intake

In postpartum dairy cows, DMI from 1 to 8 wk of lactation is generally not adequate to meet the energy requirements for both maintenance and milk production (Smith et al., 1978; Staples et al., 1990; Carroll et al., 1990; Blum et al., 1999). The most important periods are early (1-12 wk) and peak (4-6 wk) lactation when the demands for energy are highest (Peters and Ball, 1987; Butler and Smith, 1989; Lucy et al., 1992a). The energy requirement of a cow

producing 35 kg of milk daily is 3 times more than the energy requirement for body maintenance (Butler and Smith, 1989). Two groups of cows fed high energy and low energy diets consumed feed at less than 2% of body weight and progressively increased for the first 16 wk of lactation before starting to decline (Staples et al., 1990). Appetite is the greatest limitation to large milk yields because it takes a longer time to reach peak DMI (Reid et al., 1979). Furthermore, energy density of the diet and gut fill can regulate DMI (Forbes, 1970; Baile and Forbes, 1974).

Body Weight

In postpartum dairy cows, maximum loss of body tissue occurs starting from the second week pre-lactation to 5 wk of lactation (Komaragiri et al., 1998). In the first 14 wk of lactation, cows produce high milk yield at the expense of body tissue, but regain body weight towards the end of lactation (Hart et al., 1979).

Changes in the body weight, specifically body protein, of an animal could be an indicator of the cow's metabolic status (Zurek et al., 1995). About 25% of body protein and unlimited amount of body fat can be lost in lactating cows without affecting basic physiological functions (Zurek et al., 1995). Postpartum Holstein cows mobilize a mean of 54 kg of body fat and 21 kg of body protein over a 5-wk period (Komaragiri et al., 1998). Amino acids mobilized from muscle

are converted by gluconeogenesis to glucose for milk synthesis during early lactation (Trenkle, 1981; Zurek et al., 1995).

Lactating dairy cows losing weight in the early postpartum period had more services per conception and lower conception rates when compared with animals gaining weight in some studies (Ducker and Morant, 1984; Butler and Smith, 1989) but not in other studies (Gardner, 1968; Stevenson and Britt, 1979; Carstairs et al., 1980; Carroll et al., 1990). Cows losing weight at breeding have decreased fertility, whereas those gaining weight at breeding have improved fertility (Swanson, 1989) suggesting that the amount or rate of body weight loss may contribute to the inconsistency of published results.

Body Condition Score

A loss in body condition score (BCS) is correlated with high fat mobilization (Hart et al., 1979) and EB (Butler and Smith, 1989; Spicer et al., 1990). Typically, dairy cows will loose 1.0 BCS on a 9 point scale from parturition to wk 5 postpartum after which BCS remains constant until wk 12 postpartum (Spicer et al., 1990). On the average, one unit of change in BCS corresponds to about 55 kg of empty body fat (Komaragiri et al., 1998). A drop of 0.75 to 1.0 in BCS (on a 5-point scale) by high producing and by thin cows resulted in anestrus and lower fertility (Studer, 1998). Average daily milk production did not differ between cows that calved with BCS $\geq$ 3.5 compared with cows at $\leq$ 3.5 on a 5-point scale (Ruegg et al., 1992). Reproductive function may

be more sensitive to changes in BCS than milk production, a prioritized physiological event in early postpartum.

Energy Balance

The equation that defines the general relationship between dietary energy intake and energy utilization is defined as follows: Daily EB = Net Energy Intake – Net Energy Required, where "Net Energy Intake" represents energy in the ration consumed and "Net Energy Required" represents net energy needed for maintenance and lactation. More specifically, net energy intake is calculated as the average daily DMI multiplied by the net energy concentration of the diet. Net energy required for daily maintenance (NE_m) of the animals in Mcal/day can be estimated using the equation: $NE_m = 80 \times BW^{0.75}$ (kg) /1000 (NRC, 1989). Daily energy for milk production in Mcal/day is calculated using the formula of Tyrell and Reid (1965): Milk yield (kg) x [92.239857(% milk fat) + 49.140211(% solid-not fat) – 56.393297]/1000 where milk yield is average daily yield for the week, and milk composition based on weekly milk analysis. This equation, based on milk composition reflects the metabolic status of the cow more accurately than the conventional method of measuring milk yield alone (Butler and Smith, 1989).

The energy requirements of high yielding cows in early lactation cannot be satisfied through dietary intake alone, thus body reserves must be mobilized. This deficiency between dietary energy intake and energy utilized for lactation results in negative EB, a condition that often lasts for several weeks (Coppock et

al., 1974; Sejrsen and Sorensen, 1982; De Kruif and Mijten, 1992). Negative EB generally reaches its maximum during the first 2 wk of lactation and its recovery period is variable (Butler and Smith, 1989). On the average, most cows are in negative EB for the first 42 to 70 d of lactation (Berghorn et al., 1988; Villa-Godoy et al., 1988; Staples et al., 1990). Several groups (Thatcher and Wilcox, 1973; Whitmore et al., 1974; Stevenson et al., 1983; Butler and Smith, 1989; Nebel and McGilliard, 1993) concluded that high milk yield affects conception rates by delaying the resumption of ovarian activity and thus limiting the number of estrous cycles before breeding. Furthermore, due to accelerating output of energy for milk production compared to energy intake from feed, cows grouped in terms of timing of postpartum ovarian recrudescence show that late responders and anestrus cows at wk 2 of lactation have greater negative EB (Staples et al., 1990). In contrast, other studies suggest that variation in EB is determined more by calories ingested than by milk yield (Villa-Godoy et al., 1988; Villa-Godoy et al., 1990). In support of the latter suggestion, correlations between EB and DMI range from 0.6 to 0.7 whereas correlations between EB and milk yield have ranged from – 0.3 to + 0.2 (Villa-Godoy et al., 1988; Spicer et al., 1990; 1993b).

Most cows resume reproductive activity while still energetically deficient and undergoing complex metabolic changes (Spicer et al., 1990; 1993b; Zurek et al., 1995). Negative EB is directly related to interval to first postpartum ovulation (Butler et al., 1981; Berghorn et al., 1988; Butler and Smith, 1989; Canfield et al., 1990; Carroll et al., 1990; Staples et al., 1998). This relationship becomes significant by the first 2 to 3 wk postpartum and determines the onset of ovarian

activity (Butler et al., 1981; Allrich et al., 1987; Butler and Smith, 1989; Grummer and Carroll, 1991). The days to EB nadir is correlated with days to first postpartum ovulation (Canfield et al., 1990, r = 0.75; Canfield and Butler, 1991, r = 0.85). Lactating cows average 13.6 d to negative EB nadir and 27 d to first ovulation while non-lactating cows average 4.0 d to negative EB nadir and 14.3 d to first ovulation (Canfield and Butler, 1991). Also, average EB during wk 1 to 4 postpartum correlates with days to first ovulation (r = − 0.57) (Spicer et al., 1993b). In contrast, others (Villa-Godoy et al., 1988; Spicer et al., 1990) were unable to relate mean EB between wk 1 and 12 of lactation with the duration of postpartum anestrus. The discrepancy between these studies may be explained by the fact that non-lactating cows were included in the correlations of Canfield and Butler (1991) but not in the calculations of other studies (Villa-Godoy et al., 1988; Spicer et al., 1990). Nonetheless, first ovulation and initiation of estrous cycles usually occur when lactating dairy cows are still in negative EB.

Diet Supplementation

Energy density of the diet of early lactating dairy cows can be increased in several ways in an attempt to maximize milk production and speed early return to reproductive functions. Enhancing energy intake will prevent ketosis, a condition caused by excessive fat mobilization. Because excessive amounts of carbohydrates (starch) can cause rumen acidosis, supplementation of direct fed

microbials, fat, and (or) protein are among the most common methods employed to augment energy deficiency in early postpartum cows.

Direct Fed Microbials. Supplementation of diets with yeast, *Saccharomyces cerevisiae,* at various stages of lactation is shown to increase net digestion of fiber in the forestomach and lead to increased energy output (Robinson, 1997). Furthermore, the addition of yeast cultures may improve ruminal fermentation that potentially could increase DMI and milk yield (Dann et al., 2000).

Several studies have been conducted to evaluate the effects of yeast culture supplementation under different conditions on several production variables. Supplementation of the diet with yeast culture at 5×10^9 organisms in multiparous Friesian dairy cows (n = 32) during wk 7 to 12 of lactation increased DMI by an average of 1.2 kg/d and fat corrected milk (FCM) yield by 1.4 L/d (Williams et al., 1991). In another study, yeast culture (Biomate Yeast Plus™) at concentration listed above, was top-dressed in the diet of primiparous cows (n = 24). It resulted in higher (29.5 vs. 28.7 kg/d) and earlier peak (wk 7 vs. 11) milk yield compared with control cows (Wohlt et al., 1991). The same preparation of yeast culture when supplemented to multiparous Holstein cows (n = 36) at 0, 10 or 20 g/d, resulted in significant improvements in DMI (23.8, 24.7, and 25.0 kg/d) and milk yield (37.7, 40.7, and 41.4 kg/d) (Wohlt et al., 1998).

Mid-lactation cows assigned a diet with 10 g/d of yeast culture for 4 wk exhibit significantly higher milk yield (26.2 vs. 25.4 kg/d), FCM (23.6 vs. 21.6

kg/d), and milk fat (0.90 vs. 0.78 kg/d) than control cows (Piva et al., 1993). Similarly, when postpartum Holstein cows (n = 46) fed mixed diets supplemented with yeast culture (57 g/d) for 23 d prepartum and 56 d postpartum, they had higher DMI, milk yield and milk components than control cows (Robinson and Garrett, 1999). Early lactating cows (n = 20) fed yeast (10 g/d) for 60 d have increased DMI and milk fat percentage (3.51 vs. 3.37%) but a depressed milk protein percentage (Adams et al., 1995). Yeast cultures at 60 g/d top-dressed to diets of lactating Jersey dairy cows (n = 25) from 21 d prepartum to 140 d postpartum increased DMI significantly, decreased rate of body weight loss and peak milk yield was earlier than for control cows although percentages of milk fat or protein were not altered (Dann et al., 2000). Collectively, these results indicate that supplementing yeast culture to the diet of lactating cows improves DMI, milk yield, and milk fat.

Studies using other yeast concentrations have not improved production variables. Holstein cows (n = 20) in early lactation fed total mixed ration top-dressed with 90 g/d of yeast culture for 10 wk had similar mean daily DMI, milk production, milk composition, and body weights as compared to control cows (Arambel and Kent, 1990). Yeast culture (56 g/d) plus *Aspergillus orizae* (3 g/d) fed to multiparous Holstein cows (n = 521) in early lactation for 130 d showed no improvement in milk yield, 3.5% FCM, or percentages of milk fat or protein and in addition, decreased the percentages of lactose and SNF in milk (Higginbotham et al., 1994). Two concentrations of yeast culture (5.3×10^{10} and 5.1×10^{10} cfu/d per cow) supplemented to diets of lactating Holstein cows (n = 306) failed to

affect DMI, milk production, 3.5% FCM, milk fat and protein production and percentage (Swartz et al., 1994). Cows (n = 20) supplemented with yeast culture (57 g/d) for 14 d pre- and postpartum also did not alter DMI, milk yield and milk component yields (Robinson, 1997). Cows supplemented with 15 g/d of yeast and 20 g/d of Biomate Yeast Plus™ failed to increase DMI, milk yield and milk composition (Soder and Holden, 1999). A comprehensive field trial involving 46 Virginia dairy herds tested the effect of supplementing diets with microbials and yeast. There was no difference detected in fat and protein yields and protein percentage between treated and control cows (McGilliard and Stallings, 1998). Although supplementing yeast culture in the diets of lactating cows made ruminal fermentation more stable, exhibiting less variation in ammonia concentrations and microbial numbers, it did not affect total volatile fatty acids (VFA) or viable yeast concentrations in ruminal fluid (Harrison et al., 1988). The wide range of responses to supplemental yeast products maybe due to the amount and timing of yeast supplementation, and (or) the number of cows in each treatment group. Further studies are needed before recommendations can be made in terms of the ideal amount of yeast and range of conditions for supplementation that would be effective in increasing DMI, milk yield and percent milk fat or milk protein.

Fats. Fats supplemented in diets include (1) oilseeds that contain 18 to 20% fat such as cottonseeds and whole soybeans; (2) rendered animal fats that contain nearly 99% fat such as tallow, poultry fat, choice white grease and yellow grease; and (3) granulated fats, also called protected, bypass, prilled, specialty or

ruminally inert fats that contain about 80 to 99% fat (Grummer, 1992). Inert, saturated and Ca soaps of fatty acids are preferable because they are less soluble in the rumen, and thereby will not impair rumen function (Grummer, 1992). In the following paragraphs, the effects of supplemental dietary fat on DMI, milk production and reproductive function will be reviewed.

Several studies showed that DMI either decreases or is not affected with fat supplementation. Prilled fat supplemented at 5% of diet DM (Megalac®, Dwight and Church, Co., Inc, Princeton, NJ) to lactating Holstein cows (n = 46) for 13 wk decreases DMI (Jerred et al., 1990). Similarly, supplemental dietary prilled fatty acids (Dairy Fat Prills, BP Nutrition Ltd., Wincham, Norwich, Chesire, UK) at 0, 3, 6, or 9% DM of diets for 21 d to mid-lactating dairy cows (n = 16) decreases DMI (Ferguson et al., 1990). The same result was obtained when postpartum cows (n = 42) were supplemented with prilled fatty acids at 3% DM (Energy Booster-100®, Milk Specialties Co., Dundee, IL) during the first 4 wk of lactation (Beam and Butler, 1998). Supplementation with calcium soaps of rapeseed fatty acids (Infoss, 62-064, Plewiska, Poland) at 4% DM to diet of cows (n = 12) on their 9 wk of lactation resulted in lower milk protein but similar DMI compared to control cows (Kowalski et al., 1999). Other fat supplementation studies showed that feeding ruminal inert fat or calcium salt of long chain fatty acids (Ca-FA, Megalac®, Church & Dwight Co.) at 2% DM (Spicer et al., 1993b) and at 3% DM (Komaragiri et al., 1998) for 12 wk postpartum has no effect on DMI of Holstein cows (n = 14 and 22, respectively). Similarly, DMI of postpartum cows (n = 45) was not changed with tallow supplementation at 7.6%

or 15.3% of DM for 42 d starting at parturition (Beam and Butler, 1997). Also, supplementing partially hydrogenated tallow at 0, 2, 4, 6% of DM (Alifet, Alifet USA, Inc., Cincinnati, OH) for 21 d to lactating cows (n = 4, days in milk = 84) (Drackley and Elliott, 1993) or at 1 to 2% DM for 135 d to postpartum cows (n = 63, day in milk = 0) (Salfer et al., 1995), did not have effect on DMI. Moreover, postpartum cows (n = 4) at 49 d in lactation supplemented with 5% tallow (Max-Fat Corp., Green Bay, WI) for 21 d had lower DMI (Elliott et al., 1993). Generally, fat supplementation (animal and granulated fats) at > 3% of DMI decreased DMI and thus should be kept at 3% or less of diet DM.

In terms of milk production, cows (n = 4) at 49 DIM supplemented with the Ca - FA at 3 or 6% of diet DM (Megalac®, Church and Dwight, Co.) for 14 d had higher FCM and milk fat percentage (Schauff and Clark, 1992). Similarly, cows (n = 120) fed Ca - FA at 2.6% DM (CSFA, H. Nagel, Hamburg) from parturition to 120 d postpartum produced more milk and milk fat for 60 d and higher FCM for 90 d (Sklan et al., 1991). Likewise, dietary prilled fatty acids (Dairy Fat Prills, BP Nutrition Ltd., Wincham, Norwich, Chesire, UK) at 3% DM, supplemented for 21 d to diets of mid-lactating cows (n = 16) increased milk production, FCM, percentage of milk fat but had no effect on percentage of milk protein (Ferguson et al., 1990). However, Ca - FA supplementation at 5% DM (Megalac®, Dwight and Church, Co., Ltd., Princeton, N.J.) for 13 wk to cows (n = 46) does not affect milk yield, FCM or milk protein (Jerred et al., 1990). In another study, cows (n = 22) fed Ca - FA (Megalac®, Church & Dwight Co.) at 3% DM from −2 to 12 wk of lactation, produced 2.7 kg/d more milk than control cows, but milk composition

was not affected (Komaragiri et al., 1998), whereas feeding 2% of Ca - FA (Megalac®, Church and Dwight Co.) to Holstein cows (n = 14) for 12 wk postpartum had no effect on milk yield or composition (Spicer et al., 1993b). Also, supplementation of diets with 2.5 or 5% tallow (Max-Fat Corp., Green Bay, WI) for 21 d for multiparous Holstein cows (n = 4) at 49 d in milk (DIM) did not affect production of milk, 4% FCM, milk fat, milk protein and SNF while percentages of milk fat, protein and SNF were lower (Elliot et al., 1993). Increasing the amount of partially hydrogenated tallow (Alifet, Alifet USA, Inc., Cincinnati, OH) (0, 2, 4, 6% of DM) supplemented for 21 d in cows at 84 DIM did not have a significant effect on milk production, milk fat content while decreasing milk protein and SNF (Drackley and Elliott, 1993). Similarly, inclusion of partially hydrogenated tallow (93.8% fat) at 1 to 2% of DM to diets of cows (n = 63) did not influence milk yield or 3.5% FCM and milk composition during the first 151 d of lactation (Salfer et al., 1995). Collectively, Ca - FA or dietary prilled (inert fat), which are less soluble in the rumen and thus will not modify rumen functions, increases milk fat percentage and milk yield in four of six studies summarized whereas dietary tallow which can be rumenally modified had no effect on milk fat percentage or milk yield in the majority of studies summarized. The differences in these results may be explained in part to differences in the number of cows evaluated or types and amount of fat supplemented. Also, the magnitude of production response may not be apparent, if the study is short term, and (or) near peak lactation because the additional source of energy may have been diverted to increase milk production.

Benefits of feeding supplemental fat on the reproductive function of postpartum cows have been variable. Fat supplementation resulted in higher plasma cholesterol concentrations (Kronfeld et al, 1980: sunflower seed at 15% DM; Storry et al., 1980: protected lipid; Hawkins et al., 1985: cottonseed at 18.5% DM; Skaar et al., 1989: 5% prilled fat on DM basis) and higher P_4 production (Talavera et el., 1985; Carroll et al., 1990; Spicer et al., 1993b: 2% prilled fat on DM basis). The increase in P_4 production due to fat supplementation may be due to an increase in its precursor, cholesterol (Hawkins et al., 1985; Talavera et al., 1985; Grummer and Carroll, 1991; Spicer et al., 1993b). Since in vitro, bovine granulosa cells in the presence of lipoprotein cholesterol produced higher amounts of progesterone (P_4) than controls without added cholesterol (Savion et al., 1982). Furthermore, inclusion of prilled fat (Dairy Fat Prills, BP Nutr. Ltd., Wincham Norwich, Chesire, UK) at 2% of DM gave beneficial effects on conception rate in three different herds (Ferguson et al., 1990). In contrast, inclusion of partially hydrogenated tallow (93.8% fat) at various levels during the first 151 d of lactation failed to influence reproductive variables (Salfer et al., 1995). The difference in some of the results may be attributed to the type of fat or the base ration to which fat was supplemented. For example, cows fed a ration rich in oils such as whole cottonseed may not benefit as much as cows fed a ration low in oil content. Support for this latter statement comes from a study in postpartum beef cows where 30% supplemental whole cottonseed increased mean concentrations of P_4 and doubled the lifespan of corpus luteum as compared to control cows (Williams, 1989).

Protein. Feeding an adequate amount of crude protein to lactating cows is necessary because during the first week of lactation about 16 kg of empty body weight, 4 kg of total fat and 3 kg of total protein are being mobilized (Roche et al., 2000). However, feeding high protein diets to postpartum cows increased blood urea concentration and has been associated with decreased fertility presumably via toxic concentrations of ammonia altering the hypothalamus-pituitary-ovarian functions in dairy cows (Ferguson and Chalupa, 1989; Swanson, 1989; Roche et al., 2000). Feeding excess crude protein like rumen degradable protein elevates plasma urea concentrations and has decreased first service conception rate to 31% as compared to 48% for control cows (Canfield et al., 1990). Also, Holstein cows fed a 20% CP diet had higher plasma urea nitrogen concentrations during the first month of lactation (Howard et al., 1987). Generally, plasma or milk urea nitrogen concentrations above 19 mg/dl decrease uterine pH and reduce fertility in dairy cows (Butler, 1998). In support of this concept, cows (n = 181) fed high CP (23.1%) with moderate rumen undegradable protein (RUP) (5.8%) had higher plasma urea N concentrations (25 vs. 20.1 mg/dl), lower first breeding pregnancy rates (24 vs. 41%) and lower overall pregnancy rates (53 vs. 75%) compared with cows fed moderate CP (17.7%) and high RUP (6.8%) (McCormick et al., 1999). The link between increased urea concentrations and fertility has been demonstrated using bovine endometrial cells cultured with increasing urea concentrations. These conditions resulted in altered pH and increased prostaglandin F2α secretion that may have interfered with embryo survival (Gilbert et al., 1996). Cows fed a low CP (12.7%) diet had

higher serum P_4 concentrations than control cows fed high CP (16 or 19%) diet (Jordan and Swanson, 1979). But in some studies, high protein diets (20% CP) did not changed (Barton et al., 1996) or decreased (Sonderman and Larson, 1989) plasma P_4 concentrations of lactating cows. Also, days open, services per conception and percentage cows pregnant were not affected by increasing CP to 20% in the diet (Howard et al., 1987). Possibly, these conflicting results are due to varying levels of rumen degradable protein because negative EB is intensified due to the energy cost of detoxifying ammonia (Chalupa, 1984; Butler, 1998).

Furthermore, high protein (17 to 20% CP) concentrations in the diet of lactating cows stimulate high milk production in early lactation (Kung and Huber, 1983; Howard et al., 1987; Butler, 1998). Cows (n = 20) fed 19% CP compared to 16% CP had higher average milk yields (38.0 vs. 42.4 kg), milk crude protein percentage (3.08 vs. 2.89%) and milk protein yield (1.29 vs. 1.15 kg/d) during the first 6 wk postpartum (Komaragiri and Erdman, 1997). Also, early lactating cows (n = 39, 4 to 14 wk postpartum) fed diets with high CP (17.4%) content increased milk production, 4% FCM, milk fat and milk protein compared to a diet with less CP (15.2%) (Kalscheur et al., 1999). The increasing amount of rumen undegradable protein (RUP) (35.5, 41.4 and 46.5%) in the diet linearly increased milk production and FCM whereas milk fat and milk protein percentages declined but not when fed in mid (19 - 29 wk) and late (44 to 74 wk) lactations (Kalscheur et al., 1999). However, in 35 dairy herds supplemented with blended animal marine protein (Prolak, H.J. Baker and Sons, Inc.) at 2 to 4% of DM, only 19 herds had higher milk yield. Overall, supplemented cows gave milk similar in

protein and lower in fat content than control cows (Ferguson et al., 2000). Furthermore, Holstein cows fed three dietary levels of undegraded crude protein (32, 28, and 18% DM) during the first 3 months of lactation had similar yields of milk, milk protein and milk fat (Bruckental et al., 2000). This variability in responses to feeding elevated amounts of crude protein to lactating cows may be attributed to the timing and duration of protein supplementation, type and amount of protein in the base ration, variability in blood concentrations of urea, and statistical precision that varies with the number of cows used in each study.

Volatile Fatty Acids. In ruminants, among the VFA, propionate contributes up to 60% of the substrate for gluconeogenesis (Manns and Boda, 1967; Manns et al., 1967). Increasing ruminal propionate concentration by feeding monensin increased GnRH-induced secretion of LH (Randel and Rhodes, 1980; Rutter et al., 1983). Lower rates of propionate production or greater acetate - propionate ratio may reduce glucose production that in turn may serve as a signal to diminish LH secretion. Alternatively, because glucose is the major source of energy for the ovary (Rabbie et al., 1997) and glucose enhances bovine thecal cell steroidogenesis (Stewart et al., 1995), a reduced blood glucose concentration may serve as a signal to diminish ovarian steroid production and indirectly diminish LH secretion. Lactating cows utilize propionate more rapidly than do dry cows because milk lactose synthesis requires a substantial amount of glucose that is met largely by gluconeogenesis (Corse and Eliot, 1970; Wiltrout and Satter, 1972). The negative effect of increasing propionate molar proportion

in the rumen is the decrease in milk fat yield and percentages, due to reduction of fermentation in the rumen, allowing more starch to flow to the small intestine where some may be absorbed as glucose (Orskov et al., 1969; Bauman et al., 1971).

Monensin is a polyether carboxylic ionophore antibiotic produced by *Streptomyces cinnamonensis* that generally modifies rumen fermentation by increasing relative proportion of ruminal propionate (Russel et al., 1988; Lean et al., 1994; Duffield et al., 1999). Monensin in the form of controlled-release capsule (335 mg/d) administered to cows (n = 16) during first week of lactation had higher glucose concentration than control cows (Abe et al., 1994). Also, Holstein cows (n = 98) fed monensin at 300 mg/d from 2 to 4 and 6 to 8 wk of lactation increased glucose from 3.38 to 3.53 ± 0.28 and 3.54 to 3.69 ± 0.22 mmol/L, respectively (Phipps et al., 2000). Although in another study, monensin when fed to lactating dairy cows at 150, 300 or 450 mg/d during the first 28 d postpartum did not influence serum glucose but showed lower milk fat percentage as compared to control cows (Thomas et al., 1993). Similarly, cows (n=20) dosed once with controlled-release capsule of monensin (Rumensin®, Elanco Animal Health, Wiri, Auckland, New Zealand) have an average of 0.015 kg/d less milk fat than control cows (Hayes et al., 1996), and monensin fed at 300 mg/d reduced percent milk fat in Holstein cows (n = 4) (Ramanzin et al., 1997). Also, lactating dairy cows (n = 98) fed monensin at 300 mg/d, reduced milk fat and milk protein content (Phipps et al., 2000). But in another study using 1061 lactating cows, milk fat and milk protein production were not influenced by

feeding monensin at 32 g for 7 days (Lean et al., 1995). Differences maybe attributed to number of animals used, dose levels of monensin, duration of treatment and (or) stage of lactation when treatments were initiated.

Supplementing corn silage, corn grain, and soybean meal based diets fed to postpartum dairy cows with volatile fatty acids did not improve milk production or milk composition (Klusmeyer et al., 1987). When, lactating cows were gradually infused with butyrate (0, 200, 400, or 600 g/d) to replace acetate and propionate, blood glucose concentration decreased and plasma urea concentration increased indicating that more amino acids were used for gluconeogenesis when the supply of propionate was decreased (Huhtanen et al., 1993). Butyrate supplementation did not affect milk yield but increased milk fat and milk protein concentrations whereas lactose declined suggesting that decreasing propionate increases ketogenesis and decreases gluconeogenesis (Huhtanen et al., 1993). In another study, drenching lactating cows with 349 g calcium propionate in 200 ml molasses elevated plasma glucose concentrations by 11% for less than 3 h and may prevent ketosis in early lactation (Jonsson et al., 1998).

Several studies found some negative effects of volatile fatty acid infusion to dairy cows. Fistulated Holstein cows infused with propionic acid for a period of 14 d had decrease milk fat concentrations by (- 4.5 g/kg) and milk fat yield (-111 g/d) compared to isoenergetic mixture of volatile fatty acids (Hurtaud et al., 1998). Also, propionic acid infusion decreases milk yield (- 1.3 kg/d) but increases milk protein content (1.5 g/kg) (Hurtaud et al., 1998). Propionic acid

infusion (1.0 mmol/kg body weight for 30 min) in lactating cows on d 5 and 30 postpartum does not elevate plasma glucose concentrations (Sartin et al., 1985). It appears that increasing ruminal propionate by the methods mentioned above generated variable results in terms of milk production variables. Some of these effects may reflect short– term physiological responses that may differ from longer-term responses.

Postpartum Hormonal Profiles and Metabolic Signals of Early Lactating Dairy Cows

The early postpartum period is the recovery phase of the hypothalamus-pituitary-ovarian axis as well as the reproductive tract (Malven, 1984; Butler and Smith, 1989). There are several sequences of biological events that the hypothalamic-pituitary-ovarian axis undergoes for the reestablishment of normal reproductive cyclicity after parturition, this includes the recovery from high concentrations of estrogen, P_4 and glucocorticoids, return to optimal gonadotropin secretion, follicular growth and ovulation with estrus expression and maintenance of corpus luteum to sustain pregnancy (Malven, 1984; Peters and Lamming, 1984; Schallenberger, 1985; Butler and Smith, 1989).

It is the consensus among researchers that energy demand for reestablishment of early postpartum ovarian function is confounded and antagonistic to lactation (Marion and Gier, 1968; Stevenson and Britt, 1979; Butler et al., 1981; Butler and Smith, 1989). In terms of nutrient partioning,

lactation as a physiological event takes precedence during early postpartum (Staples et al., 1990), shifting the metabolic status of cows from an anabolic to a highly catabolic state (Ronge et al., 1988). This antagonism has been compounded by breeding and selection for generations for increased milk yield, changing the endocrine profiles of cows so that blood concentrations of hormones favor lactation (Barnes et al., 1985; Kazmer et al., 1986; Bonczek et al., 1988; Harrison et al, 1990; Nebel and McGilliard, 1993). The high demand of energy for milk secretion during early lactation results in negative EB and the return towards positive energy balance (positive EB), which is necessary to resume the normal hypophyseal-ovarian functions and consequently normal ovarian activities, as evidenced by ovulation and luteal function (Coppock et al., 1974; Butler et al., 1981; Nebel and McGilliard, 1993).

Gonadotropin Secretion

Gonadotropin - releasing hormone. Early postpartum cows undergo resumption of gonadotropin-releasing hormone (GnRH) secretion, and consequently regain the ability of the pituitary to generate follicle stimulating hormone (FSH) and luteinizing hormone (LH) pulses at normal frequency, which is necessary for the reestablishment of ovarian follicular growth and ovulation (Malven, 1984; Peters and Lamming, 1984; Schallenberger, 1985). The pituitary responsiveness to GnRH increases rapidly after parturition, and the recovery time of a normal LH secretion pattern takes about 10 to 20 d (Lamming et al.,

1981; 1982; Butler and Smith, 1989). Several studies indicated that GnRH plays a role in influencing LH pulse patterns, FSH secretion, estradiol (E_2) production and ovulation in postpartum dairy cows. Cows injected with a single dose of 200 µg of GnRH during the first 10 d postpartum show a significant increase in plasma LH (Foster et al., 1980). Anestrous dairy cows repeatedly treated with exogenous GnRH (2.5 or 5 µg) at 2−h intervals for 48−h develop LH pulse pattern, an LH surge, ovulation and normal luteal function (Lamming et al, 1982). Cyclic cows receiving 100 µg of GnRH show transient increases in FSH (83% of the time) followed consistently by increases in serum E_2, which generally reach peak concentrations concomitant with peak diameter of the dominant follicle before the subsequent estrus occurs (Pursley et al., 1993). Intramuscular injection of 100 µg of GnRH to dairy cows at 12 to14 d postpartum results in palpable corpus luteum (CL) and plasma $P_4 \geq 1.0$ ng/ml (Zaied et al., 1980), and an injection of 200 µg of GnRH before d 10 postpartum caused an increase in plasma LH (Foster et al., 1980). However, the effectiveness of GnRH to induce ovulation depends on the presence of large follicles at the time of treatment (Garverick et al., 1980). Most early postpartum cows under negative EB are too impaired to resume ovulation (Zurek et al., 1995).

Luteinizing hormone. In postpartum dairy cows, basal plasma LH concentrations starts to increase as the frequency of the episodic pattern of pulsatile LH release increases from 1 to 2 pulses per 6 h. This pattern of LH secretion leads to a preovulatory type of LH surge that could result in ovulation

(Carruthers and Hafs, 1980; Peters et al., 1981; Lamming et al., 1981; Butler and Smith, 1989). The blood plasma level of LH after parturition increases during the first to third week postpartum (Echternkamp and Hansel, 1973; Ingalls et al., 1973; Fernandes et al., 1978; Goodale et al., 1978; Schallenberger et al., 1978; Kesler et al., 1979; Peters, 1978; Webb et al., 1980; Bolt and Rollins, 1983).

It has been hypothesized that in lactating dairy cows, the hypothalamic locus primarily modulates LH secretion during negative EB (Beam and Butler, 1999), and that negative EB delays the time of first ovulation through inhibition of LH pulse frequency (Butler, 2000; Roche et al., 2000). After maximal negative EB, the mean plasma LH concentrations and the number of episodic LH peaks increase, and first ovulation occurs for most cows (Coppock et al., 1974; Carruthers and Hafs, 1980; Butler and Smith, 1989; Canfield and Butler, 1990). Comparing frequent sampling series immediately before and after the negative EB nadir in lactating cows, LH pulse frequency, LH baseline and mean LH increased, whereas LH pulse amplitude tended to decrease (Canfield and Butler, 1990). In addition, negative EB may modulate ovulation through ovarian sensitivity to LH signaling because ovulation occurred later in lactating cows compared to cows milked only for a day but have similar LH pulse frequency (Canfield and Butler, 1991).

Increasing EB by supplementing rumen inert fat (Megalac®, Church and Dwight Co., Inc.) to cows at about 2.2% of DM, does not influence the basal smoothed mean concentrations and amplitude of LH sampled every 10 min for 8 h on d 10 postpartum (Lucy et al., 1991b). Also, increasing the protein content of

diets of lactating cows by feeding two levels of rumen degradable CP (16 and 19%) exhibits similar LH pulse amplitude and frequency (Canfield et al., 1990). Furthermore, concentrations of LH are similar in cows fed high and low energy diets (Lucy et al., 1992a). Thus, EB may influence LH secretion but manipulation of the diet for early lactating cows seems to have no effect on LH secretion.

Follicle-stimulating hormone. Plasma concentrations of FSH increase within the first 3 to 5 d (Butler and Smith, 1989) or 5 to 10 d (Lamming et al, 1981) of lactation. Also, treatment of postpartum dairy cows with GnRH on d 10 postpartum increases FSH concentrations (Schallenberger et al., 1978; Foster et al., 1980). In contrast, FSH concentrations in postpartum cows did not change during the first 14 d postpartum in other studies (Schallenberger et al., 1978; Bolt and Rollins, 1983; Hansel and Convey, 1983). Interestingly, Dobson (1978) observed that FSH concentrations increased between 0 to 20 d postpartum in half of the 6 cows evaluated.

Cows supplemented with each of three levels of fat (3.3, 5.2, and 7.1%) (Energy Booster-100®, Milk Specialties Co., Dundee, IL) in their diet have similar plasma FSH concentrations from d 1 to 14 postpartum (Beam and Butler, 1998). Averaged across diet groups, mean FSH levels were highest from d 1 to 5 postpartum and thereafter decrease from d 5 to 11 postpartum (Beam and Butler, 1998). Regardless of diet, all cows experienced a wave of follicular development during the second week postpartum (Beam and Butler, 1997). Because dairy cows exhibit unchanged plasma FSH concentrations after 14 d postpartum and

increasing energy content of the diet of cows does not alter FSH concentrations, it is unlikely that insufficient FSH secretion is the limiting factor for dairy cows to return to ovarian activity (Kesler et al., 1977; Spicer and Echternkamp, 1986; Beam and Butler, 1997). A similar conclusion has been made regarding nutritionally induced anestrous beef cows (Vizcarra et al., 1997; Bossis et al., 1999, 2000).

Ovarian Steroid Secretion

Estradiol. Estrogen concentrations gradually increase in serum beginning at wk 3 to 5 prepartum with marked increases during the last 2 wk of pregnancy followed by a rapid decrease to low concentrations (1 to 8 pg/ml) at parturition until d 7 postpartum (Convey, 1973; Henricks et al., 1972; Echternkamp and Hansel, 1973; Sasser et al., 1979; Beam and Butler, 1997). This is followed by an increase just before the first postpartum estrus (Henricks et al., 1972; Echternkamp and Hansel, 1973; Beam and Butler, 1998). During the initial period of pituitary refractoriness at early postpartum anestrus, a minimum threshold frequency of endogenous LH secretion is necessary to stimulate feedback that would allow E_2 to evoke its unimpaired action (Schallenberger and Prokopp, 1985). Postpartum cows with dominant follicles that emerged after the EB nadir exhibit enhanced production of E_2 and greater ovulation success (Beam and Butler, 1997). Consequently, cows in their first postpartum follicular wave with lower peak plasma E_2 (1.0 vs. 5.0 pg/ml) are accompanied by ovulation failure (Beam and Butler, 1997).

Increasing EB of early postpartum cows by supplementing diets with a moderate amount of added fat results in higher peak plasma E_2 during the first follicular wave and a shorter interval to first ovulation compared to cows fed a low or a higher fat supplement (Beam and Butler, 1997). Furthermore, considering all the cows in this study, there is a slow increase in E_2 after d 7 postpartum but thereafter concentrations were similar (Beam and Butler, 1998). Adding a moderate amount of fat in the diet may be beneficial for increasing E_2 and postpartum estrus expression, but this area must be explored further to generate conclusive results.

Progesterone. In postpartum dairy cows, an increase in P_4 secretion is an indication that gonadotropin secretion has recovered (Terqui et al., 1982; Butler and Smith, 1989). Serum P_4 concentrations are normally low (< 1.0 ng/ml) at parturition due to regression of the corpus luteum of pregnancy before parturition (Spicer and Echternkamp, 1986; Savio et al., 1990). The function of the CL in postpartum cows is not optimal during the first estrous cycle compared to second estrous cycle as evidenced by lower mean peak P_4 concentration in blood plasma (5.95 ± 3.4 vs. 8.79 ± 3.3 ng/ml, Staples et al., 1990), (3.7 vs. 4.7 ng/ml, Stevenson and Britt, 1979) and P_4 area (105.4 ± 13.9 vs. 151.8 ± ng/ml·d, Spicer et al., 1990; 106.4 ± 16.1 vs. 134.2 ± 17.4 ng/ml·d, Spicer et al., 1993b).

P_4 concentrations of early postpartum cows are modulated by the energy status of the animals (Spicer et al, 1990, 1993; Butler, 2000). P_4 concentrations are associated positively with fertility (Folman et al., 1973), increased pregnancy

rate and reduced days open (Sklan et al., 1991), and negatively with caloric deficit (Villa-Godoy et al., 1988; Spicer et al., 1990; Butler, 2000). In addition, negative EB reduces the weight of CL (Apgar et al., 1975) and luteal tissue, which decreases steroidogenic activity of luteal cells, and thus decreasing P_4 synthesis (Villa-Godoy et al., 1990). Moreover, a reduced concentration of P_4 in serum could be due to faster clearance of P_4 (Villa-Godoy et al., 1988).

There was a weak positive correlation ($r = 0.3$) between P_4 concentration and the second and third postpartum luteal activity with early EB (9 d postpartum) based on energy intake and milk yield (Villa-Godoy et al., 1988). In the same study, the postpartum interval to nadir and magnitude of nadir of energy balance interacted to reduce P_4 within the second and third postpartum diestrus ($r = 0.69$). Similarly, serum P_4 and EB are positively correlated ($r = 0.26$ to 0.29, Spicer et al., 1990, 1993b).

The potential benefits of feeding supplemental fat to early postpartum cows maybe to enhance utilization of cholesterol for P_4 synthesis (Grummer and Carrol, 1991). Postpartum cows fed 5% (DM basis) Ca-FA (Megalac®, Church and Dwight, Co.) had higher mean plasma P_4 concentrations in the second and third postpartum estrous cycle compared to control cows (Carroll et al., 1990). In dairy cows fed rumen inert fat at 2.6% DM (Calcium soaps of fatty acids, H. Nagel, Hamburg), P_4 concentrations are higher in the luteal phase before breeding although ovarian cyclicity commenced later as indicated by a longer time required to reach 2 ng/ml of P_4 in plasma (Sklan et al, 1991). Dairy cows fed Ca-FA at 2% DM (Megalac®, Church and Dwight, Co.) between wk 5 to 12 have

greater luteal phase P_4 secretions than cows fed the control diet (Spicer et al., 1993b). Similarly, supplementation with Ca-FA (Megalac®, Church and Dwight, Co.) at 2.2% of DM and 15.7% degradable intake protein (DIP) to postpartum cows (n = 55) for 120 d doubles the number of corpora lutea, reduces the time to first rise in P_4 and higher plasma P_4 than control cows fed 0% fat and 11.1% DIP (Garcia-Bojalil et al., 1998). Luteal phase P_4 concentrations tend to be higher in dairy cows fed a high fat and low escape protein diet (3% tallow and 5% escape protein) compared to other diets of varying source of fat and protein but similar in energy and crude protein content (Son et al., 1996). Contrary to these findings, the ability of postpartum cows to produce lipoproteins and stimulate P_4 synthesis is not different between cows supplemented with fat and control cows (Carroll et al., 1992). Collectively these studies indicate that fat supplementation increases P_4 secretion and thus may be beneficial to hasten the onset of next breeding cycle and to maintain pregnancy.

Metabolites and Metabolic Hormones

Cholesterol. Cholesterol is a precursor for ovarian steroidogenesis derived from cellular de novo synthesis from acetate or uptake of plasma lipoprotein (Grummer and Carrol, 1988; Savion et al., 1992). In vivo, increased granulosa cell P_4 production after the LH surge may be dependent on the supply of sterol from follicular fluid lipoproteins (Dieleman and Blankenstein, 1984). Consistent with this statement, lipoprotein cholesterol increases P_4 production by

bovine granulosa cells (Savion et al., 1982) and luteal cells (Carroll et al., 1992) in vitro. Thus, strategies designed to increase plasma cholesterol to increase the supply of cholesterol to the ovary and consequently increase luteal P_4 secretion may increase reproductive efficiency because P_4 concentrations at about > 4 ng/ml at the peak of luteal phase prior to insemination is positively correlated with conception (Folman et al., 1973) (see also *Progesterone* section). Also, plasma cholesterol concentrations at > 130 mg/dl in cows were positively correlated with numbers of recoverable embryos from superovulation (Kweon et al., 1986).

In postpartum dairy cows, plasma concentrations of cholesterol are lower at 10 to 33 d (134 ± 5 mg/dl) postpartum compared to 79 to 100 d postpartum (199 ± 9 mg/dl) (Carroll et al., 1990). In a more detailed study, postpartum cows fed Ca-FA (Megalac®, Dwight and Church, Co.) at 2% of diet DM experienced a 69% increase in total plasma cholesterol between wk 5 to 12 compared to only 23% in cows fed the control diet (Spicer et al., 1993b). Similarly, Holstein cows fed 5% of diet DM Ca-FA (Megalac®, Dwight and Church, Co.) have higher mean plasma cholesterol (204 ± 5 mg/dl) over 100 d postpartum compared with animals not receiving supplemental fat (159 ± 4 mg/dl) (Carroll et al., 1990). Moreover, there is a positive correlation (r = 0.47) between P_4 and cholesterol during the early (1-12 wk) postpartum period (Spicer et al., 1993b). The increase in plasma P_4 is possibly due to an increase in the capacity of the ovary to uptake cholesterol as precursor for steroidogenesis or decreased P_4 clearance (Grummer and Carroll, 1988; Spicer et al., 1993b). Mean plasma concentrations of cholesterol of early lactating cows were higher when fed sunflower seed at

15% DM (Kronfeld et al., 1980), protected lipid (Storry et al, 1980), cottonseed at 18.5% DM (Hawkins et al., 1985), tallow (Schauff et al., 1992), and inert fat at 2.0% DM (Spicer et al., 1993b, Son et al., 1996; Moallem et al., 1997). Collectively, these studies provide clear evidence that plasma cholesterol concentration increases with time postpartum and that feeding supplemental lipids can magnify this increase.

Nonesterified Fatty Acids. Some authors suggested that plasma nonesterified fatty acids (NEFA) may be a potential indicator of the overall status of postpartum cows since NEFA is negatively correlated ($r = -0.39$ to -0.71) with EB (Harrison et al., 1990; Canfield and Butler, 1991; Lucy et al., 1992a; Beam and Butler, 1998). Also, NEFA decreases as EB becomes positive (Sechen et al., 1989, 1990). Plasma NEFA concentrations can be used as indicators of energy status of cows with a BCS of around 3.0 (on a scale of 1 to 5) at parturition but not for thin (i.e., BCS = 2.2) cows (Nachtomi et al., 1986) or heavy (i.e., BCS = 3.8) cows (Fronk et al., 1980). The pattern of plasma concentrations of NEFA in postpartum cows decrease each week from 612 μeq/L at wk 1 to 237 μeq/L at wk 9 (Staples et al., 1990). Similarly, NEFA decreases between 1 and 10 wk postpartum from 527 to 171 μmoles/L (Smith et al., 1978). There is a linear decrease in free fatty acid concentrations from 5 to 30 d postpartum (Sartin et al., 1989). Lactating Holstein cows fed high-energy diets have plasma NEFA levels less lower than cows fed low energy diets suggesting that plasma NEFA levels reflects the amount of fat being mobilized in response

to a dietary induced negative EB (Lucy et al., 1992a). Changes in plasma NEFA concentrations in lactating cows supplemented with concentrate at 3 to 5.5 kg/d are closely related to EB (Coulon et al., 1985). Thus, the decreasing plasma NEFA concentrations with week postpartum may be an indication that the cows were progressing towards a positive EB and (or) experiencing a decrease in lipolysis due to depletion of body fat stores. Furthermore, the decrease in plasma NEFA levels may also be due to an increased utilization of NEFA for milk fat synthesis due to increasing milk production with week postpartum.

Fat supplementation increases plasma NEFA due to incomplete uptake of fatty acids after lipoprotein lipase hydrolysis of very low-density lipoprotein triglycerides although fat supplementation increases net adipose tissue triglyceride hydrolysis (Grummer and Carroll, 1991). Holstein cows fed 15% protected tallow (encapsulated lipid in formaldehyde treated soybean meal, Alta Lipids USA Ltd., Boise, ID) exhibit higher plasma NEFA (684 μmoles/L) compared both to control cows (527 μmoles/L) and to cows fed 30% protected tallow (496 μmoles/L) during wk 1 postpartum but not in the succeeding weeks (5 to 15 wk), where both cows fed 15 and 30% fat have higher NEFA than control cows (Smith et al., 1978). Cows supplemented with 30% tallow had lower plasma NEFA during wk 1 of lactation of probably because of lesser body fat mobilization resulting from a sufficient amount of energy from supplemented fat. Although an elevation of NEFA usually is paralleled by an increase in plasma ketone concentrations, adding fat to the diet of lactating cows elicited an

antiketogenic effect probably due to a glucose-sparing effect (Grummer and Carroll, 1991).

Somatotropin (bST) treated cows that developed negative EB on d 3 of a 7 d trial have higher basal plasma NEFA by d 2 and 3 than control cows (Houseknecht et al., 1995). Similar works show that lactating cows treated with bST had increased milk yield (12 – 31 %) without a commensurate increase in DMI, giving rise to a negative EB status, which in turn elevates plasma NEFA (Bauman et al. 1988; Sechen et al., 1989, Bauman, 1992). Plasma NEFA concentrations are not associated with days to first ovulation or to first visual estrus (Harrison et al., 1990). Similarly, cows categorized as early, late and non-responders based on the time and presence of CL show similar NEFA concentrations (Staples et al., 1990). Thus, the usefulness of NEFA as a measure of return to postpartum reproductive competence is not apparent (Staples et al., 1990). Probably plasma NEFA is primarily used as supplementary energy source by the mammary gland for milk synthesis (Tyrell et al., 1988; Bauman et al., 1988) and serves as a precursor for milk fat (Hart, 1978) in early lactating cows in negative EB. In parallel, NEFA levels are not a reliable indicator of the onset of nutritional anestrus in beef heifers nor the return of estrous cyclicity in anestrous beef heifers refed to gain body weight (Bossis et al., 1999, 2000).

Leptin. Leptin is a newly discovered 16-kDa protein produced from adipocytes, with plasma concentrations that parallel the amount of fat reserves

and may regulate feed intake (Houseknect et al., 1997). Leptin from adipocytes is thought to pass from the circulation to the cerebrospinal fluid and into the hypothalamus and may affect satiety and possibly GnRH secretion (Blache et al., 2000). Because leptin is so new, little work has been done in farm animals. In ad libitum-fed dairy cows, plasma leptin concentrations are positively correlated to BCS during late lactation (i.e., 261 d, Ehrhardt et al., 2000). Preliminary data indicate that lactating dairy cows have lower plasma leptin levels than non-lactating cows (Block et al., 2000) and that dairy cows with early first postpartum ovulations have greater plasma leptin levels than cows with later first postpartum ovulations (Kadokawa et al., 2000). In underfed ewes, plasma leptin concentrations decreased as much as 56% and the variations in plasma leptin concentrations were contributed mainly by BCS (35%) and nutritional status (17%) (Delavaud et al., 2000). Furthermore, expression of leptin mRNA is decreased by 2-d food deprivation in cattle (Tsuchiya et al., 1998; Amstalden et al., 2000). Leptin may be an important signal for regulation of feed consumption that indirectly can influence reproductive functions. However, whether systemic leptin concentration changes during early lactation in dairy cattle needs to be further elucidated.

Insulin and Glucose. Insulin and glucose are modulators of energy utilization in ruminants (Trenkle, 1981). Koprowski and Tucker (1973) established that insulin increased approximately 2- to 3-fold between 4 and 12 wk of lactation and remains similar thereafter. Similarly, mean plasma insulin

concentrations increase 1.4-fold from 8.8 ± 0.5 μU/ml on d 1-20 to 11.9 ± 0.6 μU/ml on d 41-56 postpartum (Smith et al., 1978). In early lactation, insulin concentration is low probably due to the low concentration of plasma glucose (Vicini et al., 1991). Mean plasma concentrations of glucose increase 15% with time postpartum from 37.7 ± 0.7 mg/100 ml on d 1−20 to 43.3 ± 0.8 mg/100 ml on d 41− 56 with days postpartum (Smith et al., 1978). A comparable result was obtained by Harrison et al. (1990), except that blood glucose concentrations were lower during the second week postpartum compared with the values obtained by Smith et al. (1978). In contrast, Sartin et al. (1989) found that plasma glucose does not vary from d 5 to 30 postpartum in dairy cows.

Glucose is a known stimulator of insulin secretion, but in ruminants the correlations between glucose and insulin are low (Horino et al., 1968; McAtee and Trenkle, 1972; Trenkle, 1981). This is because most carbohydrates are fermented in the rumen and thus little or no increase in blood glucose occurs after feeding (Trenkle, 1981). Early lactating cows mainly in negative EB rapidly utilize glucose for milk lactose synthesis, which results in lower plasma concentrations of both glucose and insulin as compared to cows at later stages of lactation (Smith et al., 1976; Hart et al., 1978). There is a weak positive relationship between daily EB with plasma insulin (r = 0.13) between d 1 to 30 of lactation (Beam and Butler, 1998). Correlations between milk yield and plasma insulin are negative and ranged from r = - 0.3 to - 0.8 (Koprowski and Tucker, 1973; Hart et al., 1979). Also, weak negative correlations exist between insulin and milk fat production (r = - 0.26), percentage (r = - 0.19) and ruminal acetate:

propionate ratios (r = - 0.71) (Walker and Elliot, 1972). The decrease in insulin maybe an indirect result of lower rumen acetate – propionate ratio. A lower ratio will trigger lipolysis and allow for fat to be used for milk fat synthesis in early lactation. Cows selected for high milk yield have significantly lower insulin concentrations (15.3 and 15.2 μU/ml) than cows selected for low milk yield (162.3 and 77.1 μU/ml) on d 30 and 90 of lactation (Hart et al., 1980). Similarly, several studies indicate that insulin secretion decreases in cows selected for high milk yield (Hart et al., 1978; Bonczek et al., 1988; Sartin et al., 1988).

Lower availability of glucose and insulin may directly decrease LH pulsatility (Butler and Smith, 1989; Canfield and Butler, 1990, 1991) or limit ovarian responsiveness to gonadotropins (Spicer et al., 1990; Spicer and Echternkamp, 1995; Stewart et al., 1995). According to Porestky (1997), the effect of insulin on ovarian tissues is similar to pituitary gonadotropins, which may include effects on steroidogenic enzymes, modulation of gonadotropin receptor number, and non-specific enhancement of cell viability. The ovarian action of insulin in cattle has been reviewed (Spicer and Echternkamp, 1995). In vitro, insulin augments FSH-induced P_4 production and increases proliferation of granulosa cells from small (1-5 mm) and large (> 8 mm) follicles (Langhout et al., 1991), and stimulates aromatase activity of bovine granulosa cells (McArdle et al., 1989; Saumande, 1991; Spicer et al., 1993a; Gong et al., 1994; Spicer and Chamberlain, 1998). In vivo, exogenous insulin increases E_2 concentrations in follicular fluid of superovulated cattle (Simpson et al., 1994). Furthermore, insulin is luteotropic in vivo (McCann, 1984) and in vitro (Savion et al., 1982;

Chakravorty et al., 1993). Thus, reduced insulin due to negative EB may negatively affect ovarian luteal function by reducing follicular steroidogenesis and (or) luteal P_4 secretion (Villa-Godoy et al., 1990). Insufficient DMI of early lactating cows increases glucose utilization and reduces insulin secretion, which correspondingly decreases milk fat yield and may impair ovarian functions. Increasing gluconeogenic precursor like propionate during early lactation may ensure sufficient glucose supply and increase insulin secretion necessary for optimal milk fat synthesis and ovarian function.

Insulin - like growth factor - I. Insulin-like growth factor-I (IGF-I) is a mediator of many biological functions in dairy cows. It mediates the positive action of growth hormone (GH) and GH-releasing factor (GRF) on milk production (Bauman and McCutcheon, 1986; Etherton and Bauman, 1998). For example, dairy cows treated with daily sc injections of 10 μg of GRF (1-29) NH_2 / kg body weight for 57 d increased milk production by 14% and IGF-I concentrations by 40 % compared to control animals (Abribat et al., 1990). In lactating dairy cows, IGF-I concentrations are low during the first few weeks postpartum but increase twofold to eightfold through wk 12 postpartum (Abribat et al., 1990; Spicer et al., 1990, 1993b). Daily EB in postpartum dairy cows is positively correlated with serum IGF-I ($r = 0.43$, Spicer et al., 1990; $r = 0.41$; Beam and Butler, 1998). Several authors (Butler and Smith, 1989; Canfield and Butler, 1990; Spicer et al., 1990; Canfield and Butler, 1991; Spicer et al., 1993b)

postulated that the increasing EB with time postpartum increases ovarian responsiveness to gonadotropins via increased IGF-I concentrations.

In vitro, IGF-I is a potent mitogenic and steroidogenic stimulator of bovine granulosa cells (Savio et al., 1990; Gong et al., 1994; Spicer et al., 1993a; Spicer and Chamberlain, 1999) and thecal cells (Stewart et al., 1995; Spicer et al., 1997; Spicer and Chamberlain, 1998). Incubation of cultured granulosa cells from small (< 5 mm) and large (≥ 8 mm) follicles for 24 h with 100 ng/ml of IGF-I leads to no effect on FSH–induced E_2 production (Spicer et al., 1993a; Gong et al., 1994), whereas the same 24 h treatment with IGF-I enhances FSH–induced P_4 production (Spicer et al, 1993a). In contrast, 48 h treatment with 30 or 100 ng/ml of IGF-I consistently increases (by 5- to 10-fold) FSH-induced E_2 production by bovine granulosa cells (Spicer et al., 1993a; Spicer and Stewart, 1996; Spicer and Chamberlain, 1999). In bovine thecal cells, IGF-I at 100 ng/ml increases the number of LH binding sites and enhances LH-induced P_4 and androstenedione production (Stewart et al., 1995; Spicer and Chamberlain, 1999).

In cattle, follicular fluid IGF-I concentrations are correlated positively (r = 0.3 to 0.7) with follicular diameter (Spicer et al., 1988; Echternkamp et al., 1990; Spicer and Enright, 1991). Also, a decrease of plasma IGF-I concentrations results in slower growth rate of preovulatory follicles (Spicer and Enright, 1991; Lucy et al., 1992b). However, IGF-I concentrations do not differ between dominant and subordinate follicles in lactating dairy cows (Stewart et al., 1996). Dairy cows with elevated concentrations of plasma IGF-I develop ovulatory

dominant follicles during the first follicular wave postpartum (Beam and Butler, 1997). Moreover, a positive relationship exists between concentrations of E_2 and P_4 in follicular fluid and plasma IGF-I in lactating Holstein cows (Lucy et al., 1992a). Similarly, plasma E_2 and IGF-I during the first follicular wave are positively correlated ($r = 0.71$) (Beam and Butler, 1998). Consistent with the latter observations, intraovarian infusion of exogenous IGF-I increases E_2 concentrations in follicular fluid of small (1-5 mm) but not large (> 5mm) follicles (Spicer et al., 2000). Thus, in vivo evidence, like in vitro evidence, supports the notion that IGF-I regulates follicular function in cattle.

Cows in positive EB during the first 12 wk postpartum have greater concentrations of IGF-I in serum and luteal-phase P_4 secretion than cows in negative EB (Spicer et al., 1990). Correlation coefficients among weekly averages revealed positive correlations (i.e., $r = 0.31$) between serum P_4 and IGF-I (Spicer et al., 1990). As mentioned earlier, cows fed low energy–diets experience slower follicular growth and decreased plasma and follicular IGF-I concentrations compared to cows fed high-energy diet (Lucy et al., 1992a). Cows that have lower concentrations of serum IGF-I experience failure of the first wave dominant follicle to ovulate regardless of whether or not they were fed a ration supplemented with prilled lipid (7% of DM, Energy Booster–100, Milk Specialties, Co., Dundee, IL) (Beam and Butler, 1998). Thus, increased ovarian function and gonadotropin responsiveness as EB increases may be explained by increased systemic concentrations of IGF-I (Spicer et al., 1990; Nebel and McGilliard, 1993).

Insulin-like growth factor-binding proteins (IGFBPs). IGFBPs bind IGF-I and -II with high affinity, prolong the half-life of IGF-I and II, and transport IGF-I and -II to cells or tissues (Monniaux et al., 1997; Cohick, 1998; Lucy et al., 2000). Ultimately, the availability of IGF-I and -II for ovarian cell mitogenesis and steroidogenesis may be dictated by the presence of IGFBPs (Spicer and Echternkamp, 1995).

There have only been a few attempts to measure systemic IGFBPs in early lactating dairy cows. Several IGFBPs were identified in serum of lactating cows namely a 43-kDa IGFBP and 39-kDa IGFBP (IGFBP-3), a 34-kDa IGFBP (IGFBP-2), a 29-kDa IGFBP and a 24-kDa IGFBP by ligand blotting (Cohick et al., 1992). Vicini et al. (1991) reported that in early lactation, systemic IGFBP-2 levels are higher in animals that were in negative EB. Also, lactating cows deprived of feed for 2 d have increased serum IGFBP-2 (McGuire et al., 1995a). In restricted-fed postpartum beef cows with lower BCS and in anestrus, the circulating amount of IGFBP-2 at 2 d postpartum is greater while IGFBP-3 concentration is lower compared to cows that resumed estrous cyclicity (Roberts et al., 1997). Also, lactating Holstein cows under hyperinsulinemic-euglycemic clamps for 4 d have 73% lower IGFBP-2 and 73% lower 26-kDa IGFBP at the end of the clamp using western immunoblotting (McGuire et al., 1995b). Thus, IGFBP-2 may be a reflection of the energy status of early lactating cows. In contrast, Holstein heifers under negative EB for four estrous cycles exhibited higher serum IGFBP-2 and unchanged IGFBP-3 levels (Vandehaar et al., 1995).

Midlactation cows fed with restricted amounts of energy and protein (simulating an energy deficiency normally seen in early lactation) did not exhibit reduced IGFBP-2 (McGuire et al., 1992). Clearly additional work is needed to clarify how the various IGFBPs change during early lactation.

Postpartum Ovarian Follicular Changes in Early Lactating Cows

Follicular Dynamics

Follicular dynamics is defined as a continued growth and regression of antral follicles that will eventually lead to development of a preovulatory follicle (Lucy et al., 1992b). During a typical estrous cycle in cows, two or three waves of follicular growth and development occurs, wherein the preovulatory follicle is derived from the last wave (Spicer and Echternkamp, 1986; Lucy et al., 1992b; Ginther et al., 1996; Roche et al., 2000). The three distinct processes that occur during a follicular wave are recruitment, selection and dominance as defined by Hodgen (1982). Recruitment is the process whereby a cohort of follicles from the pool of antral follicles (4 mm) begins to grow for about 2 to 4 d due to increased FSH stimulation (Hodgen, 1982; Walters and Schallenberger, 1984; Lucy et al., 1992b). FSH functions include cAMP production, cell proliferation, antrum formation, aromatase activity and production of E_2 (Webb et al., 1999).

Selection is the process whereby a single follicle emerges from the pool of recruited follicles and achieves competence for ovulation (Hodgen, 1982; Lucy et

al., 1992b). Selection of the dominant follicle takes about 36 to 48 h following initiation of follicular wave (Ginther et al., 1996), and is associated with increased concentrations of E_2 and decreased concentrations of the low molecular weight IGFBPs (i.e., IGFBP-2, -4 and -5) (Stewart et al., 1996; Mihm et al., 2000).

Dominance is associated with increased levels of both LH receptor and 3β-hydroxysteroid dehydrogenase mRNA and protein in granulosa cells and increased levels of LH receptor mRNA and protein in the theca interna cells of the dominant follicles (Stewart et al., 1996; Bao et al., 1997; Bao and Garverick, 1998).

Dominance, as detected by ultrasonography, is the phase when the selected follicle (> 10 mm) inhibits the recruitment of a new group of follicles as evidenced by the absence of growing follicles > 5 mm (Fortune et al., 1991; Lucy et al., 1992; Roche et al., 2000). It takes 5 to 7 d for the dominant follicle to develop to the ovulatory size of about > 20 mm (Fortune, 1994; Ginther et al., 1996). Increased LH pulsatility induced by low luteal P_4 secretion maintains the growth of the dominant follicle (Sirois and Fortune, 1990, Savio et al., 1993) with basal FSH to maintain the dominant follicle (Fortune, 1994; Ginther et al., 1998). Reduction of pituitary secretion of FSH is a result of negative feedback of E_2 and (or) inhibin produced by the ovulatory follicle (Webb and Morris, 1988; Staigmiller et al., 1992; Roche et al., 2000) and the support of FSH to subordinate follicles is lessened. At this time, dependence of ovulatory follicles on FSH is transferred to LH (Cambell et al., 1995; Webb et al., 1999). Thus, in cattle, there is a requirement for FSH for growth between approximately 4 to 9 mm in diameter

whereas LH pulses are required for follicle development beyond 9 mm in diameter (Webb et al., 1999).

During the estrous cycle, as explained above, the first wave dominant follicle regresses and the development of a second wave dominant follicle occurs (Ginther et al., 1989). If the second wave dominant follicle becomes atretic, then a third wave dominant follicle will develop and will eventually ovulate (Taylor and Rajamahendran, 1991).

Postpartum Ovarian Changes

The patterns of follicular growth in the postpartum period are not consistent or predictable (Savio et al., 1990). Lactation and negative EB during the early postpartum may contribute to the deviations from the normal patterns of follicular dynamics (Lucy et al., 1992a). During the postpartum period, nutrient partitioning may change reproductive processes such as absence of follicular growth (inactive ovaries) and development of follicular and luteal cysts, which are abnormally large follicles (≥ 25 mm) (Marion and Gier, 1968; Stevenson and Britt, 1979; Spicer and Echternkamp, 1986; Lucy et al., 1992a). The inadequate energy intake during the early postpartum period, leads to insufficient LH release, causing the ovary to fail to undergo normal follicular growth (discussed earlier under LH) (Imakawa et al., 1986; Lucy et al., 1991b). Furthermore, negative EB manifests itself in delayed ovarian activity by impinging on pulsatile LH release (Butler and Smith, 1989).

First wave dominant follicles are bigger and plasma E_2 concentrations are lower in lactating cows than in non-lactating cows (Lucy et al., 1992b). Also, low levels of plasma P_4 delay the normal timing of ovulation following luteal regression and contribute to formation of follicular cysts due to absence of atresia of large follicles (Lucy et al., 1992a). Due to decreased E_2 secretion of preovulatory follicles in lactating cattle, there is a reduction in the intensity of estrus expression and (or) insufficient LH release before ovulation (Lucy et al., 1992a). This event may be a consequence of negative EB in dairy cows during early lactation that consequently leads to decreased LH secretion and a delay in return to estrus as reviewed by Butler and Smith (1989). The diameter of the dominant follicle increases when cows are in positive EB (Lucy et al., 1991a). Cows in negative EB balance have preovulatory follicles that grow more slowly compared to the growth rate in cows in positive EB cows (Lucy et al., 1990). Fewer small follicles (3 to 5 mm) and more large follicles (6 to 9 mm or > 15 mm) are observed in cows fed protected fat (Lucy et al., 1991b; 1992a). But cows (n = 54) supplemented with 0.55 kg/d of calcium salts of long chain fatty acids (Koffolk Inc., Petach Tikva, Israel) have lower percentage (40%) of E_2 active follicles than control cows (67%) between 60 to 90 d postpartum (Moallem et al., 1999).

First Postpartum Ovulation

The first postpartum ovulation is an indicator of the resumption and completion of ovarian follicular development and recovery of hormonal conditions

in lactating cows (Butler and Smith, 1989; Butler, 2000). The interval to first ovulation postpartum ranges from 17 to 42 d (for review see Butler and Smith, 1989). Also, duration of the first postpartum estrous cycle ranges from 6 to 28 d (Butler et al., 1981; Fonseca et al., 1983). Length of the ovarian cycles is normal (i.e.,18 to 24 d) if the ovulatory dominant follicles are identified before d 10 postpartum (Savio et al., 1990). Otherwise, only 40% of dairy cows exhibit a normal length first estrous cycle (Staples et al., 1990). First postpartum ovulations are associated with high frequency of silent estruses (i.e., 40 – 80%) in lactating dairy cows (Whitmore et al., 1974; Savio et al., 1990; Spicer et al., 1990; Staples et al., 1990).

Occurrence of first postpartum ovulation is influenced by EB. Averaged over the first 3 to 4 wk of lactation, EB is significantly correlated to days to first ovulation (Butler et al., 1981; Spicer et al., 1993). On the average, first ovulation occurs 25 d before zero EB is attained (Berghorn et al., 1988), or approximately 10 d after maximal negative EB (Butler et al., 1981). In contrast, a study in which cows were grouped based on average EB during their first 12 wk of lactation, the "positive" EB cows were in negative EB for only the first 2 wk postpartum and "negative" EB cows that were in negative EB for the first 7 wk postpartum, ovulated at the same time (Spicer et al., 1990).

Adding fat in the diet of postpartum dairy cows did not affect interval to uterine involution, interval to first, second or third ovulation and estrus, conception rate at first service, services per conception, or days open (Carroll et al., 1990; Spicer et al., 1993). Furthermore, pregnancy rates (77%) were similar

between control cows and cows fed calcium soaps of fatty acids (Koffolk Inc., Petach Tikva, Israel) at 2.2% DMI (Moallem et al., 1997). Similarly, conception rates (56 vs. 43%) were not different between cows fed (0 vs. 5%) prilled fat (Carroll et al., 1990). However, first service conception rates are higher in Holstein cows fed prilled fat at 2% of DMI (BP Nutrition, Ltd., Wincham, Norwich, Chesire, United Kingdom) than control cows in 150-d herd trials (Ferguson et al., 1990). As mentioned earlier, type of fat supplemented or duration of supplementation (see fat supplementation section) may explain why reproductive responses to adding fat to the diet have been variable.

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CHAPTER III

Direct Fed Propionibacteria: Effects on Energy Balance, Plasma Hormone Concentrations, and Reproduction in Early Postpartum Dairy Cows*

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ABSTRACT

The objective of this study was to determine the effects of direct-fed bacteria (Propionibacteria) on energy balance, milk yield and composition, metabolites and hormones of early lactating dairy cows. Nineteen pluriparous Holstein cows were individually fed a total mixed ration from -2 to 12 wk postpartum. Each treated cow (n=10) received 17 g of Propionibacteria culture daily. Daily feed intake and milk production and weekly body weight were recorded. Blood samples were collected twice a week for measurement of plasma cholesterol, non-esterified fatty acids (NEFA), leptin, insulin,

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glucose, insulin-like growth factor-I (IGF-I), insulin-like growth factor binding proteins (IGFBPs), and progesterone (P_4) concentrations. Cows supplemented with Propionibacteria culture improved energy balance (EB) only at first week of lactation but decreased EB at wk 7 and 10. Treatment did not affect milk production and dry matter intake during the 12-wk study, but DMI on a per kg BW basis was lower in treated cows. Cows fed Propionibacteria had greater percentages of milk protein and solids-not-fat (SNF) than control cows during the first week of lactation, but not percentages of milk fat and lactose. Plasma glucose, insulin, cholesterol, and IGF-I concentrations were not significantly affected by supplemental feeding of Propionibacteria culture, but these variables increased with week postpartum. Plasma IGFBP-2 and IGFBP-5 decreased while IGFBP-3 increased with week postpartum. Plasma NEFA concentrations at wk 1 were significantly lower in control than treated cows but not thereafter. Leptin was significantly higher in treated cows than control cows throughout the study. Peak luteal phase concentrations of P_4 and area under the P_4 curve during first and second postpartum estrous cycle were similar between the two groups of cows. Interval to first and second postpartum ovulations did not differ between treated and control cows. Average diameter of largest (F1), second largest (F2), third largest (F3) follicles, and number of small (3 to 5 mm), medium (6 to 9 mm) and large ($\geq$ 10 mm) follicles did not vary between Propionibacteria-treated and control cows, but diameter of F3 follicles increased with week postpartum. Feeding Propionibacteria culture to early lactating dairy cows

improved some production and metabolic variables during the first week of lactation, but did not improve reproductive function.

INTRODUCTION

Many high-producing dairy cows are unable to consume enough feed to meet energy demands during early lactation, resulting in a state of negative energy balance. Energy balance (EB) is quantified using measures of dry matter intake (DMI), milk production (quantity and composition) and body weight (BW), and may be associated with reproductive efficiency. In lactating dairy cows, EB during the first few weeks postpartum is positively related to concentrations of plasma progesterone (P_4) during the first postpartum estrous cycle (Berghorn et al., 1988; Villa-Godoy et al., 1988; Spicer et al., 1990). Cows exhibiting estrus with subsequent formation of a functional corpus luteum that secretes greater P_4 levels have the best chance of maintaining pregnancy (Villa-Godoy et al., 1988). In addition, cows that express estrus before first postpartum ovulation have greater EB than cows that do not express estrus (Berghorn et al., 1988; Spicer et al., 1990). Negative EB is therefore a likely cause for poor reproductive efficiency in lactating dairy cows (Kimura et al., 1987; Sklan et al., 1991). Because plasma cholesterol (Carroll et al., 1990; Spicer et al., 1993b), insulin (Koprowski and Tucker; 1973; Smith et al., 1976) and insulin-like growth factor-I (IGF-I) (Spicer et al., 1990, 1993b) increase, whereas plasma non-esterified fatty acids (NEFA) decrease (Staples et al., 1990; Canfield and Butler, 1991; Beam

and Butler, 1998) with increasing week of lactation, these hormones and metabolites are primary candidates for transmitting the metabolic status of a cow to its reproductive axis. Indeed, concentrations of cholesterol and IGF-I in blood of cattle are modified by variations in fat, protein and (or) energy intake, and increase as EB increases (Kronfeld et al., 1980; Grummer and Davis, 1984; Ronge et al., 1988; Spicer et al., 1990; 1993b). Moreover, insulin and IGF-I stimulate mitogenesis and steroidogenesis of bovine ovarian cells in vitro (Schams et al., 1988; McArdle et al., 1989; McArdle et al., 1991; Saumande et al., 1991; Spicer et al., 1993a; Gong et al., 1994; Spicer and Chamberlain, 1998), and thus, negative EB may affect ovarian activity by decreasing luteal P_4 production (Hawkins et al., 1985; Talavera et al., 1985; Grummer and Carroll, 1988; Spicer et al., 1993b). Recent studies also implicate leptin as a possible metabolic mediator of reproduction by inhibiting bovine granulosa and theca cells steroidogenesis (Spicer and Francisco, 1997, 1998).

Propionate, a ruminal volatile fatty acid, acts as a precursor for hepatic glucose production. Propionate infusion at 200 g in the abomasum of heifers for 21 d increases glucose concentration compared to control animals (Rutter et al., 1983). Drenching rations of lactating cows with calcium propionate elevates plasma glucose concentration (Jonsson et al., 1998). Cows fed monensin in the form of controlled-release capsule (335 mg/d) during the first week of lactation had higher glucose concentration than control cows (Abe et al., 1994). Similarly, feeding monensin at 300 mg/d during the first 2 mo of lactation increased plasma glucose (Phipps et al., 2000). Conversely, preventing reabsorption of glucose in

renal tubules decreases plasma glucose and insulin levels in dairy cows (Amaral-Phillips et al., 1993). Also, infusion of butyrate, a ruminal volatile fatty acid that inhibits the use of propionate for gluconeogenesis into the rumen of lactating cows, decreases plasma glucose concentrations (Huhtanen et al., 1993). Whether plasma insulin, IGF-I, cholesterol, and other metabolites are altered by changes in ruminal propionate is unknown.

Propionibacteria are natural inhabitants of the rumen that compose 1.4 % of the ruminal microflora and produce propionic acid in the rumen (Oshio et al., 1987). Directly feeding Propionibacteria may increase hepatic glucose production via increased ruminal propionate production and absorption. Theoretically, the efficiency of propionate as a source of energy in the form of ATP is 109% compared to glucose (McDonald et al., 1987). The efficiency of utilization for maintenance of propionic acid is 0.86 vs. 0.59 for acetate and 0.76 for butyrate (McDonald et al., 1987). Thus, the present study was conducted to test the hypothesis that manipulating rumen microflora by supplementing diet with direct-fed microbial, such as Propionibacteria culture, will increase plasma concentration of glucose, IGF-I, insulin and (or) other metabolic modulators of reproductive function. These changes would then increase plasma P_4 concentrations and shorten days to first postpartum estrous cycle.

MATERIALS AND METHODS

Experimental Design and Sample Collection

Twenty Holstein pluriparous cows were assigned randomly to one of two dietary groups: total mixed ration (TMR) without Propionibacteria (control, n = 10) or TMR plus Propionibacteria (Treated, n = 10) from –2 wk to 12 wk postpartum. Cows calved between November 26, 1997 and March 26, 1998. Cows were allowed free access to feed and water. Each treated cow received 17 g of Propionibacteria culture (AgTech Inc., Waukesha, WI) daily, top-dressed into 1 to 2 kg of the TMR. Cows were individually fed and housed in a stanchion barn and grouped by treatment to prevent potential transfer of Propionibacteria from treated to control cows. Half of each group of cows was placed across one another and separated from each other by two unoccupied stalls. Each day, control and treated cows were separated and each group was placed in one of the two adjacent dry lots for two 4- to 5-h intervals (900 to 1200 and 2100 to 300). One Propionibacteria-treated cow was taken out of the study due to a foot problem. Weekly BW were recorded and body condition (BCS) of the cows were evaluated at wk 4 and 10 postpartum using a five point scale: 1 = very thin to 5 = excessively fat (Sniffen and Ferguson, 1995).

The TMR was composed of sorghum\sudan silage, alfalfa hay, whole cottonseed, and concentrate (Table1). Energy concentration of the diet was

formulated to support daily milk production of 50 kg (NRC, 1989). Cows were fed from 1300 to 500 and 800 and 2000 daily. Daily feed intake was recorded and the diet was sampled weekly and composited monthly for analysis.

Cows were milked twice daily (0300 and 1500 h) and milk yield was recorded. Milk samples were collected weekly during successive a.m. and p.m. milkings and analyzed for percentage of milk fat, protein, lactose, and solids-not-fat (SNF), somatic cell count (SCC) and milk urea nitrogen (MUN). The average days for the first week postpartum milk collection was 4.9 ± 1.7 d for control cows and 4.7 ± 1.9 d for treated cows. Milk production was corrected for percentage milk fat and identified as fat corrected milk (FCM).

Blood samples (10 ml) were collected at 0830 h, twice weekly at 3 to 4 d intervals via coccygeal venipuncture. After collection in tubes containing EDTA, blood was centrifuged at 1200 x g for 20 min (5°C) and plasma was decanted and stored frozen at –20°C for subsequent analysis. The average days for the first week postpartum blood collection was 5 ± 2 d for control cows and 4 ± 2 d for treated cows.

The ovaries of the cows were scanned weekly by transrectal ultrasonography using a linear array ultrasound scanner equipped with 7.5 MHz rectal probe (Corometrics Medical Systems, Inc., Wallingford, CT) starting at 3 wk and continuing through 12 wk postpartum to measure follicles and determine presence of corpus luteum. Follicles on the right and left side of the ovaries were measured and categorized as the largest follicle (F1), the second largest (F2) and the third largest follicle (F3) during 3 to 12 wk postpartum. Furthermore,

follicles were categorized as small (3-5 mm), medium (6-9 mm) and large (≥ 10 mm).

Assays

IGF-I concentrations in plasma were determined in all samples by radioimmunoassay (RIA) after acid-ethanol extraction (16 h at 4°C) as described previously (Echternkamp et al., 1990). Intraassay and interassay coefficients of variation were 13% and 15%, respectively.

Plasma P_4 concentrations were determined in all samples using a solid phase RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) without extraction as previously described (Stewart et al., 1996). Intraassay and interassay coefficients of variations were 8.7% and 8.6%, respectively.

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

Plasma concentrations of glucose were determined in all samples using Glucose kits (Roche Diagnostic Systems, Inc., NJ) and a clinical analyzer (Cobas FARA II, Roche Analytical Instrument, Montclaire, NJ). This procedure was based on the enzymatic reaction of hexokinase coupled with glucose-6-phosphate dehydrogenase. The intraassay coefficient of variation was 2.3 %.

Concentrations of total plasma cholesterol were determined in all samples using an enzymatic method using a total cholesterol kit reagent (Sigma, St. Louis, MO) as previously described (Williams, 1989; Spicer et al., 1993b). Enzymatic reagents contain cholesterol esterase, cholesterol oxidase and peroxidase to yield the final product, quinoneimine. Standard curves were constructed between 0 and 400 mg/dl. Intraassay and interassay coefficients of variation were 2.2 % and 1.4 %, respectively.

NEFA concentrations were determined in plasma samples collected on wk 1, 6 and 12 postpartum by an enzymatic method using NEFA-C kits (Waco Chemicals USA, Inc., VA) and a clinical analyzer (Cobas FARA II, Roche Analytical Instrument, Montclaire, NJ). This enzymatic method utilizes acyl-CoA synthethase and acyl-CoA oxidase to produce 3-methyl-N-ethyl-N- (B-hydroxyethyl)-aniline (MEHA). The intraassay coefficient of variation was 4.5 %.

Concentrations of leptin were measured in plasma samples collected on wk 2 to 12 postpartum using a Multi-species RIA kit (LINCO Research, Inc., St. Charles, MO) according to the manufacturer's recommendations with minor modifications (Maciel et al., 2001). Briefly, on day 1, 100 µl of first antibody were added to all tubes, except total count (TC) and non-specific binding (NSB) tubes. Then tubes were vortexed, covered and incubated for 24 h at 4°C. The standard curve was modified to include 1, 2, 3, 5, 10 and 20 ng/ml of human leptin standard. On the second day, 100 µl of the tracer (^{125}I-human leptin) was added to all tubes then incubated for another 24 h at 4°C. On the third day, 1.0 ml of precipitating reagent was added to all tubes except TC tubes and incubated for

20 min at 4°C. Tubes were centrifuged at 3,000 x g for 30 min, the supernatant was decanted and the precipitate was counted using a Gamma Counter. The sensitivity of the assay as defined as 95% of total binding was 0.85 ± 0.08 ng/ml.

Ligand Blots

Plasma samples of the cows collected on wk 1, 6 and 12 postpartum were analyzed for insulin-like growth factor binding proteins (IGFBPs) using one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Echtenkamp et al., 1994; Stewart et al., 1996). Briefly, 4 μl of each sample was mixed with 21 μl of non-reducing denaturation buffer (62.5 mM Tris-HCL, 2% SDS, 25% glycerol and 0.01 % bromphenol blue without mercaptoethanol) (BIORAD, Hercules, CA). Proteins were denatured at 100°C for 3 min and centrifuged at 4700 x g for 3 min and loaded to wells of 12 % polyacrylamide gels. Control lanes were added with 25 μl wide range color marker (MW 6,500 - 205,000, Sigma, St. Louis, MO) and a mixture of 4 μl bovine follicular fluid and 21 μl of the sample buffer. Gels were run for 18 to 20 h and bands were transferred using nitrocellulose papers (Midwest Scientific, St. Louis, MO) for 2.5 to 3.0 h and hybridized with ^{125}I-IGF-II (about 15,000 cpm/0.1 ml; total volume = 6 ml) at 4°C for 12 h in a platform shaker. Nitrocellulose blots were first washed with Tris-buffered saline (TBS) and 0.1% Tween, and then washed again with TBS alone. Nitrocellulose was dried and placed on X-ray film

for 14 d at –80°C. X-ray films were developed and bands measured using a densitometer (Molecular Analyst, BIORAD, Hercules, CA).

EB Calculations

EB was calculated by using net energy intake as the average daily DMI multiplied by the net energy concentration of the diet. Net energy required for daily maintenance of the animals was derived using the equation 80 x $BW^{0.75}$ (kg) /1000 (NRC, 1989). Daily energy for milk production was calculated using the formula (Tyrell and Reid, 1965), milk yield (kg) x [92.239857(% milk fat) + 49.140211(% SNF) – 56.393297]/1000 where milk yield is the average daily yield for the week, and milk composition based on weekly milk analysis. This equation reflects the metabolic status of the cow more accurately than the conventional method of measuring milk yield alone (Butler and Smith, 1989).

Maximum Progesterone and Area under Progesterone Curve Calculations

Days of first and second ovulations were defined as the days of first and second rise in plasma $P_4 \geq 1.0$ ng/ml that were maintained for two or more additional sampling days. Based on these criteria, 79.8 % of the cows (8 of 10 control and 7 of 9 treated cows) had a "first ovulation" and 57.8 % of the cows (7 of 10 control and 4 of 9 treated cows) had a "second ovulation" during the 12-wk study. Data for the third postpartum ovulation were not included in the analysis

because of the small number of cows that exhibited a third estrous cycle within 90 d postpartum (4 of 10 control and 2 of 9 treated cows).

The maximum P_4 concentrations and area under P_4 curve were used to evaluate luteal activity of the early postpartum cows. Peak P_4 concentration was defined as the maximum concentration of P_4 achieved during diestrus of the first and second postpartum estrous cycles of the cows. Multiple regression analysis was utilized to fit a curve through the initial and final nadirs of each estrous cycle, and an integral of the equation generated the P_4 area under curve.

Statistical Analysis

Milk production and composition, EB, BW, BCS, DMI, plasma hormones, metabolites and IGFBPs and class and size of follicles were analyzed as a completely randomized design for repeated measures, utilizing the mixed model (Littell et al., 1996): $Yijk = U + Di + C(D)ij + Wk + (D \times W)ik + eijk$ where U = overall mean, D = diet, C(D) = cow within group, W = week postpartum, (D x W)ik = diet by week post partum interactions, and e = residual error. Similarly, area of P_4 under the curve, maximum P_4 and interval to first and second postpartum ovulations were analyzed using the same procedure and model but cycle number was used instead of week. The model of the covariate structure for repeated measurements was an autoregressive with lag equal to one (Littell et al., 1996). If the week by diet interaction was significant, simple effects of diet were analyzed using the slice option for the LSMEANS statement. Conversely, main

effects were analyzed using LSMEANS with the DIFF option if the interaction was not significant. Relationships between EB, FCM and milk composition, plasma metabolites and hormones were calculated using Pearson correlation coefficients.

RESULTS

Energy Balance, Body Weight and Body Condition Score

EB was not influenced ($P > 0.10$) by the interaction of treatment x week postpartum, but EB was affected ($P < 0.001$) by week postpartum and treatment ($P < 0.10$) (Figure 1). Generally, both groups of cows had a positive EB starting at 8 wk of lactation. Specifically, postpartum weeks 1, 3 and 6 differed ($P < 0.05$) from their succeeding week by -3.1, -2.5, and -2.27 Mcal/d, respectively (Figure 1). Average EB of postpartum cows tended to differ ($P < 0.10$) between cows fed with Propionibacteria (-1.596 ± 0.72 Mcal/d) and control cows (0.196 ± 0.69 Mcal/d) during the 12-wk feeding period, particularly on wk 1, 7 and 10 (Figure 1).

BW was not affected ($P > 0.50$) by the interaction of treatment x week postpartum. During the first 12 wk of lactation, average BW of postpartum cows tended to differ ($P < 0.10$) between Propionibacteria-treated (667.1 kg ± 19 kg) and control (616.2 ± 18 kg) cows. Also, weekly BW differed ($P < 0.001$) among

weeks postpartum. In both groups of cows, BW decreased between wk 1 and wk 3, but did not change between wk 5 and wk 12 of lactation (data not shown).

The interaction ($P > 0.50$) between treatment x week postpartum did not affect average BCS. Also, treatment had no effect ($P > 0.50$) on BCS, measured at wk 4 and 10 postpartum. The BCS ranged from 2.5 to 3.75 and averaged 2.69 ± 0.08 for control cows and 2.68 ± 0.06 for Propionibacteria-treated cows. Average weekly BCS increased significantly ($P < 0.01$) from wk 4 to wk 10 (2.53 ± 0.07 vs. 2.86 ± 0.06) in both groups of cows.

Dry Matter Intake and Fat Corrected Milk

The interaction of treatment and week postpartum did not affect ($P > 0.50$) DMI or FCM. Also, DMI did not differ ($P > 0.10$) between Propionibacteria-treated and control cows. Averaged over the 12-wk period, control and Propionibacteria-treated cows consumed 23.97 ± 0.48 and 23.37 ± 0.50 kg/d, respectively. DMI consistently increased ($P < 0.05$) from wk 1 to wk 12 of lactation (Figure 2). The DMI intakes at wk 1, 2, 3, 4, and 7 postpartum were less ($P < 0.01$) than their succeeding week (Figure 2). However, DMI expressed as g per kg BW was lower ($P < 0.01$) in Propionibacteria-treated (35.3 ± 0.8 g/kg) than control (38.9 ± 0.9 g/kg) cows starting from wk 3 to 12 postpartum.

Daily milk yield (DMY) and FCM production did not differ ($P > 0.50$) between the control and treated cows over the 12-wk study. DMY and FCM averaged 40.9 ±1.4 and 34.49 ± 0.86 kg/d for control cows and 39.9 ±1.4 and

35.16 ± 0.89 kg/d for Propionibacteria-treated cows, respectively. There were weekly changes ($P < 0.001$) in FCM production of the cows (Figure 2). Between wk 1 and 3 of lactation, FCM production increased ($P < 0.05$) from 29 ± 1.3 to 36 ±1.3 kg/d, whereas between wk 9 and 11, FCM production decreased from 36.6 ± 1.3 to 31.9 ±1.3 kg/d. Postpartum changes in DMY were similar to changes in FCM production (data not shown).

Milk Protein and Solids-Not-Fat

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

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

Milk Fat and Lactose

There was no interaction ($P > 0.50$) between treatment and week postpartum on percentages of milk fat and lactose. The average milk fat percentage did not differ ($P > 0.10$) between Propionibacteria-treated ($3.2 \pm 0.08\%$) and control ($3.02 \pm 0.08\%$) cows. There was a significant ($P < 0.001$) week postpartum effect on milk fat percentage, which generally decreased as week postpartum progressed. Specifically, milk fat percentage on wk 5, 6 and 10 differed ($P < 0.05$) from their succeeding week (Figure 5).

There was no treatment effect ($P > 0.10$) nor interaction ($P > 0.50$) between treatment and week postpartum on the percentage of lactose in milk. Percentage of lactose averaged $5.0 \pm 0.02\%$ and $4.98 \pm 0.02\%$ in Propionibacteria- treated and control cows, respectively over the 12- wk period and did not differ ($P > 0.10$) between the two groups of cows. However, week affected ($P < 0.001$) percentage of lactose in milk such that percentage of lactose in milk increased from wk 1 to 2, maintained until wk 8, and decreased thereafter (Figure 5).

Milk Urea Nitrogen

There was no treatment effect ($P > 0.50$) nor interaction ($P > 0.50$) between treatment and week postpartum on milk urea nitrogen (MUN). Concentrations of MUN averaged 20.15 ± 0.42 and 20.26 ± 0.66 mg/dl in control

and Propionibacteria-treated cows, respectively and did not differ ($P > 0.50$). However, MUN concentrations changed ($P < 0.001$) with week postpartum. There was a 25% increase ($P < 0.001$) in MUN levels between wk 2 and 3 postpartum from (Figure 6). After wk 3, MUN levels did not change (Figure 6).

Glucose and Insulin

Neither plasma glucose ($P > 0.10$) nor plasma insulin ($P > 0.50$) concentrations were affected by the interaction of treatment x week postpartum. Cows fed Propionibacteria had an average plasma glucose concentration of 60.00 ± 0.91 mg/dl compared to that of control cows (60.02 ± 0.88 mg/dl; $P > 0.50$). Concentrations of glucose in plasma increased ($P < 0.01$) with wk postpartum such that glucose concentration at wk 2 was 4.5% greater ($P < 0.02$) than wk 1 (Figure 7). Plasma glucose levels did not significantly change after wk 2 postpartum.

Average concentration of insulin in plasma of cows fed Propionibacteria was similar ($P > 0.10$) to that for control cows (0.39 ± 0.02 vs. 0.42 ± 0.02 ng/ml). Concentrations of insulin in plasma increased ($P < 0.001$) with week postpartum such that insulin concentrations at wk 2 differed from wk 1 ($P < 0.10$) (Figure 7). Plasma insulin concentrations increased gradually thereafter such that over the 12-wk period, plasma insulin increased twofold (0.25 ± 0.03 ng/ml in wk 1 to 0.51 ± 0.03 ng/ml in wk 10) (Figure 7).

Cholesterol

Serum cholesterol was not affected ($P > 0.50$) by treatment or by the interaction of treatment x week postpartum. Plasma cholesterol concentrations averaged 179.7 ± 7.1 mg/dl in Propionibacteria-treated and 178.5 ± 6.9 mg/dl in control cows. However, plasma cholesterol concentrations increased ($P < 0.001$) with week postpartum (Figure 8). Cholesterol levels increased over twofold between wk 1 and 6 ($P < 0.01$) and plateaued during the remaining weeks of the study (Figure 8).

Non-Esterified Fatty Acids and Leptin

There was a significant ($P < 0.01$) treatment x week postpartum interaction on plasma NEFA concentrations. Plasma NEFA concentrations in Propionibacteria-treated cows at wk 1 postpartum were greater ($P < 0.01$) than in control cows (Figure 9). Plasma NEFA concentrations decreased ($P < 0.001$) with week postpartum for both groups of cows, although the decrease was more rapid and dramatic in Propionibacteria-treated than control cows (Figure 9).

Treatment x week interaction ($P > 0.50$) and week postpartum ($P > 0.50$) did not affect plasma leptin concentrations (Figure 10). However, plasma leptin concentrations tended to differ ($P < 0.10$) in Propionibacteria-treated cows compared to control cows over the 12-wk study and averaged 8.10 ± 1.0 ng/ml and 5.25 ± 1.0 ng/ml in treated and control cows, respectively (Figure 10).

Insulin-like Growth Factor -I

There was no treatment ($P > 0.50$) or treatment x week postpartum interaction ($P < 0.50$) on plasma IGF-I concentrations, which averaged 11.15 ± 1.4 ng/ml for control cows and 10.16 ± 1.45 ng/ml for Propionibacteria-treated for cows. However, IGF-I concentrations increased ($P < 0.001$) with week postpartum (Figure 11). Weeks 1, 4 and 11 were significantly different with their succeeding weeks ($P < 0.05$). Concentrations of IGF-I increased nearly 4-fold between wk 1 and wk 12 of lactation (Figure 11).

Insulin-like Growth Factor Binding Proteins

Plasma IGFBP-3 (40-44 kDA), IGFBP-2 (32 kDA), IGFBP-5 (30 kDA), a 28-kDa IGFBP, a 26-kDa IGFBP, and IGFBP-4 (22-kDa) concentrations were not influenced ($P > 0.50$) by the interaction of treatment x week postpartum or by treatment ($P > 0.50$). Concentrations of plasma IGFBP-3, IGFBP-2 and IGFBP-5 changed ($P < 0.05$) with week postpartum. Plasma IGFBP-3 concentration was 15 to 16% lower ($P < 0.001$) at wk 1 than wk 6 and 12 whereas both plasma IGFBP-2 and IGFBP-5 were 19 to 33% greater ($P < 0.001$) at wk 1 than wk 6 and 12 (Figure 12). Plasma concentrations of the 28-kDa IGFBP, 26-kDa IGFBP and IGFBP-4 did not vary with week postpartum ($P > 0.50$) and averaged 1.09 ± 0.1, 0.95 ± 0.1 and 1.16 ± 0.1 arbitrary densitometric units (ADU) /4 µl for

Propionibacteria-treated cows and 1.24 ± 0.1, 1.1 ± 0.1 and 1.23 ± 0.1 ADU/4 µl for control cows.

Peak Progesterone and Area under the Progesterone Curve

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

There was no treatment x postpartum estrous cycle interaction ($P > 0.10$) on peak P_4 concentrations. Peak P_4 did not differ ($P > 0.10$) between control (4.0 ± 0.2 ng/ml) and Propionibacteria-treated (3.5 ± 0.3 ng/ml) cows. Also, peak P_4 in the first (3.8 ± 0.2 ng/ml) and second (3.7 ± 0.3 ng/ml) postpartum estrous cycle did not differ ($P > 0.50$).

Postpartum Interval to Ovulation

Interaction of treatment and postpartum estrous cycle did not influence ($P > 0.10$) average days to ovulation. Average days to first and second ovulations (i.e., first and second rise in plasma $P_4 \geq 1.0$ ng/ml) did not differ ($P > 0.10$)

between control (30.1 ± 7.4 and 57.7 ± 7.4 d) and Propionibacteria-treated (44.8 ± 7.7 and 68.11 ± 8.4 d) cows. Interval to first and second ovulation averaged 37.4 ± 5.3 d and 62.9 ± 5.6 d, respectively. Average days to first postpartum ovulation were not significantly correlated with average EB during wk 1 ($r = 0.40$, $P > 0.10$), wk 1-3 ($r = 0.18$, $P > 0.50$) or wk 1-12 ($r = -0.09$, $P > 0.50$).

Postpartum Follicular Class and Numbers

Average diameters of F1, F2 and F3 follicles were not influenced ($P > 0.50$) by the interaction of treatment x week postpartum or by treatment ($P > 0.50$). Diameter of F1 and F2 follicles did not change ($P > 0.10$) with week postpartum whereas diameter of F3 follicles tended to increase ($P < 0.10$) with week postpartum (Figure 13). Diameters of F3 follicles on wk 3, 4 and 5 were less ($P < 0.05$) than on wk 7, 8, 9, 10, and 11 postpartum (Figure 13).

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

Correlations

Simple correlation coefficients among weekly averages (n = 228) of EB, FCM, DMI, milk fat and protein, plasma hormones and metabolites are shown in Table 2. EB was positively correlated ($r = 0.68$, $P < .001$) with DMI but negatively correlated with FCM ($r = -0.38$, $P < 0.001$), milk fat ($r = - 0.64$, $P < 0.001$), and milk protein ($r = - 0.35$, $P < 0.001$). Also, EB was positively correlated with IGF-I concentrations ($r = 0.47$, $P < 0.001$) and plasma P_4 concentrations ($r = 0.36$, $P < 0.001$). DMI was positively correlated with MUN ($r = 0.28$, $P < 0.001$), P_4 (0.40, $P < 0.001$), cholesterol ($r = 0.69$, $P < 0.001$), IGF-I ($r = 0.43$, $P < 0.001$), insulin ($r = 0.37$, $P < 0.001$) and glucose ($r = 0.45$, $P < 0.001$). Plasma IGF-I concentrations were also positively correlated with insulin ($r = 0.31$, $P < 0.001$), cholesterol ($r = 0.42$, $P < 0.001$) and P_4 ($r = 0.37$, $P < 0.001$). Plasma P_4 concentrations were positively correlated with cholesterol ($r = 0.38$, $P < 0.001$) and glucose ($r = 0.35$, $P < 0.001$) but not insulin ($r = 0.08$, $P > 0.23$). Plasma leptin concentrations were negatively correlated ($r = -0.17$, $P < 0.06$) with plasma IGF-I concentrations.

DISCUSSION

This study found that feeding Propionibacteria increased plasma NEFA and leptin concentrations, and percentages of milk protein and SNF during wk 1 postpartum. Treatment did not affect BCS, FCM, percentages of milk fat and lactose, or concentrations of plasma cholesterol, insulin, glucose and IGF-I.

Treated cows had lower DMI on a per kg BW basis. Also, Propionibacteria-treated cows did not show improvement in reproductive measures such as average days to ovulation, progesterone peak and area under the curve, and follicle size and numbers compared to control cows.

Several dietary supplements including fat (Carroll et al., 1990; Spicer et al., 1993b; Beam and Butler, 1997, 1998, 1999) and yeast (Piva et al., 1993; Robinson and Garrett, 1999) have been given to postpartum dairy cows in early lactation to increase EB, but have yielded inconsistent results. We found that EB of lactating cows improved during the first week of lactation with feeding Propionibacteria but not in the succeeding weeks of supplementation. Also, NEFA were significantly higher during wk 1 postpartum in Propionibacteria-treated cows, which may indicate that these cows mobilize more fat because of lower DMI or were in better BCS and thus had more fat to mobilize than control cows (Fronk et al., 1980; Nachtomi et al., 1986). In support of the latter suggestion, plasma leptin levels were greater in Propionibacteria-treated than control cows and lower DMI on a per kg BW basis throughout the first 12 wk postpartum. Because leptin is produced by adipocytes, and leptin concentrations rise in parallel with BCS (Ehrhardt et al, 2000; Delavaud et al., 2000), treated cows may have had higher body fat and therefore have had a higher fat mobilization capability as indicated by higher plasma NEFA in these cows. This may explain why Propionibacteria-treated cows had greater EB and percentage milk protein and SNF than their control counterparts during wk 1 of lactation (Hart et al., 1979; Bauman et al., 1988; Tyrell et al., 1988).

Both treated and control cows improved EB as week postpartum progressed, but control cows attained a better EB with week postpartum than treated cows although the difference was only significant on wk 7 and 10. Because both groups of cows had similar DMI (in kg/cow/d) and produced comparable FCM and milk components, Propionibacteria-treated cows in the succeeding weeks postpartum probably were not able to compensate for higher maintenance requirements due to having greater BW than control cows. But, DMI on a per kg BW basis was lower in treated cows. Several works have established that DMI and FCM affects EB, and the results in this study concur with other studies that report positive correlations between DMI and EB ($r = 0.68$) (Coppock et al., 1974; De Kruif and Mitjen, 1992) and negative correlations between FCM and EB ($r = -0.38$) (Butler et al., 1981; Villa-Godoy et al., 1988; Spicer et al., 1990; 1993b). Interestingly, DMI expressed on a per kg BW basis revealed that Propionibacteria-treated cows had lower intakes than controls. Previous studies have implicated leptin as modulator of feed intake (Houseknect et al., 1997). Whether the greater levels of leptin in treated cows is influencing DMI will require further study.

Although no previous study has evaluated the effect of supplemental Propionibacteria on DMI and milk yield, some studies in which cows were fed supplemental probiotic yeast have found improvement in DMI, FCM and milk composition (Williams et al., 1991; Adams et al., 1995; Wohlt et al., 1998; Dann et al., 2000) whereas other studies have not (Arambel and Kent, 1990; Piva et al., 1993; Swartz et al., 1994; Robinson, 1997; Soder and Holden, 1999). In the

present study, DMI and FCM were not different between control and Propionibacteria-treated cows but improved with week postpartum. Reasons for differences among studies mentioned are uncertain but may be attributed to the timing (stage of lactation) and duration of probiotic supplementation.

Percentages of milk protein and SNF were higher in cows fed Propionibacteria vs. control cows on wk 1 of lactation whereas percentages of milk fat and lactose did not differ between treatments. Similarly others have found that infusion of propionate aimed to increase glucose in lactating cows resulted in higher percentage milk protein (Huhtanen et al., 1993; Hurtaud et al., 1998). Higher percentages of milk protein and SNF in treated cows during the 1 wk postpartum is likely due in part to their higher plasma NEFA concentrations, because several studies showed that NEFA during early postpartum is primarily used for milk synthesis (Hart et al., 1979; Bauman et al., 1988; Tyrell et al., 1988). Also, higher NEFA levels during wk 1 postpartum in treated cows may be attributed to better BCS (as explained above) and better EB during wk 1 postpartum than control cows. Given that fat mobilization at early lactation is a mechanism for dairy cows to cope with an energy deficiency due to higher metabolic demands of lactation (Coppock et al., 1974; Sejrsen and Neimann-Sorensen, 1982; De Kruif and Mitjen, 1992), the greater plasma NEFA concentrations at early lactation compared to the succeeding weeks may indicate that dietary intake of both groups of cows was not sufficiently meeting their energy requirement. In this study, percentage milk fat and lactose did not differ between Propionibacteria-treated and control cows and this is contrary to the

outcomes of other probiotic studies that showed higher milk fat when diets were supplemented with yeast for 60 d (Adams et al., 1995) or 4 wk (Piva et al., 1993) starting at mid-lactation. The effect of feeding supplemental Propionibacteria on percentage milk fat during midlactation remains to be determined. However, several authors found no significant effect of 10 to 90 g/d yeast supplementation on milk composition at early- and mid-lactation (Arambel and Kent, 1990, Higginbotham et al., 1994; Swartz et al., 1994; Robinson, 1997; McGilliard and Stallings, 1998). Thus, timing and duration of probiotic supplementation may also influence milk components.

In the present study, plasma glucose, insulin and cholesterol concentrations increased with week postpartum. There was a commensurate increase in plasma insulin concentration as the concentration of glucose increased during early postpartum. Similar trends were observed by other workers (Koprowski and Tucker, 1973; Smith et al., 1976). The lower insulin concentration during the first few weeks of lactation may be reflective of the amount of glucose in blood plasma as well as DMI of cows. In support of this statement, positive relationships between insulin and glucose ($r = 0.35$) and between DMI and insulin ($r = 0.37$) or glucose ($r = 0.44$) were observed in the present study. Theoretically, supplementation of Propionibacteria should increase propionate and thus increase glucose concentration via gluconeogenesis, but supplemental Propionibacteria in the present study did not affect glucose concentrations. Thus, the amount of the Propionibacteria in the ration may not have been adequate or the timing of supplementation may need

to be changed. Alternatively, any increase in plasma glucose due to increased propionate production may have gone undetected if the mammary gland utilized it. Because VFA and rumen microbes were not measured during the study, the absence of an increase in glucose cannot be fully explained.

Cholesterol concentration increased with week postpartum in the present and previous studies (Carroll et al., 1990; Spicer et al., 1993b) and may be used as a possible indicator of the energy status of the animals. In support of this suggestion, plasma cholesterol is positively correlated with EB ($r = 0.44$ to 0.41) in the present and previous studies (Spicer et al., 1993b). Because cholesterol is utilized by the ovarian cells for steroidogenesis (Savion et al., 1982; Grummer and Carrol, 1988; Carroll et al., 1992), it is likely that the increase in P_4 as time postpartum progresses is due in part to increases in plasma cholesterol concentrations (Spicer et al., 1993b); the positive correlation between P_4 and cholesterol ($r = 0.38$) supports this suggestion.

IGF-I was first implicated as a possible mediator of return to ovarian function in dairy cattle in 1990 (Spicer et al., 1990). Similar to other studies (Ronge et al., 1988; Abribat et al., 1990; Hoshino et al., 1991), we observed that EB and plasma IGF-I increased in parallel between wk 1 and 12 postpartum in dairy cows and were positively correlated ($r = 0.47$). Because IGF-I is a potent trophic mitogenic and steroidogenic factor in the ovary (for review see Spicer and Echternkamp, 1995), perhaps increased plasma IGF-I levels increase ovarian responsiveness to gonadotropins as suggested previously (Canfield and Butler, 1990; 1991; Spicer et al., 1990, 1993b). In further support of this suggestion,

systemic IGF-I levels were positively correlated with plasma P_4 in the present (r = 0.37) and previous (r = 0.17 to 0.32) (Spicer et al., 1990; 1993b) studies.

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

In the present study, IGFBP-3 increased with week postpartum and is probably regulated by EB. However, IGFBP-3 did not change in midlactation cows after 2 d of feed deprivation (McGuire et al., 1995a), postpartum beef cows with low BCS (Roberts et al., 1997), and Holstein heifers under negative EB (Vandehaar et al., 1995). The difference maybe due to the longer period of observation that enabled us to track changes of IGFBP-3 with week postpartum. Several more IGFBPs were identified in this study including 28-kDa IGFBP, 26-kDa IGFBP and IGFBP-4, which did not change with week postpartum. The 26-kDa IGFBP is likely a glycosylated form of IGFBP-4 (Liu et al., 1993; Carr et al.

1994; Stanko et al., 1994) whereas the 28-kDa IGFBP is likely a variant of IGFBP-5 (Stanko et al., 1994; Stewart et al., 1996).

In previous studies (Villa-Godoy et al., 1988; Spicer et al., 1990, 1993b), luteal function assessed by area under the P_4 curve increased between the first and second postpartum estrous cycles but not in this study. Similarly, the first estrous cycle of postpartum dairy cows had lower mean peak P_4 concentrations in blood plasma compared to the second estrous cycle (Stevenson and Britt, 1979; Spicer et al., 1990; Staples et al., 1990) but not in the present study. The reason for these discrepancies may lie in the fact that first ovulation did not occur until 37.4 $\pm$ 5.3 d postpartum in the present study, whereas in the previous studies mentioned, first postpartum ovulation occurred much earlier (i.e., 17 to 24 d). Because P_4 was positively correlated with cholesterol ($r = 0.38$) and first ovulation occurred after the maximum plasma cholesterol level was achieved, it is likely that P_4 concentrations (i.e., luteal function) after the first and second postpartum ovulations in the present study were already maximal. Factors associated with increased luteal function (i.e., ovarian competence) include plasma levels of IGF-I and cholesterol. Both IGF-I (Spicer et al., 1990; Lucy et al., 1992; Nebel and McGilliard, 1993) and cholesterol (Kronfeld et al, 1980; Hawkins et al., 1985; Carroll et al., 1990; Spicer et al., 1993b; Son et al., 1996) are responsive to dietary manipulation, and thus improved nutritional strategies to maximize these factors will likely improve reproductive efficiency.

In the present study, correlations between days to first ovulation and average EB during wk 1, wk 1 to 3 or wk 1 to 12 were not significant. This is in

agreement with Villa-Godoy et al. (1988) but contrary to the findings of others (Butler et al., 1981; Butler and Smith, 1989; Spicer et al., 1993b). Spicer et al. (1993b) found that average EB during the first 4 wk of lactation was negatively correlated to postpartum interval to first ovulation (r = -0.52). Butler et al. (1981) found a similar correlation (r = -0.60). The absence of a significant correlation in the present study may be due to the fact that cows exhibited longer average interval to first postpartum ovulation and thus were already moving towards positive EB at this time.

Diameter of the first and second largest follicles and number of follicles did not vary in the present study. This finding differs from that of Lucy et al. (1991a) who found that number of small follicles (< 5 mm) decreased while number of large follicles (> 10 mm) increased with increasing days postpartum, and EB was related to changes in follicular populations. Also, cows supplemented with lipids in an attempt to improve EB have follicles with larger diameters (Lucy et al., 1991b). The discrepancy may be explained by the difference in time frame of follicle measurement between studies. Lucy et al. (1991a) measured follicles for only a period of 7 to 25 d between 0 to 25 d postpartum whereas in this study follicle diameters were measured weekly between 3 and 12 wk postpartum. Also, because follicular populations change with EB, the difference in the magnitude of EB between groups of cows among studies may have contributed to the discrepancy in the results.

CONCLUSION

Collectively, supplemental Propionibacteria improved some production variables during wk 1 of lactation but did not have a significant impact on these and the reproductive variables measured in succeeding weeks of lactation. Supplemental feeding of Propionibacteria culture to dairy cows starting 2 wk before parturition and continuing through 12 wk of lactation increased plasma leptin levels and decrease DMI on a kg/BW basis but did not affect FCM. Percentage milk protein and SNF were higher in Propionibacteria-treated cows than control cows during 1 wk postpartum. This increase is possibly due to higher plasma NEFA concentration during the 1 wk of lactation in Propionibacteria-treated cows. However, supplemental feeding of Propionibacteria did not alter percentages of milk fat and lactose.

Plasma glucose, insulin and IGF-I concentrations were similar between Propionibacteria-treated and control cows. In addition, supplemental feeding of Propionibacteria did not alter plasma cholesterol, a necessary precursor for ovarian P_4 production. Similarly, P_4 peak and area under the curve and interval to first and second postpartum ovulations were similar in both treated and control cows. EB was not correlated to first postpartum ovulation due to longer interval to first postpartum ovulation where EB was moving towards positive status.

Propionibacteria supplementation did not affect IGFBPs. But, systemic IGFBP-2 and IGFBP-5 decreased while IGFBP-3 increased with week postpartum. What regulates the levels of IGFBPs of postpartum cows will require further study but likely depend on the stage of lactation and EB.

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

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

*Robt Morgan, Inc., Paris, IL contains

Free fatty acids - 93%, MIUs - 2.0% and Cane Molasses flavor - 5.0%

** 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 2. Correlation coefficients among average weekly (n=228) energy balance (EB), dry matter intake (DMI), fat corrected milk (FCM), body weight (BW), milk fat (FAT), milk protein (PROT), milk lactose (LACT) milk urea nitrogen (MUN), progesterone (P4), cholesterol (CHOL), insulin-like growth factor-I (IGF-I), insulin (INS), glucose (GLU) and leptin (LEP, n = 114) during the first 12 wk of lactation in dairy cows (n = 19).

				Milk				Plasma					
Parameters	FCM	DMI	BW	FAT	PROT	LACT	MUN	P4	CHOL	IGF-I	INS	GLU	LEP
EB	-0.38**	0.68**	-0.18**	-0.64**	-0.35**	0.22**	0.14*	0.36**	0.44**	0.47**	0.32**	0.23**	-0.11
FCM		0.37**	0.17**	0.48**	-0.22**	-0.02	0.21**	-0.01	0.29**	-0.08	0.08	0.20**	0.04
DMI			0.08	-0.33**	-0.44**	0.22**	0.28**	0.40**	0.69**	0.43**	0.37**	0.44**	-0.03
BW				0.06	0.11	-0.18**	-0.09	0.07	0.07	0.14*	0.16*	0.17*	-0.06
FAT					0.20**	-0.23**	-0.16*	-0.26**	-0.29**	-0.22**	-0.13*	-0.08	0.11
PROT						-0.33**	-0.43**	-0.19**	-0.45**	-0.24**	-0.26**	-0.18**	-0.08
LACT							0.26**	0.06	0.30**	0.20**	-0.02	0.21**	-0.05
MUN								0.08	0.43**	-0.00	0.11	-0.04	0.21*
P4									0.38**	0.37**	0.08	0.35**	0.06
CHOL										0.42**	0.31**	0.41**	-0.03
IGF-I											0.31**	0.43**	-0.17¥
INS												0.35**	0.05
GLU													0.08

** *P < 0.01*
* *P < 0.05*
¥ *P < 0.06*

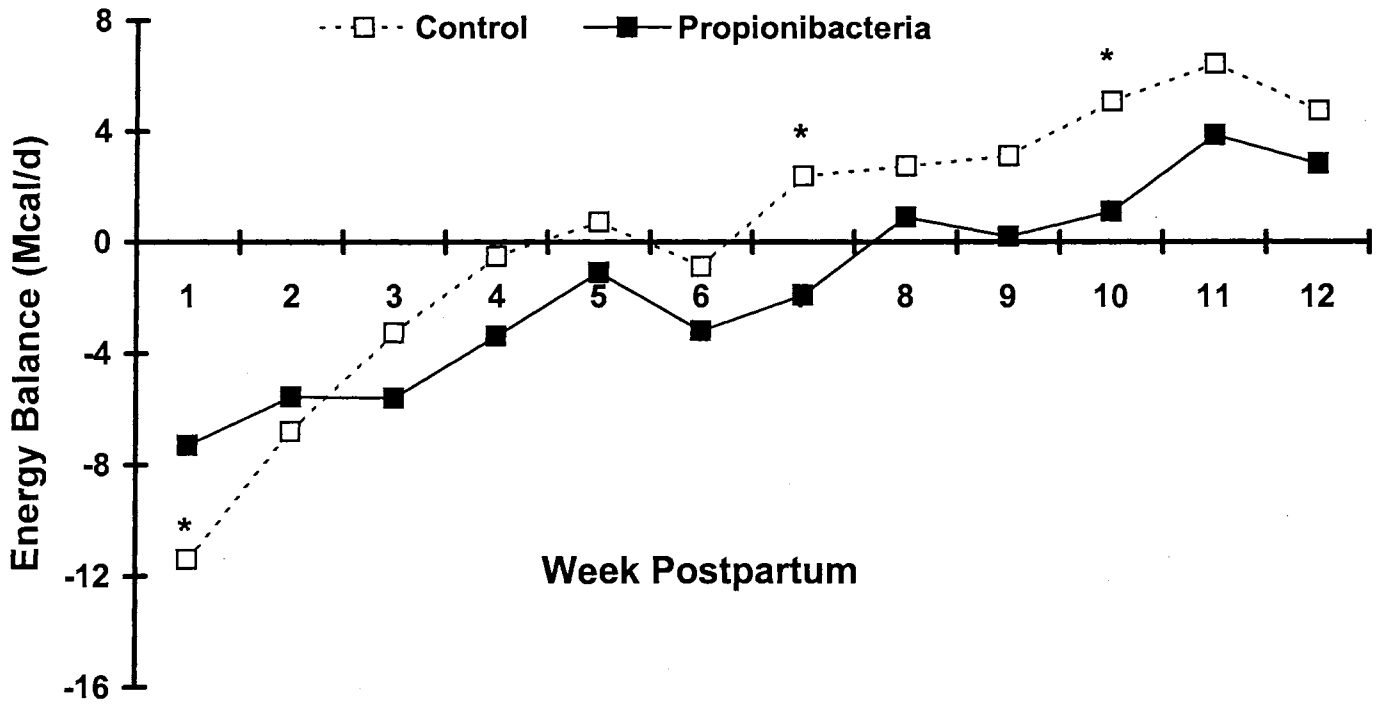

Figure 1. Weekly changes in energy balance of postpartum cows fed Propionibacteria (n=9) and control (n=10) diets during the first 12 wk postpartum. SEM was 1.48 Mcal/d for control and 1.54 Mcal/d for treatment cows. *Mean within week differs ($P < 0.10$) from Propionibacteria mean.

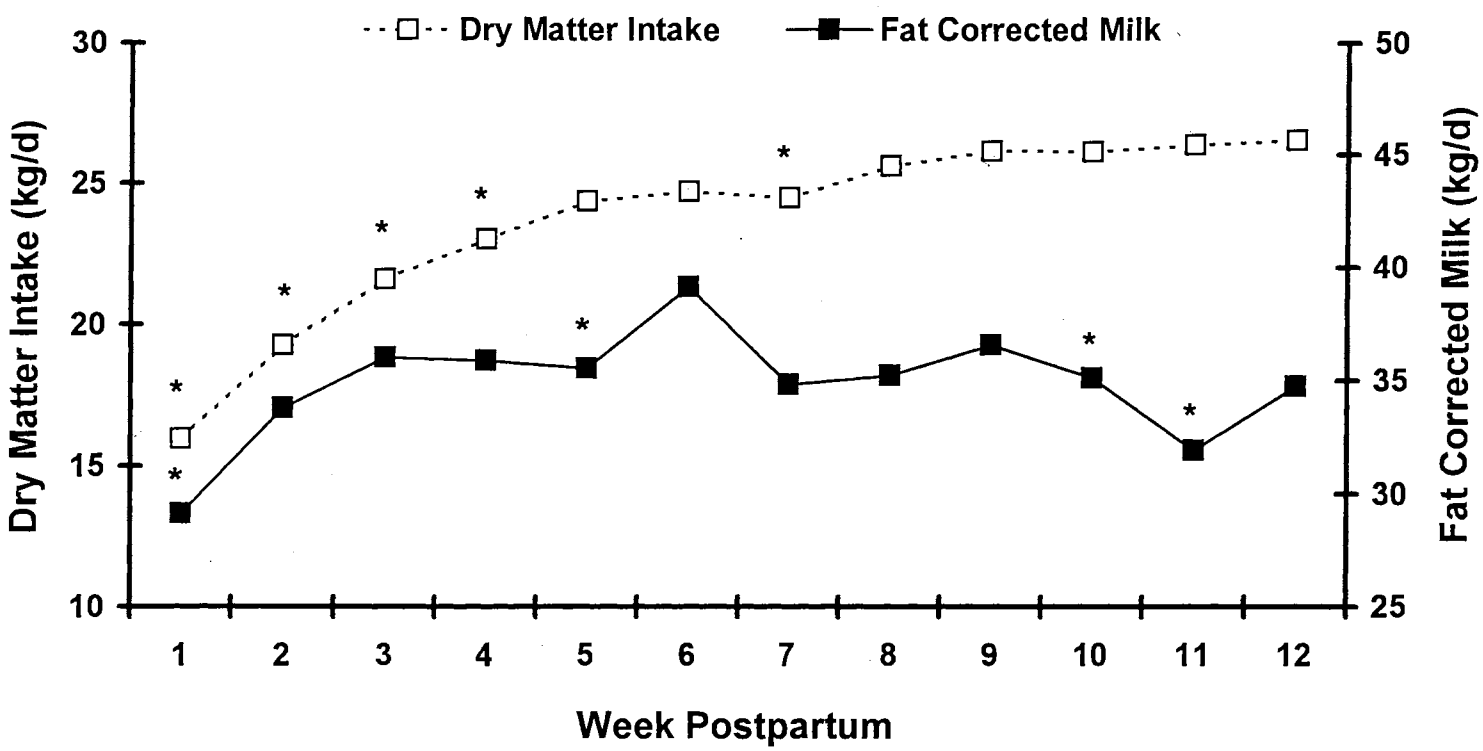

Figure 2. Weekly changes in dry matter intake (DMI) and fat corrected milk (FCM) of postpartum cows. Data from cows fed control and Propionibacteria diets were pooled (n=19) because treatment x week interaction and treatment were not significant. *Mean is significantly different from the next succeeding week ($P < 0.001$). Pooled SEM = 0.63 kg/d for DMI and 1.30 kg/d for FCM.

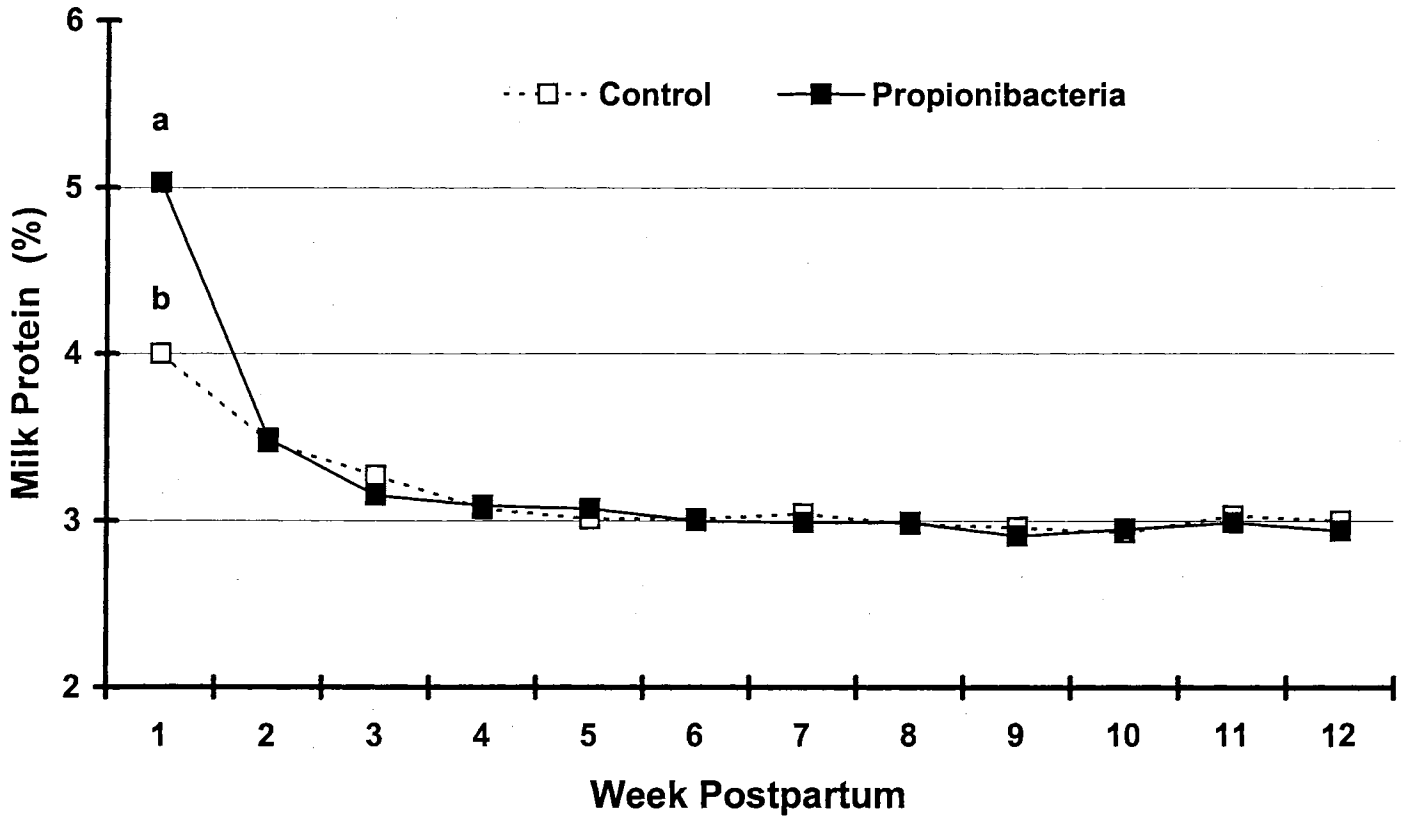

Figure 3. Weekly changes in percent milk protein of postpartum cows fed Propionibacteria (n=9) and control (n=10) diets during the first 12 wk of lactation. [a,b]Means with different superscript within week differ ($P < 0.01$). SEM = 0.14 % for control and 0.15 % for treatment cows.

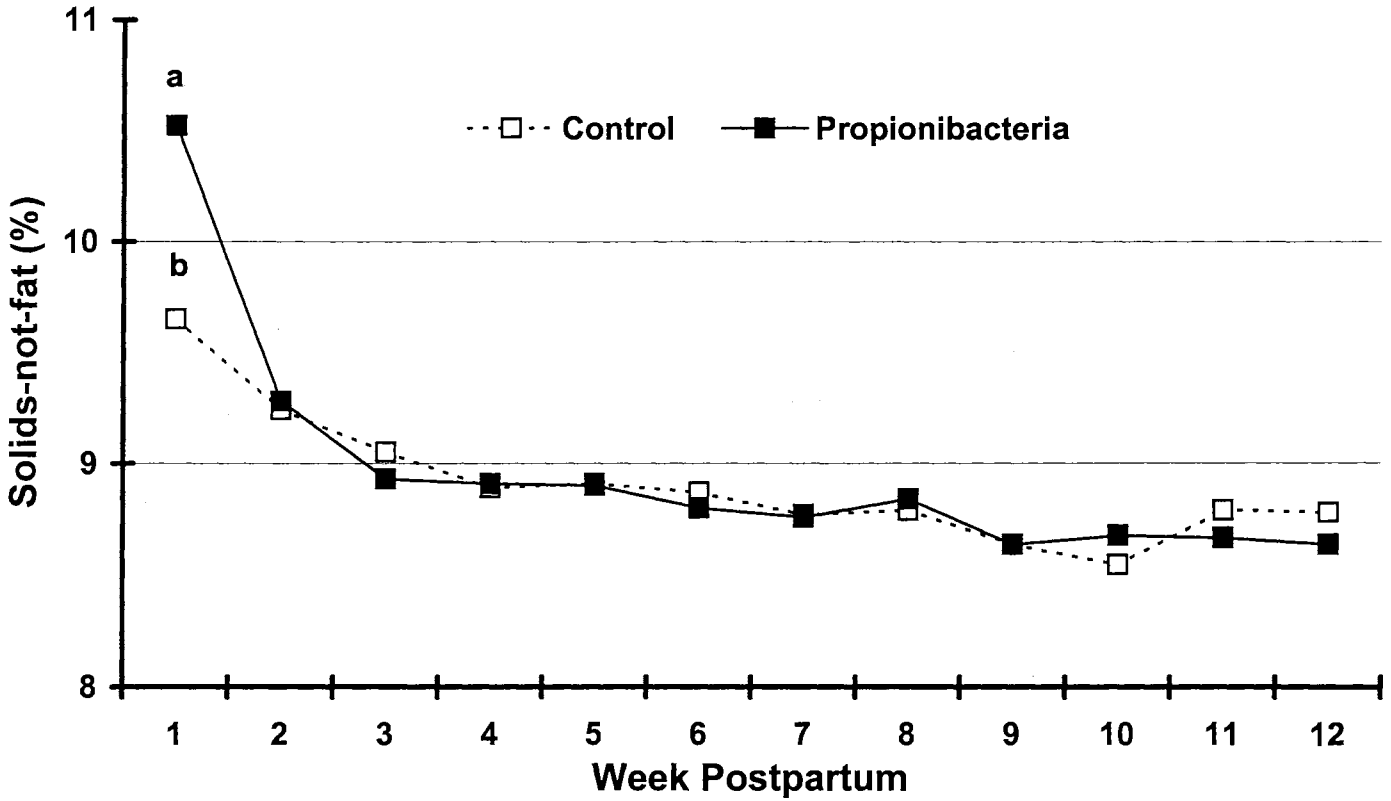

Figure 4. Weekly changes in percent solids-not-fat of postpartum cows fed Propionibacteria (n=9) and control (n=10) diets during the first 12 wk of lactation. [a,b]Means with different superscript within week differ ($P < 0.05$). SEM = 0.14% for control and 0.15% for treatment cows.

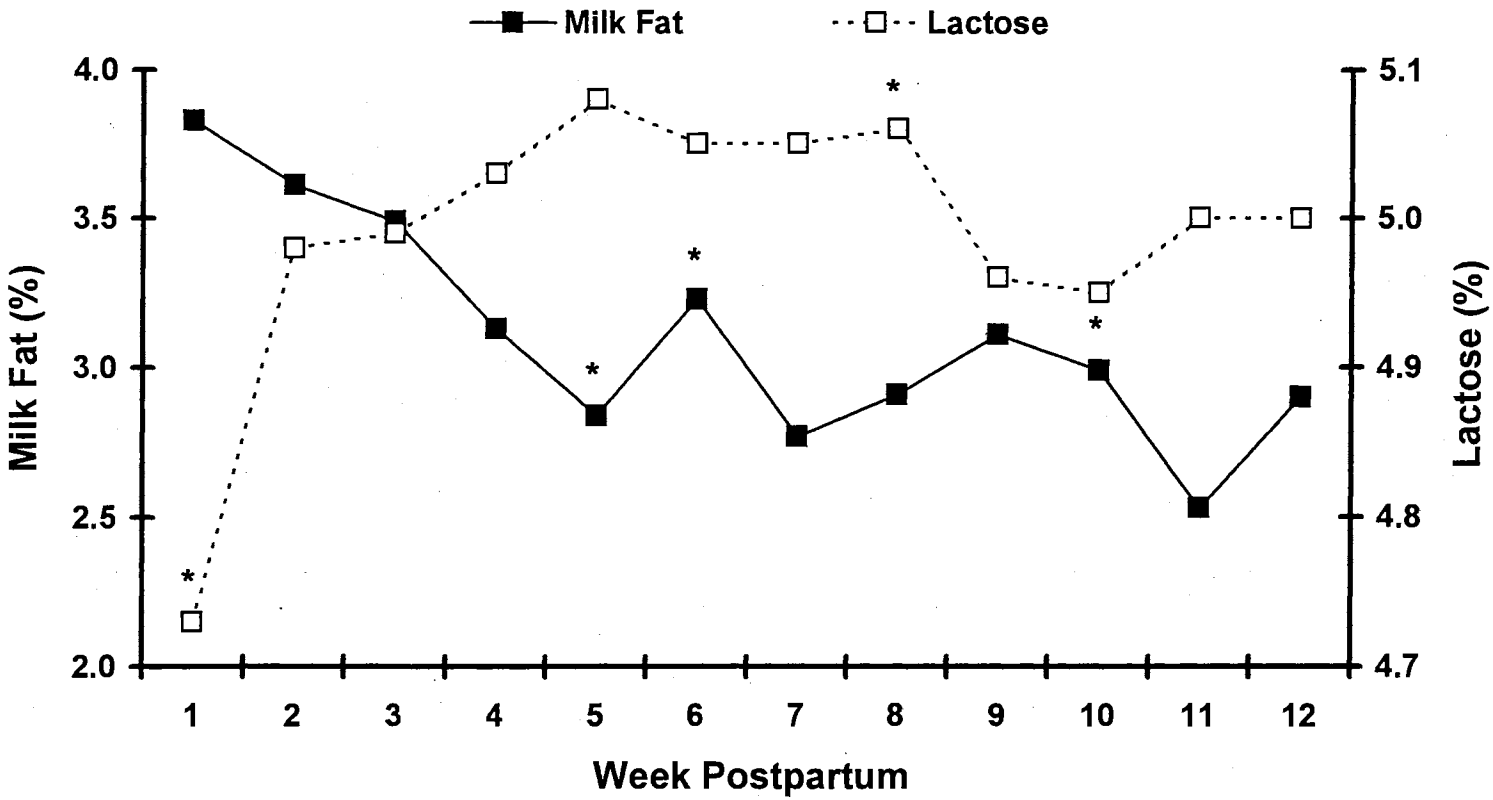

Figure 5. Weekly changes in percent milk fat and lactose of postpartum cows. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction and treatment were not significant. *Mean is significantly different from the next succeeding week ($P < 0.05$). Pooled SEM = 0.16 % for milk fat and 0.04 % for lactose.

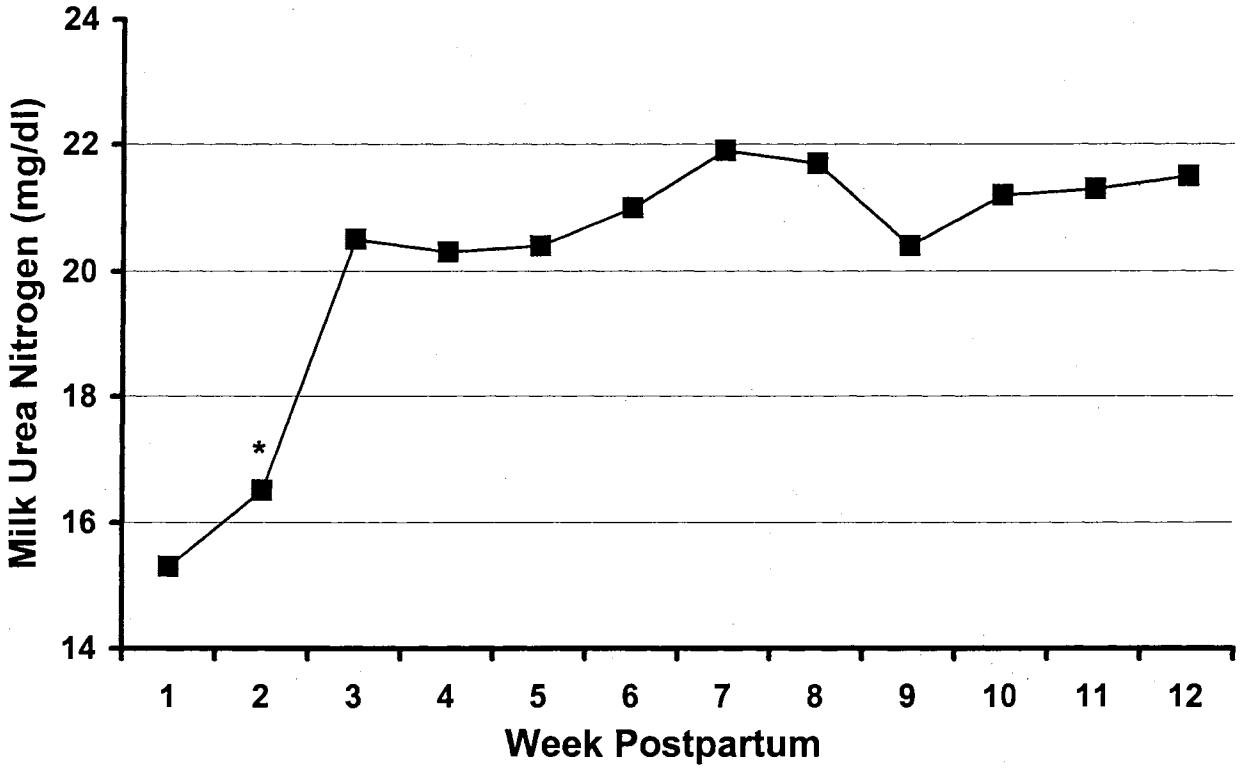

Figure 6. Weekly changes in milk urea nitrogen of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction and treatment were not significant. * Mean is significantly different from the next succeeding week ($P < .001$). Pooled SEM = 0.82 mg/dl.

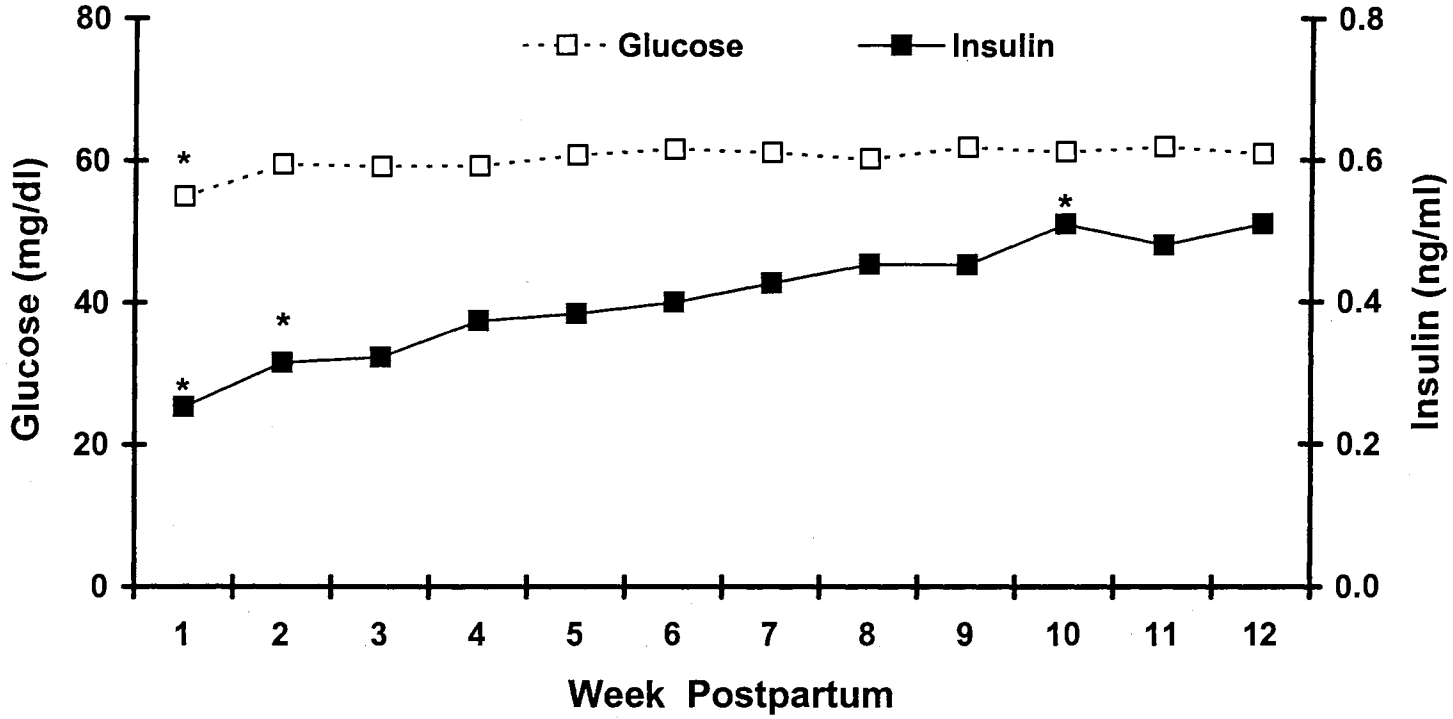

Figure 7. Weekly changes in plasma glucose and insulin concentration of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction and treatment were not significant. * Mean is significantly different from the next succeeding week ($P < 0.001$ for insulin and $P < 0.01$ for glucose). Pooled SEM = 0.034 for insulin ng/ml and 1.22 mg/dl

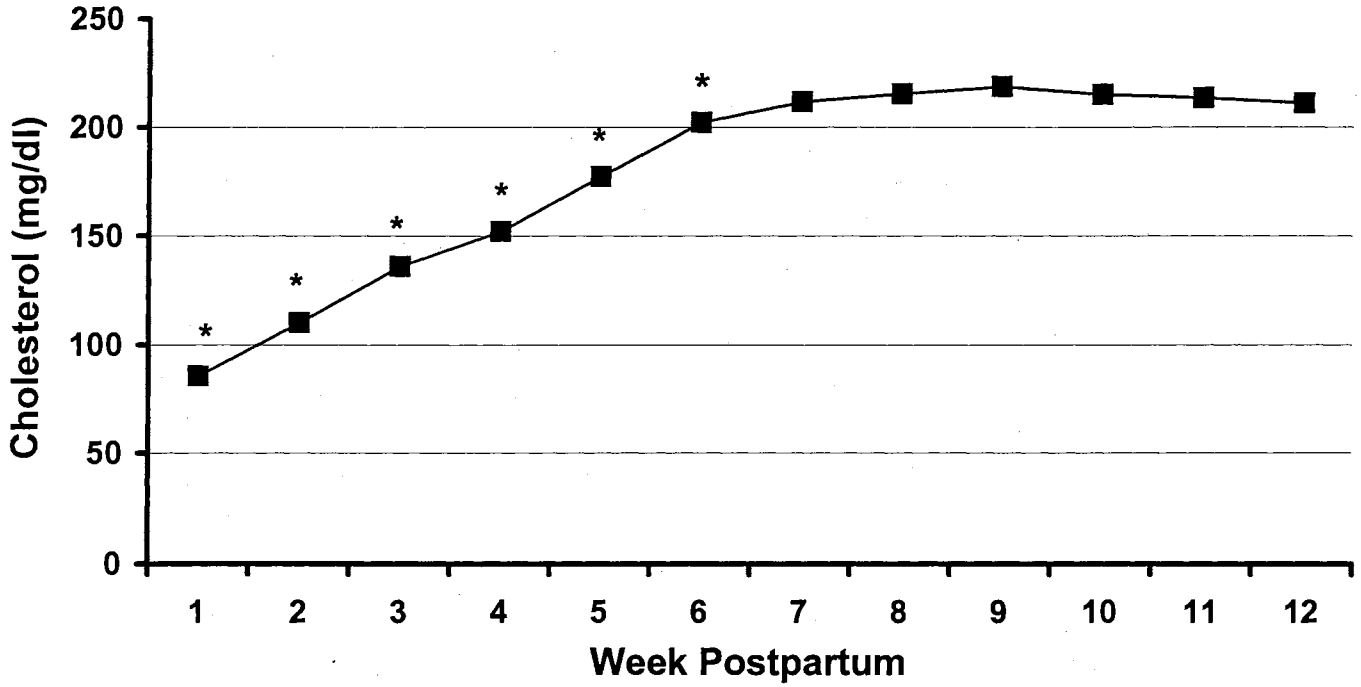

Figure 8. Weekly changes in plasma cholesterol concentrations of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction and treatment was not significant. * Mean is significantly different from the next succeeding week ($P < 0.01$). Pooled SEM = 6.6 mg/dl.

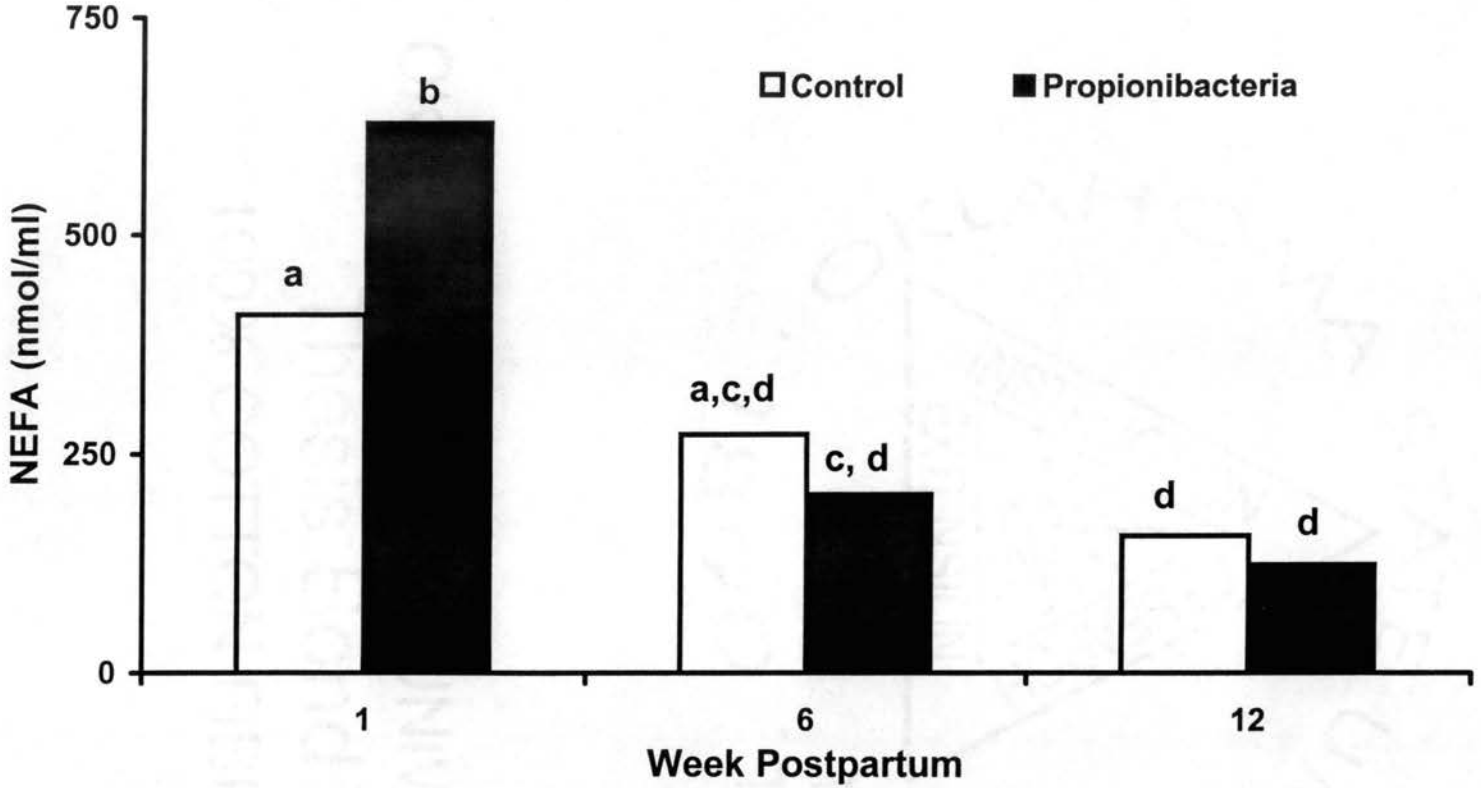

Figure 9. Changes in plasma non-esterified fatty acids (NEFA) concentrations of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria (n=9) and control (n=10) diets. Means without a common superscript differ ($P < 0.01$). Pooled SEM = 47 nmol/ml for control and 49 nmol/ml for treatment cows.

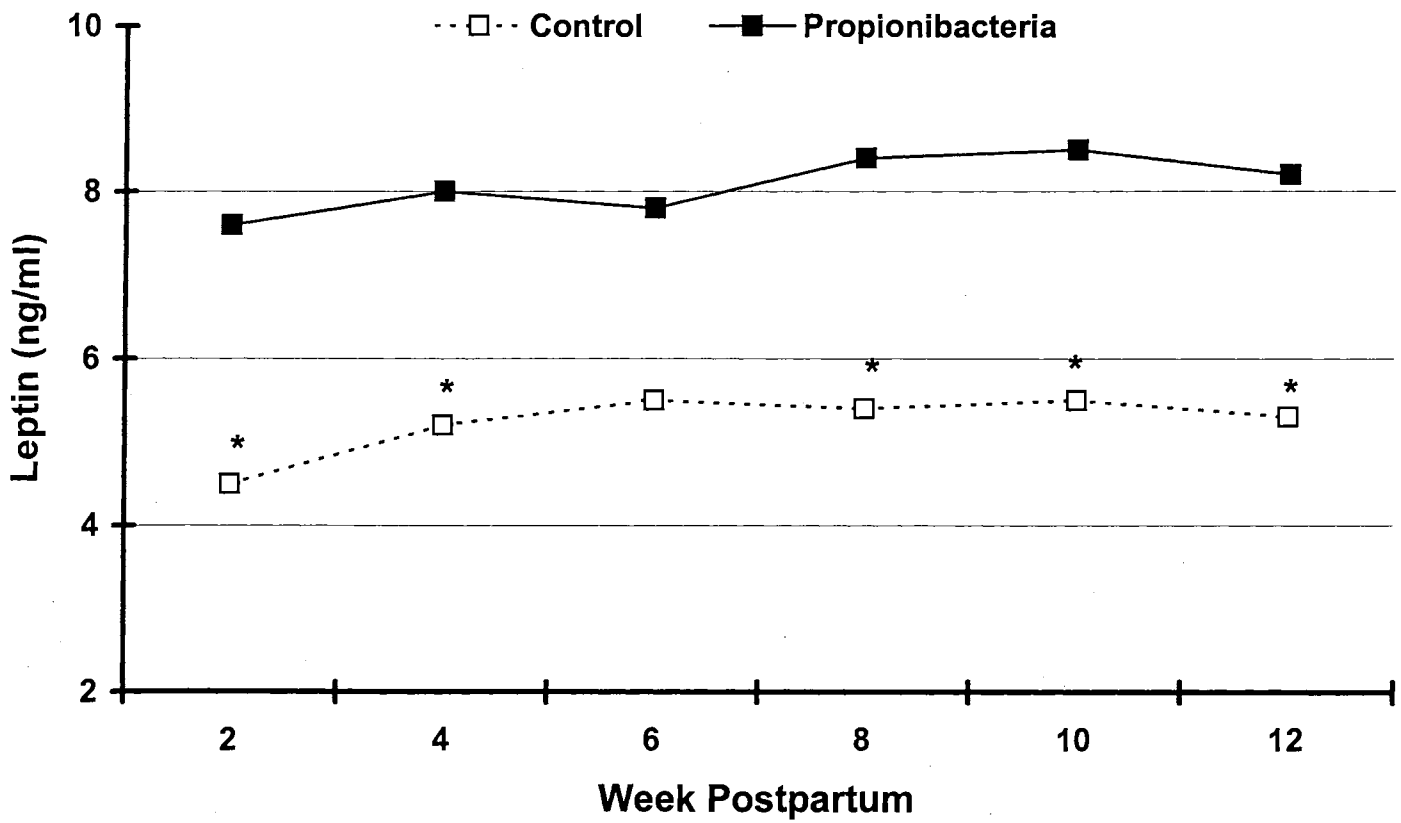

Figure 10. Weekly changes in plasma leptin concentrations of postpartum cows fed Propionibacteria (n=9) and control (n=10) diets during the first 12 wk of lactation. *Mean within week differs ($P < 0.10$) from Propionibacteria mean. Pooled SEM = 1.1 ng/ml for control and 1.1 ng/ml for Propionibacteria treated cows.

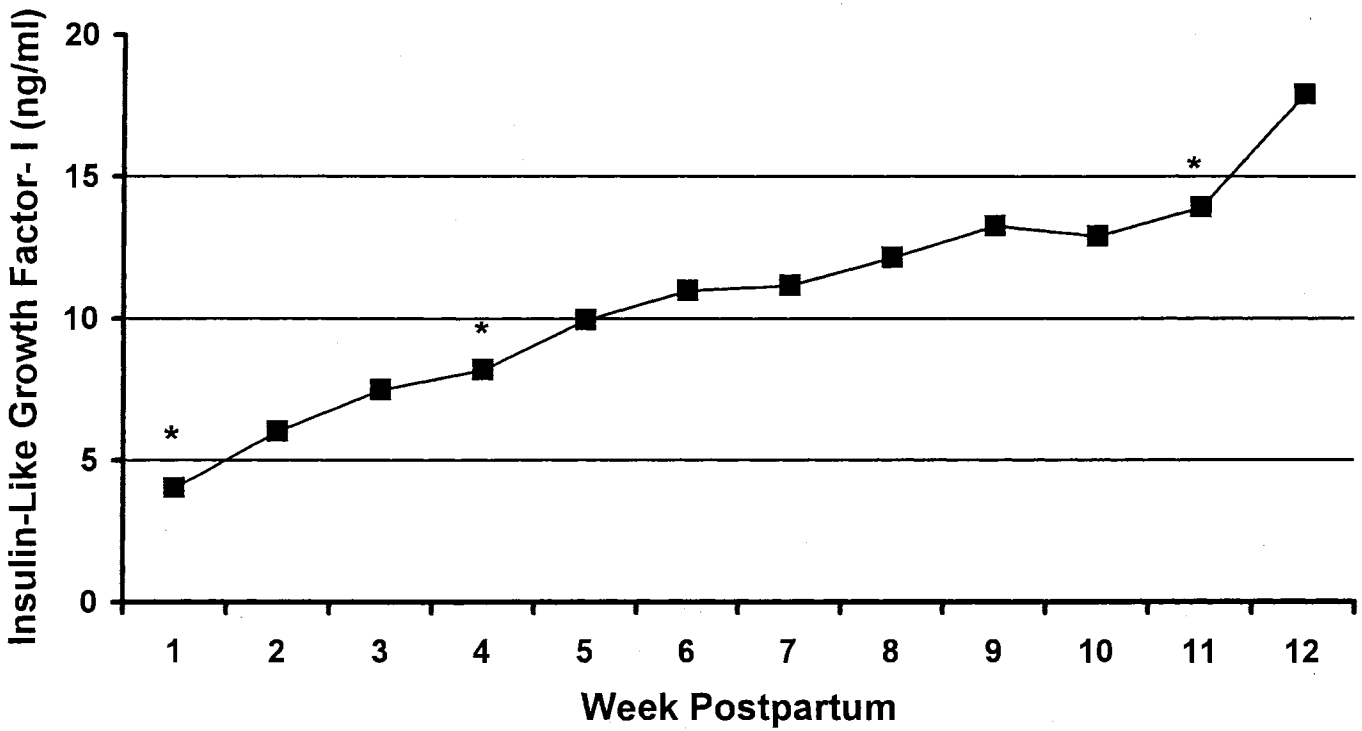

Figure 11. Weekly changes in plasma insulin-like growth factor-I concentrations of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction and treatment were not significant. *Mean is significantly different to the next succeeding week ($P < 0.001$). Pooled SEM = 1.4 ng/ml.

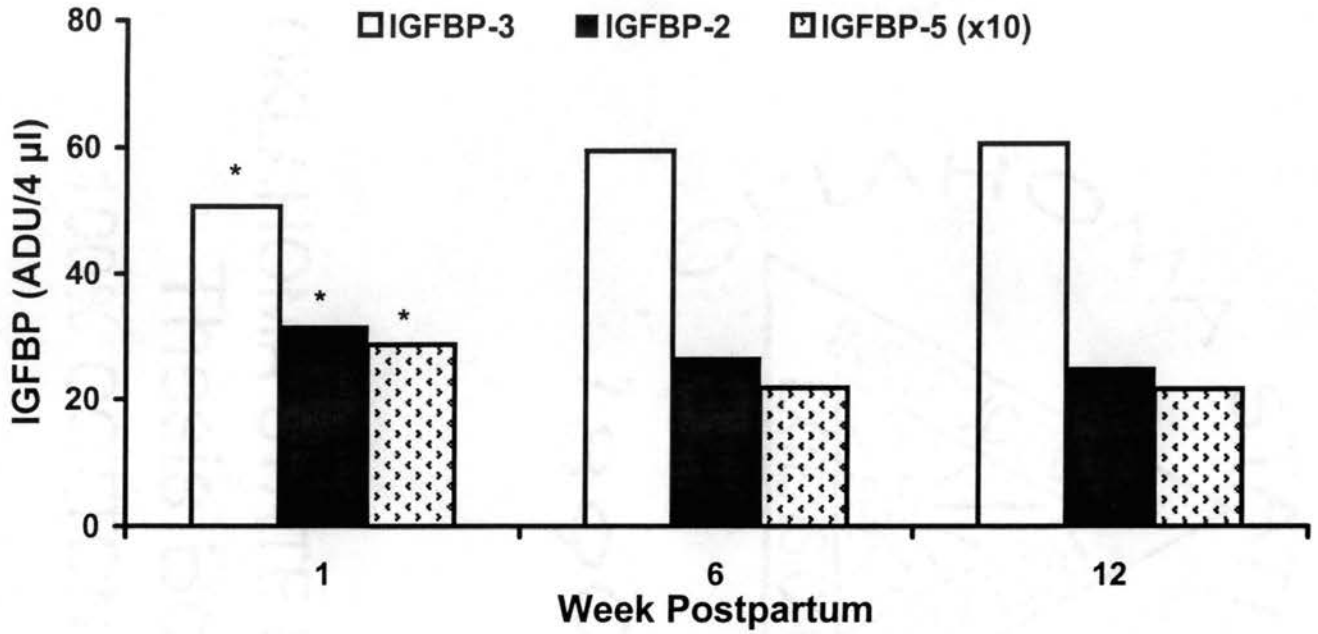

Figure 12. Changes in plasma insulin-like growth factor-binding proteins (IGFBP-3, IGFBP-2, and IGFBP-5) of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction and treatment were not significant. * Mean differs from wk 6 and 12 within a specific IGFBP ($P < 0.05$). Pooled SEM for IGFBP-3 = 2.7 ADU/4 ul, IGFBP- 2 = 1.9 ADU/4 ul and IGFBP-5 = 2.7. ADU/4 ul. ADU = arbitrary densitometric unit

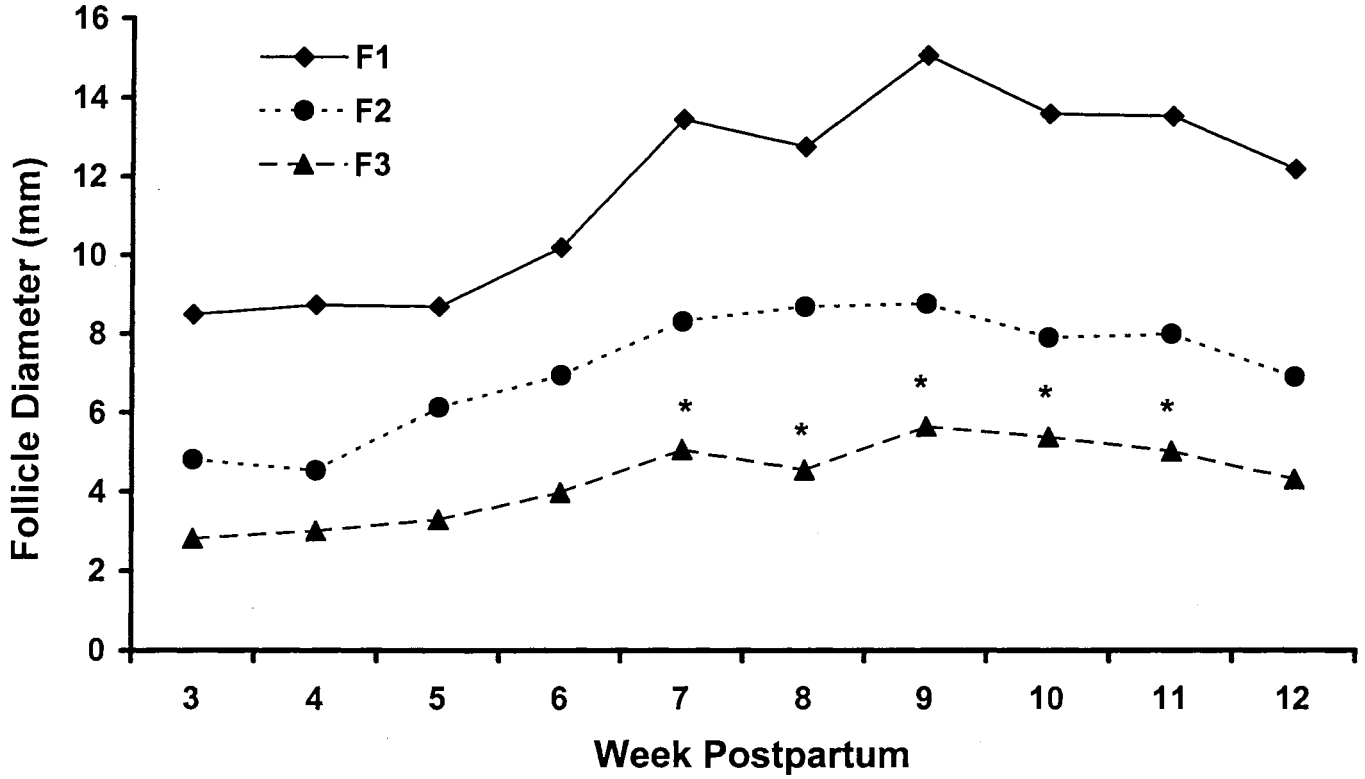

Figure 13. Changes in follicle diameter classified as F1 (largest), F2 (second largest) and F3 (third largest) of postpartum cows during the first 12 wk of lactation. Data from cows fed Propionibacteria and control diets were pooled (n=19) because treatment x week interaction was not significant. * Means of wk 3, 4 and 5 differ from means of wk 7, 8, 9, 10 and 11 ($P < 0.05$). Pooled SEM for F1 = 0.3 mm, F2 = 0.9 mm and F3 = 0.7 mm.

CHAPTER IV

Models Predicting Plasma Cholesterol and Progesterone Concentrations and Interval to First and Second Ovulations Using Production, Metabolic and Endocrine Variables in Early Postpartum Dairy Cows

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ABSTRACT

The objective of this study was to establish relationships among metabolic and endocrine factors that may influence ovarian activity during early lactation. Nineteen pluriparous Holstein cows were individually fed a total mixed ration during the first 12 wk postpartum. Cows were bled twice each week for determination of plasma concentrations of insulin (INS), glucose (GLU),

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cholesterol (CHOL), insulin–like growth factor-I (IGF-I), and progesterone (P_4). Feed intake and milk production were recorded daily while body weights (BW) and milk composition were recorded weekly. The relationships among plasma CHOL and P_4, and days to first and second postpartum ovulation were modeled with energy balance (EB), dry matter intake (DMI), milk yield and composition, plasma metabolites and hormones using backward elimination technique of multivariate regression analysis. The variables EB, DMI, fat corrected milk (FCM), BW and milk composition contributed significantly to predicting both plasma CHOL and P_4 concentrations. These variables consistently predicted both plasma CHOL and P_4 as indicated by the small difference between predicted and observed values throughout the 12 wk postpartum period. The models using hormones and metabolites as variables predicted plasma CHOL concentrations more closely than they predicted plasma P_4 concentrations based on magnitude of residuals. The variables that predicted plasma CHOL concentrations were DMI x SNF (Solids-not fat) using Model 1 (production variables) and the square of GLU (GLU^2) using Model 3 (plasma hormones and metabolites) contributing 63% and 55% to total model R^2, respectively. For plasma P_4 concentrations, EB (Model 2 - production variables) and IGF-I (Model 3 - plasma hormones and metabolites) were the major predictors explaining 61% and 74% of the total model R^2, respectively. Week postpartum when included in Model 1 and 3, contributed 62% to 88% of the total model R^2. The production variables EB and percentage of milk lactose were the greatest contributors to the models predicting days to first and second postpartum ovulations, respectively.

DMI and FCM also contributed significantly to both models but to a lesser extent. Of the plasma hormones and metabolites evaluated, IGF-I^2 was the most significant predictor of days to first postpartum ovulation and GLU^2 and INS were significant predictors of days to second postpartum ovulation. Plasma IGF-I, GLU and INS have been implicated in ovarian functions and their significant contribution to these models reinforces their importance in postpartum return to ovarian competence. Further research will be needed to ascertain how some of these variables can be used in an applied way to monitor reproductive competence in early lactating dairy cows.

INTRODUCTION

The return to ovarian function of early postpartum cows requires optimal metabolic and endocrine conditions. Factors related to early postpartum ovarian competence include proposed relationships between energy balance (EB) and postpartum interval to first ovulation (Butler et al., 1981; Butler and Smith, 1989; Spicer et al., 1990, 1993a; Carroll et al., 1990; Zurek et al., 1995; Staples et al., 1998). Variables that are positively correlated with each other include plasma CHOL with plasma progesterone (P_4), conception and number of recoverable embryos (Kweon et al., 1986; Grummer and Carroll, 1988; Spicer et al., 1993b); plasma P_4 concentration with days open and pregnancy rate (Folman et al., 1973; Villa-Godoy et al., 1988), plasma insulin-like growth factor -I (IGF-I) with ovarian function (Spicer et al., 1990; 1993b; Lucy et al., 1992; Beam and Butler,

1997); and plasma glucose (GLU) with pulsatility of luteinizing hormone (LH) (Butler and Smith, 1989; Canfield and Butler, 1990, 1991). Negative correlations include milk yield with days to first postpartum ovulation (Thatcher and Wilcox, 1973; Whitmore et al., 1974; Stevenson et al., 1983; Butler and Smith, 1989; Nebel and McGilliard, 1993). Simple correlation coefficients (r) among some of these variables range from r = 0.06 to 0.64 (Villa-Godoy et al., 1988; Lucy et al., 1991, 1992; Spicer et al., 1990, 1993b; Francisco, 2001).

Because ovarian activity is not influenced by any single condition or metabolite, it is necessary to integrate relationships among various metabolic and endocrine factors. Few modeling studies have attempted to compare production variables with metabolic and endocrine variables of lactating dairy cows to predict postpartum ovarian competence. Stevenson and Britt (1980) utilized changes in concentrations of estradiol (E_2), LH, glucocorticoids, number of ovarian follicles, milk yield, energy intake, body weight and cross products of these variables between wk 1 and 2 postpartum to model first postpartum ovulation. The 9-variable model predicting days to first ovulation shows that the change from 1 to 2 wk of E_2 concentrations and number of follicles are the most important factors. The scope of Stevenson and Britt (1980) model is limited because variables were measured for only a 14 d period, thus it may not predict cows with > 14 d to first ovulation. Because most dairy breeders do not routinely breed cows at first ovulation, measurement of variables over a longer period to cover second and third postpartum ovulations would be preferable. Another model (Heuer et al., 2000) predicted average herd mean EB in early lactating

cows using various dietary inputs, body condition score, milk components (fat, protein, lactose), and beta-hydroxy-butyrate but did not include any endocrine variables. In this study, fat-protein ratio, milk fat and milk protein concentrations explained substantially the variability in EB.

In the past few years, several metabolic and endocrine modulators have been identified that affect reproductive function including IGF-I, INS, GLU, CHOL and P_4. Thus, it is necessary to expand the previous models to include these reproductive modulators to delineate their contributions in explaining the variation in postpartum reproductive competence of early lactating dairy cows. Previous studies have documented the importance of CHOL as a precursor of ovarian steroidogenesis (Kronfeld et al., 1980; Grummer and Carroll, 1988; Spicer et al., 1993a) and thus understanding the variables that contribute to changes in plasma CHOL concentrations may help understand factors that may contribute to reproductive success in postpartum dairy cows. Also, the use of some of these variables like CHOL for quick test (e.g., Johnson and Johnson Advanced Cholesterol kit®, BioScience 2000®, CholesTrack®), in assessing health and metabolic status of humans are very prevalent nowadays (Koda-Kimble and Young, 1988; Lloyd, 1991; Stehbens, 2001). Whether this practice of testing the levels of plasma metabolites can be extended to dairy cows to assess metabolic competence for return to postpartum ovulation is uncertain and will require studies to establish the importance of a given metabolite for such a use. Therefore, the goal of this study was to establish relationships among production variables and metabolic and endocrine variables that may affect ovarian

activities, via multivariate regression modeling of plasma CHOL, P_4, and days to first and second postpartum ovulation during early lactation.

MATERIALS AND METHODS

Data collection was previously described in another study (Francisco, 2001, unpublished thesis). Briefly, data were collected from nineteen pluriparous cows from 1 to 12 wk postpartum. Cows (n = 19) were individually fed a total mixed ration (TMR) consisting of sorghum silage (31.9 %), alfalfa hay (21.2 %), cottonseed (8.0 %) and concentrate (38.9 %). Nine cows were supplemented with Propionibacteria (17 g/d). The TMR was sampled weekly and composited monthly for analysis. Feed intake and milk production were recorded daily, whereas body weights were recorded weekly. Milk was collected twice daily (0300 and 1500) and analyzed weekly. Blood was collected twice each week via coccygeal venipunture, plasma harvested and assayed for plasma concentrations of P_4, GLU, INS, CHOL, and IGF-I (Francisco, 2001).

As described by Francisco (2001), EB was calculated from net energy intake as the average daily dry matter intake (DMI) multiplied by the net energy concentration of the diet. Net energy required for daily maintenance of the animals in Mcal/d was calculated from the equation: 80 x $BW^{0.75}$ (kg) /1000 (NRC, 1989). Daily energy for milk production in Mcal/d was calculated using the formula, milk yield (kg) x [92.239857 (% milk fat) + 49.140211 (% solids-not fat) – 56.393297]/1000, where milk yield is average daily yield for the week, and milk

composition which was based on analysis of weekly milk samples (Tyrell and Reid, 1965).

Multiple regression models were developed to predict plasma CHOL and P_4 concentrations and days to first and second postpartum ovulations as indicators of metabolic and reproductive status of the cows, respectively. The basic model utilized to obtain the relationships of the observed variables was:

$$\text{Log } Y_i = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k + \varepsilon_i$$

where Y_i represents a dependent variable (plasma CHOL and plasma P_4 concentrations and first and second postpartum ovulations), β_o represents the intercept, β's are the true regression for observed X's, k is the number of independent variables, and ε_i is the random error associated with the $_i$th observation.

All variables in the model were expressed as weekly means (n = 228) from wk 1 to 12 postpartum (Table 1-2). Data from control (n = 10) and treated (n = 9) cows were pooled because most of the variables did not vary by treatment (see Francisco, 2001, unpublished thesis). Variables in the model included all the possible combinations of cross products and squares of all the variables except week postpartum. The first group of variables included the "production" variables EB, body weight (BW), DMI, fat-corrected milk (FCM), and percentage of milk fat, milk protein, lactose, and solids-not fat (SNF). The second set of variables incorporated the metabolic and hormonal variables, plasma INS, GLU and IGF-I concentrations. These two sets of variables were used separately in obtaining the "best" model for predicting plasma CHOL (Models 1 and 3) and P_4 (Models 2

and 4) concentrations during the first 12 wk postpartum. Plasma CHOL and P_4 values were log transformed to increase coefficient of determination (R^2). To determine the impact of week postpartum in predicting plasma CHOL (Models 1 and 3) and P_4 (Models 2 and 4) concentrations, it was included as one of the variables in one group of models and not included in another group of models.

The same data sets were used to predict days to first (Models 5 and 7) and second (Models 6 and 8) postpartum ovulations. In the models using metabolic and hormonal variables (Models 7 and 8), plasma P_4 was not included because P_4 concentrations were used to determine if and when the cows had ovulated postpartum. Based on P_4 concentrations, there were 15 and 11 cows that showed first and second postpartum ovulation, respectively. Weekly means of the variables (Table 2) from 1 to 5 wk (n = 75) and 1 to 7 wk (n = 77) postpartum were averaged and utilized to generate the models for days to first and second postpartum ovulation, respectively, because the interval to first snd second postpartum ovulation averaged 5 and 7 wk, respectively.

The backward elimination technique of the multiple regression procedure was used to obtain the models used in the study (SAS User's Guide, 1988). Initially, the backward regression technique includes all the variables in the first model giving the highest possible R^2. This is followed by removal of the least significant variable with the lowest R^2 contribution to the model producing the second model, and so forth. Only the variables that remained significant at the $P < 0.05$ or 0.01 were included in the final model unless stated otherwise. Models

were selected based on high R^2, small residual variations between predicted and observed values, and a low number of variables included in the model.

RESULTS

Model Selection

Models for Plasma CHOL and P_4. Models 1 and 2 were generated using production variables (Table 1). The "best" fit model that predicted plasma CHOL concentrations (Model 1) had a total R^2 of 0.68 (Table 3). The relative contribution of each variable, as indicated by partial R^2, showed that the interaction of DMI x SNF is the most important component in the model contributing 63% (R^2 = 0.43). This is followed by FCM x SNF (R^2 = 0.19), which explained 28% of the composite model R^2. There were other significant variables (i.e., FCM x LACT, BW x LACT, FCM^2, FAT^2, FCM x FAT, and BW) in the model but they contributed less than 5% of the model R^2.

The "best" fit model for plasma P_4 concentrations (Model 2) included 3 production variables that were all significant ($P < 0.01$, Table 3), and had a lower R^2 (0.29) compared with Model 1 (plasma CHOL). Two more variables (i.e., FCM x FAT and DMI x FAT) were significant but contributed less than 5% of the total R^2. The difference in R^2 between Model 1 and Model 2 indicates that the variables that predicted plasma CHOL explained more of the variation than the variables that predicted plasma P_4 (Table 3).

Models 3 and 4 included only three variables (plasma insulin, glucose, and IGF-I), their cross products and squares (Table 1). The models for plasma CHOL (Model 3) and plasma P_4 (Model 4) have similar R^2 values (Table 3) when based on this combination of mean weekly measurements of hormones and metabolites (Table 1). Model 3 indicates that GLU^2 contributes nearly half (55.0%) to the total R^2 indicating that it is the most important variable in predicting plasma CHOL concentrations. This is followed by IGF-I (18.9%) and IGF-I x GLU (11.8%) with the rest of the significant variables (i.e., IGF-I x INS and INS) each contributing less than 10% to the total R^2 (Table 3). The R^2 of the model predicting plasma P_4 (Model 4) was 0.33 of which plasma IGF-I and GLU^2 variables contributed 74% and 21.8%, respectively (Table 3). $IGF\text{-}I^2$ contributed significantly but less than 5% of the total R^2.

Week postpartum, when included in Models 1 - 4, had the greatest contribution to the total R^2 of all the models, ranging from 63% to 88% (Table 4). In Model 1 (plasma CHOL), week postpartum contributed 71.6% to model R^2 while EB x FAT and FCM x FAT contributed a total of 22.8% (Table 4). Percent contribution of variables that both appeared in Model 1 without and with week postpartum vary such as DMI x SNF (63.2% vs. 2.1%), FCM x SNF (28.1% vs. 2.1%), and FCM x LACT (10.3% vs. 2.7%). The other significant variables that contributed less than 5% of the model R^2 were IGF-I and IGF x GLU. In Model 2 (plasma P_4), week postpartum contributed 67.9% of total R^2. The variable EB appeared both in Model 2 with and without week postpartum but at different contribution to the total model R^2 (3.4% vs. 61.1%). Other variables (i.e., $PROT^2$,

EB^2, EB x FCM, LACT, EB x DMI, PROT, and SNF) contributed significantly but less than 5% of the model R^2. Week postpartum, when included in Model 3 (plasma CHOL) contributed 88.2% of the total R^2. The common variables of Model 3 with and without week postpartum were plasma IGF-I (3.6% vs. 18.9%), GLU^2 (7.6% vs. 55.0%) and IGF-I x GLU (0.2% vs. 11.8%). Other significant variables that contributed less than 5% were IGF-I and IGF x GLU. When included in Model 4 (plasma P_4), week postpartum contributed 62.9% of the total R^2. Variables common in Model 4 (plasma P_4) with and without week postpartum was plasma IGF-I concentration contributing 8.1% and 74.2%, respectively. Plasma INS concentration was a significant variable in Model 4 but it contributed less than 5% of total R^2.

Models for Days to First and Second Postpartum Ovulation. Models 5 and 6 predicted days to first and second postpartum ovulation, respectively, using the average weekly means (wk 1 to 5, Model 5 and wk 1 to 7, Model 6) of production variables (Table 5). Model 5 showed that EB had the greatest contribution to total R^2 (0.21) while for Model 6, percentage of milk LACT (0.43) (Table 5). Model 6 had a higher total R^2 compared to Model 5 (0.76 vs. 0.35) having the same number of variables (Table 5). Both models had FCM and DMI as common variables. Models 7 and 8 were derived using the average of weekly means (wk 1 to 5, Model 7 and wk 1 to 7, Model 8) of plasma hormones and metabolites to predict days to first and second postpartum ovulation, respectively. The square of plasma IGF-I concentrations was the only significant

predictor for days to first postpartum ovulation (Model 7), while days to second postpartum ovulation (Model 8) showed that the square of plasma GLU and plasma INS concentrations contributed 72.7% and 27.3%, respectively, to the total R^2 (Table 5). All other variables were not significant.

If days to first and second postpartum ovulation were modeled on a weekly basis, rather than using means of wk 1 to 5 or wk 1 to 7, the production variables that influenced first postpartum ovulation were EB, FCM and percentage of milk PROT (Table 6). Plasma hormones and metabolites that influenced first postpartum ovulation were plasma CHOL concentration that successively appeared from 2 to 5 wk postpartum and plasma IGF-I concentration that emerged 3 times (Table 7). The production variables that influenced second postpartum ovulation were FCM, DMI and percentage of milk LACT (Table 8). In the model of plasma hormones and metabolites, plasma GLU concentration emerged in 5 of 7 wk as a significant variable that influenced second postpartum ovulation (Table 9).

Model Prediction

Models for Plasma CHOL and P_4. Observed plasma CHOL concentrations and predicted values using Models 1 (production variables) and 3 (plasma hormones and metabolites) are plotted by week postpartum in Figure 1. Predicted plasma CHOL values derived using Model 1 were more similar than the values using Model 3 when compared to observed values. The weekly

postpartum residuals between observed plasma CHOL and predicted values ranged from –1.0 to 0.89 (Model 1 - production variables) and -0.97 to 0.86 (Model 3 - plasma hormones and metabolites). Because the range of residuals of Model 1 and 3 were similar and Model 1 predicted plasma CHOL concentrations better than Model 3, Model 1 is better than Model 3 in predicting plasma CHOL concentrations. Generally, the residuals between observed and predicted values using the regression coefficients of Models 1 (production variables) and 3 (plasma hormones and metabolites) for predicting plasma CHOL concentrations, congregate within -0.5 to 0.5 except for some outliers (Figure 2).

Observed plasma P_4 concentrations and predicted values derived using Models 2 (production variables) and 4 (plasma hormones and metabolites) are plotted in Figure 3. Observed values and predicted values of plasma P_4 concentrations using Models 2 and 4 were similar starting from 4 wk postpartum. Residuals between observed plasma P_4 concentrations and predicted values ranged from –3.5 to 3.5 (Model 2 - production variables) and –3.4 to 3.8 (Model 4 - plasma hormones and metabolites). Even though the residuals are slightly less diverse in Model 2 than Model 4, Models 2 and 4 are comparable in predicting plasma P_4 concentration as indicated by similar R^2 (Figure 3). Generally, the residuals for Models 2 (production variables) and 4 (plasma hormones and metabolites) (Figure 4) used for predicting plasma P_4 concentrations fall between –2 to 2 and are more dispersed than residuals obtained from Models 1 and 3 used for predicting plasma CHOL concentrations (Figure 2).

Models for Days to First and Second Ovulation. The residuals between observed and predicted days to first postpartum ovulation ranged from - 21 to 32 for Model 5 (production variables) and -22 to 36 for Model 7 (plasma hormones and metabolites, Figure 5). Because the dispersion of the residuals for the two models is equivalent and the R^2 of Model 5 is greater than that of Model 7, Model 5 (production variables) is slightly better predictor of days to first postpartum ovulation than Model 7 (plasma hormones and metabolites).

The difference between predicted and observed values for days to second postpartum ovulation ranged from – 6 to 11 for Model 6 (production variables) and –15 to 9 for Model 8 (plasma hormones and metabolites) (Figure 6). Because the dispersion of the residuals for Model 6 is less than Model 8 and the R^2 of Model 6 is over threefold greater than that of Model 8, days to second postpartum ovulation is best predicted by Model 6 (production variables).

DISCUSSION

During early postpartum the increase in plasma CHOL concentrations coincides with the increase in DMI and FCM with week postpartum (Carroll et al., 1990; Spicer et al., 1993b; Francisco, 2001, unpublished thesis). DMI and milk production and its components are more important factors driving changes in plasma cholesterol than are hormones and metabolites as shown in Models 1 and 3. In addition, the percentage of SNF in milk decreases with increasing milk production during the early postpartum period (McDonald et al., 1995; Francisco,

2001). The decrease in the percentage of milk SNF in early lactation is a possible mechanism to cope with lower DMI. This will reduce energy expenditure of cows for milk production and its components (Coppock et al., 1974; De Kruif and Mijten, 1992). Previously, plasma CHOL concentrations were consistently important in predicting nutritional status of lactating and non-lactating dairy cows (Kronfeld et al., 1982) and the fact that cross products that included DMI and EB were significant contributors to variation in plasma cholesterol levels in Model 1 of the present study support these results. In Model 3, the variables that contributed to predicting plasma CHOL concentrations were plasma GLU, INS and IGF-I concentrations. This is not surprising because during early lactation plasma INS, IGF-I and GLU concentrations increase concomitantly with plasma CHOL (Carroll et al., 1990; Spicer et al., 1993b; Francisco, 2001). This is further verified by the fact that week postpartum, when included in Model 3, results in a higher R^2 than the model with just plasma hormones and metabolites.

Both production variables and plasma hormones and metabolites predicted P_4 concentrations. In Model 2, weekly EB accounted for about 61% of the variability and is the most important predictor of the model. EB had been shown in several studies to modulate plasma P_4 concentrations during early postpartum (Villa-Godoy et al., 1988; Spicer et al, 1990, 1993b; Butler, 2000). Plasma P_4 concentrations have been associated positively with fertility (Folman et al., 1973) and pregnancy rates (Sklan et al., 1991) and negatively with EB (Villa-Godoy et al, 1988; Spicer et al., 1990; Butler, 2000) and days open (Sklan et al., 1991). In addition, a negative EB reduces the weight of corpus luteum

(Apgar et al., 1975) and decreases steroidogenic activity of luteal tissue (Villa-Godoy et al., 1990). Thus, the weekly energy status of the cows measured as the difference between net energy intake and energy expended for lactation plus maintenance in the present study, served as a good predictor of return to normal ovarian activity as measured by P_4 production. In Model 4, IGF-I accounted for 74% of the variability and served as the best predictor of plasma P_4 levels in early postpartum cows. Plasma IGF-I concentrations increase with week postpartum as does plasma P_4 concentrations (Abribat et al., 1990; Spicer et al., 1990) and are positively correlated with energy status and P_4 production in early postpartum cows (Spicer et al., 1990, 1993; Lucy et al., 1992). Also, IGF-I stimulates bovine luteal cell P_4 production (Sauerwein et al., 1992; Liebermann et al., 1996). Thus, it is not surprising that a plasma IGF-I concentration is a good predictor of plasma P_4 concentration in early postpartum cows. Values predicted by both Models 2 and 4 closely matched observed plasma P_4 concentrations after wk 4 of lactation suggesting that both models were precise from 4 to 12 wk postpartum period. Because the dispersion of the residuals for Model 2 were slightly less than for Model 4, but R^2 of Model 4 was slightly greater than that of Model 2, both models appear similar in their ability to predict plasma P_4 concentrations. Thus, plasma P_4 concentration could be predicted as accurately by EB as by plasma IGF-I concentrations and further imply that IGF-I may mediate the effect of EB on luteal function as previously suggested (Spicer et al., 1990). The CHOL models were more accurate models (i.e., lower residuals and greater R^2) than the P_4 models, probably because plasma CHOL concentrations steadily increase with

week postpartum and then plateau after 8 wk postpartum compared to plasma P_4 concentrations that increase and decrease every 21 d after the first postpartum estrous cycle.

The main predictors of the models without week postpartum become secondary predictors when week postpartum was in the model. For instance, in Model 2 (plasma CHOL), the variable GLU^2 largely predicts the model (55%) without week postpartum but becomes a secondary predictor when week postpartum is included in the model (7.6%). Also, most variables that have values that run parallel with week postpartum were normally overshadowed by week postpartum. IGF-I contributed 74.2% to the variability of the P_4 model (Model 3) without week postpartum but decreased to 8.1% when week postpartum was included in the model. Week postpartum contributed more to model R^2 of plasma CHOL (Models 1 and 2) than plasma P_4 (Models 3 and 4). This can be partially attributed to the parallel and more consistent increase in plasma CHOL with week postpartum than plasma P_4 as eluded to earlier. Collectively, week postpartum contributed significantly to the variation in plasma CHOL and P_4 concentration when included in the models. However, the removal of week in the models helped to determine which production, endocrine and metabolic variables contributed the most to the models independent of week postpartum.

In Model 5, EB "best" predicted days to first postpartum ovulation. This model supports the findings of others that EB during the early postpartum period

is a critical factor to the return to reproductive cyclicity (Butler et al., 1981; Berghorn et al., 1988; Butler and Smith, 1989; Staples et al., 1998).

Out of several metabolic and hormonal variables included in the model, first postpartum ovulation was influenced most by plasma IGF-I concentrations. Plasma IGF-I concentrations steadily increase with week postpartum, and may reach a threshold that serves as a signal that first postpartum ovulation may occur. Generally, plasma IGF-I peaks around 7 to 8 wk postpartum long after which the first postpartum ovulation normally takes place (Abribat et al., 1990; Spicer et al., 1990). Furthermore, IGF-I is positively correlated with P_4 (as mentioned earlier), which is necessary to maintain pregnancy after ovulation. On a weekly basis, models for first postpartum ovulation appeared to be influenced most by plasma CHOL and IGF-I and this coincides with the results of Model 7. Plasma CHOL is a known precursor of P_4 (Grummer and Carrol, 1988; Savion et al., 1992), and necessary to maintain pregnancy after ovulation. Also, IGF-I is a potent stimulator of HDL and LDL metabolism and P_4 biosynthesis by ovarian cells (Veldhuis et al., 1987; Veldhuis and Gwynne, 1989)

Percentage milk lactose was the only production variable in Model 6 that contributed significantly to variation in interval to second postpartum ovulation. This result is indirectly substantiated by the fact that return to reproductive efficiency is influenced by the EB of the cow, and EB is partially explained by the variability of milk components according to the models of Heuer et al. (2000). Among the metabolic and hormonal variables included in Model 6, plasma GLU and INS were important indicators of return to second postpartum ovulation.

Because glucose is the main source of energy for ovarian function (Rabiee et al., 1997) and influences bovine thecal cell steroidogenesis in vitro (Stewart et al., 1995), it may play a major role in achievement of postpartum ovulation. In a study by Rabiee and Lean (2000), they found that there was a positive and highly significant cross correlation (r = 0.5) between the uptake of GLU and CHOL and suggested that GLU may promote CHOL uptake into ovarian cells or vice versa. Also, low plasma INS concentrations directly decreased LH pulsatility (Butler and Smith, 1989; Canfield and Butler, 1990). Moreover, INS administered in vivo increases estradiol concentrations in follicular fluid of superovulated cattle (Simpson et al., 1994). In cultured granulosa cells, insulin augments FSH-induced P_4 production (Langhout et al., 1991) and LDL metabolism (Veldhuis and Gwynne, 1989), increases proliferation (Langhout et al., 1991; Spicer et al., 1993a) and stimulates aromatase activity (Spicer and Francisco, 1997; Spicer and Chamberlain, 1998). Thus, lower insulin that is normally observed in early lactating cows (Koprowski and Tucker, 1973; Smith et al, 1976; Hart et al., 1978) likely affects days to return to postpartum ovulation by direct action on the ovary.

During early postpartum where lactation overrides other physiological processes (Bauman and Curie, 1980; Swanson, 1989), glucose a precursor of milk lactose may become limiting to other physiological processes such as postpartum ovulation (Staples and Thatcher, 1990). Interestingly, milk lactose was the most important production variable whereas plasma INS and GLU were the most important hormone/metabolites variables modeling days to second postpartum ovulation. The energetic efficiency of converting glucose to milk

lactose is 0.98 (McDonald et al., 1995). If early postpartum DMI is low and if a cow produces more milk, then glucose supply to the ovary may not be sufficient for a cow to return to postpartum ovulation. Results of Model 5 are consistent with this latter statement because EB, DMI and FCM contributed significantly to days to first postpartum ovulation. Contrary to this, Stevenson and Britt (1980) showed that energy intake and milk yield during the first 2 wk of lactation provided small contribution to the model predicting days to first ovulation. This is probably due to shorter period of data collection (2 wk vs. 12 wk), and the fact that EB and milk production are low and increasing during the first 2 wk postpartum. The models developed in this study covered a longer postpartum period (5 to12 wk) than the model of Stevenson and Britt (1980), which only covered 2 wk postpartum. In the present study, some of the same variables (i.e., IGF-I, CHOL, GLU and INS) emerged in the models predicting days to first and second postpartum ovulation when values for each week were modeled.

CONCLUSIONS

Relationships between plasma CHOL and P_4 with EB, DMI, milk yield and composition, metabolites and hormones and week postpartum were modeled using multivariate regression analysis. Models using EB, DMI, FCM, BW and milk composition as variables consistently predicted both plasma CHOL and P_4 quite well as shown by the minimal residuals between predicted and observed values. Models using metabolites and hormones as variables predicted plasma

CHOL more accurately than they predicted plasma P_4 based on the magnitude of residuals. The primary variables involved with predicting plasma CHOL were DMI x SNF (Model 1 - production variables) and the square of GLU (Model 3 - plasma hormones and metabolites), contributing most to the total model R^2 (63 and 55%, respectively). For P_4, EB (Model 2 - production variables) and IGF-I (Model 4 - plasma hormones and metabolites) were the primary contributors to the total model, providing 61 and 74% of the total model R^2 values, respectively. Week postpartum when included in the model was the major predictor in Models 1 to 4, although cross products of EB were significant contributors to Models 1 and 2 and IGF-I and (or) cross-products of GLU were significant contributors of Models 3 and 4.

Days to first and second postpartum ovulation were "best" predicted by production variables EB and milk lactose percentage (Models 5 and 6). Plasma metabolic and hormonal predictors for days to first and second postpartum ovulations were IGF-I^2 (Model 7 - plasma hormones and metabolites) and INS (Model 8 - plasma hormones and metabolites), respectively. Previous studies have implicated both IGF-I and insulin as important regulators of ovarian function. Data analysis from other herds or studies should be used to further validate these results.

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Table 1. Mean, SD, minimum and maximum values (n = 228) of the variables used in predicting plasma cholesterol (CHOL) and progesterone (P_4) in lactating dairy cows (Models 1 - 4).

Variable	Mean	SD	Minimum	Maximum
Model 1 and 2				
Energy Balance (EB), Mcal/d	-0.6	6.2	-19.0	18.0
Body Weight (BW), kg	641.8	56.9	527.3	779.1
Dry Matter Intake (DMI), kg/d	23.7	4.0	13.1	33.4
Fat Corrected Milk (FCM), kg/d	34.8	6.7	12.4	68.5
Milk Lactose (LACT), %	5.0	0.2	4.1	5.4
Milk Fat (FAT), %	3.1	0.8	0.8	6.6
Milk Protein (PROT), %	3.2	0.6	2.2	9.7
Solids-Not-Fat (SNF), %	8.9	0.6	7.3	14.7
Model 3 and 4				
Insulin-Like Growth Factor-I (IGF-I), ng/mL	10.6	6.0	1.4	39.2
Insulin (INS), ng/mL	0.4	0.2	0.2	1.2
Glucose (GLU), mg/dL	60.1	5.0	42.5	77.5

Table 2. Mean, SD, minimum and maximum values (n = 15) of the variables used in predicting days to first (Models 5 and 7) second (Models 6 and 8) postpartum ovulation in lactating dairy cows .

Variable	Mean	SD	Minimum	Maximum
Model 5				
Energy Balance (EB), Mcal/d	-4.1	3.4	-8.4	6.2
Body Weight (BW), kg	646.5	56	566.9	746.9
Dry Matter Intake (DMI), kg/d	21.4	2.6	19.1	29.5
Fat Corrected Milk (FCM), kg/d	33.8	3.9	28.4	42.0
Milk Lactose (LACT), %	4.9	0.1	4.7	5.2
Milk Fat (FAT), %	3.3	0.5	2.6	4.0
Milk Protein (PROT), %	3.5	0.3	3.1	4.5
Solids-Not-Fat (SNF), %	9.2	0.4	8.6	10.1
Model 7				
Cholesterol (CHOL), mg/dL	132.8	22.6	90.1	171.2
Insulin-Like Growth Factor-I (IGF-I), ng/mL	7.8	3.9	2.6	15.1
Insulin (INS), ng/mL	0.3	0.1	0.2	0.6
Glucose (GLU), mg/dL	59.6	6.0	46.2	76.4
Model 6				
Energy Balance (EB), Mcal/d	-3.4	3.5	8.0	5.0
Body Weight (BW), kg	649.6	54.5	572.7	741.3
Dry Matter Intake (DMI), kg/d	22.2	1.7	20.4	24.6
Fat Corrected Milk (FCM), kg/d	34.9	5.0	29.7	45.9
Milk Lactose (LACT), %	5.0	0.1	4.8	5.3
Milk Fat (FAT), %	3.3	0.8	2.6	4.0
Milk Protein (PROT), %	3.4	0.3	3.1	4.1
Solids-Not-Fat (SNF), %	9.2	0.4	8.6	9.7
Model 8				
Cholesterol (CHOL), mg/dL	148.0	22.1	114.6	184.3
Insulin-Like Growth Factor-I (IGF-I), ng/mL	9.8	4.1	3.1	15.3
Insulin (INS), ng/mL	0.4	0.1	0.3	0.5
Glucose (GLU), mg/dL	60.8	1.8	57.3	63.5

Table 3. Variables and proportion of R^2 in the model predicting plasma cholesterol and progesterone using weekly measurements (n = 228) of production variables, hormones and metabolitesin lactating dairy cows.

Variables*	Partial R^2	% Contribution to the Model	Total R^2
Model 1			
Plasma Cholesterol Model			**0.68**
DMI x SNF	**0.43****	63.2	
FCM x SNF	**0.19****	28.1	
Model 2			
Plasma Progesterone Model			**0.29**
EB	**0.18****	61.1	
EB x FAT	**0.05****	17.1	
FCM x LACT	**0.04****	14.2	
Model 3			
Plasma Cholesterol Model			**0.37**
GLU^2	**0.20****	55.0	
IGF-I	**0.07****	18.9	
IGF-I x GLU	**0.04****	11.8	
IGF-I x INS	**0.03****	8.8	
INS	**0.02****	5.5	
Model 4			
Plasma Progesterone Model			**0.33**
IGF-I	**0.25****	74.2	
GLU^2	**0.07****	21.8	

* Variables included are only those that contributed > 5% of the total R^2.
** $P < 0.01$

Table 4. Partial R^2 and percent contribution of week postpartum when included in plasma cholesterol and progesterone models (Models 1- 4) using weekly measurements of variables (production, hormone and metabolites) in lactating dairy cows

	Partial R^2	% Contribution*	Total R^2
Production Variables			
Model 1 - Plasma Cholesterol Model			**0.73**
Week	**0.52****	71.6	
EB x FAT	**0.09****	12.5	
FCM x LACT	**0.08****	10.3	
Model 2- Plasma Progesterone Model			**0.37**
Week	**0.25****	67.9	
BW x SNF	**0.02****	8.1	
EB x LACT	**0.03****	8.0	
Hormones and Metabolic Variables			
Model 3 - Plasma Cholesterol Model			**0.59**
Week	**0.53****	88.2	
GLU^2	**0.05****	7.6	
Model 4 - Plasma Progesterone Model			**0.41**
Week	**0.26****	62.9	
INS x GLU	**0.12****	28.0	
IGF	**0.03****	8.1	

* Variables included are only those that contributed > 5% of the total R^2.
** $P < 0.01$

Table 5. Variables and proportion of R^2 in the model predicting first (n = 15) and second (n = 11) postpartum ovulation using the average of weekly (wk 1 to 5, Model 5 and 7; wk 1 to 7, Model 6 and 8) measurements of variables (production, hormones and metabolites) in lactating dairy cows.

Variables[a]	Partial R^2	Partial R^2 (%)	Total R^2
Model 5			
First Postpartum Ovulation Model (d)			**0.35**
EB	0.21*	59.8	
DMI	0.09*	26.6	
FCM	0.05*	13.7	
Model 6			
Second Postpartum Ovulation Model (d)			**0.76**
LACT	0.43**	57.2	
FCM	0.22*	29.5	
DMI	0.10*	13.3	
Model 7			
First Postpartum Ovulation Model (d)			**0.28**
IGF-I[2]	0.28*	100.0	
Model 8			
Second Postpartum Ovulation Model (d)			**0.32**
GLU[2]	0.23[£]	72.7	
INS	0.09[£]	27.3	

[a] Variables included are only those that contributed > 5% of the total R^2.

** $P < 0.01$

* $P < 0.05$

[£] $P < 0.1$

Table 6. Variables and R^2 of the models predicting first and second postpartum ovulation in lactating dairy cows using measurements of production variables on a weekly postpartum basis.[a]

First Postpartum Ovulation

Week	Production and Milk Component Variables				R^2
1	**EB**				**0.16**
	(0.15)[b]				
2	**FAT**	**PROT**			**0.34**
	(0.06)	(0.10)			
3	**FCM**	**PROT**	**LACT**	**SNF**	**0.56**
	(0.05)	(0.10)	(0.07)	(0.12)	
4	**FCM**	**EB**			**0.25**
	(0.15)	*(0.12)*			

Second Postpartum Ovulation

Week	Production and Milk Component Variables				R^2
1	**FCM**	**DMI**	**EB**	**SNF**	**0.71**
	(0.03)	*(0.02)*	*(0.02)*	*(0.04)*	
2	**LACT**				**0.39**
	(0.05)				
3	**LACT**				**0.57**
	(0.01)				
4	**EB**	**PROT**	**SNF**		**0.87**
	(0.01)	*(0.02)*	*(0.01)*		
5	**FCM**	**DMI**			**0.44**
	(0.09)	*(0.04)*			
6	**FCM**	**LACT**	**BW**	**FAT**	**0.65**
	(0.06)	*(0.03)*	*(0.12)*	*(0.13)*	
7	**FCM**	**DMI**	**PROT**		**0.96**
	(0.01)	*(0.01)*	*(0.01)*		

[a]Multiple regression using backward elimination technique of variables that contributed to R^2 at $P < 0.15$.

[b]*(p-value)*

Table 7. Variables and R^2 of the models predicting first and second postpartum ovulation in lactating dairy cows using measurements of plasma hormones and metabolites on a weekly postpartum basis.[a]

First Postpartum Ovulation

Week	Plasma Hormones and Metabolic Variables						R^2
1	INS	GLU	GLU^2				0.38
	(0.12)[b]	*(0.03)*	*(0.03)*				
2	IGF-I	$CHOL^2$	GLU^2				0.64
	(0.03)	*(0.01)*	*(0.03)*				
3	IGF-I	CHOL					0.41
	(0.04)	(0.14)					
4	CHOL	INS	GLU	$CHOL^2$	INS^2	GLU^2	0.91
	(0.001)	*(0.001)*	*(0.02)*	*(0.001)*	*(0.001)*	(0.02)	
5	CHOL	IGF-I	$CHOL^2$				0.56
	(0.07)	(0.02)	(0.07)				

Second Postpartum Ovulation

Week	Plasma Hormones and Metabolic Variables					R^2
1	INS	INS^2	GLU^2			0.52
	(0.03)	*(0.03)*	*(0.11)*			
2	IGF	IGF^2				0.15
	(0.11)	*(0.08)*				
3	GLU					0.47
	(0.02)					
4	CHOL	INS	$CHOL^2$	INS^2		0.71
	(0.02)	*(0.02)*	*(0.02)*	*(0.03)*		
5	GLU	GLU^2				0.49
	(0.03)	*(0.03)*				
6	GLU	GLU^2				0.39
	(0.05)	*(0.05)*				
7	CHOL	GLU	$CHOL^2$	INS^2	GLU^2	0.82
	(0.03)	*(0.01)*	*(0.03)*	*(0.01)*	*(0.01)*	

[a]Multiple regression using backward elimination technique of variables that contributed to R^2 at $P < 0.15$.

[b]*(p-value)*

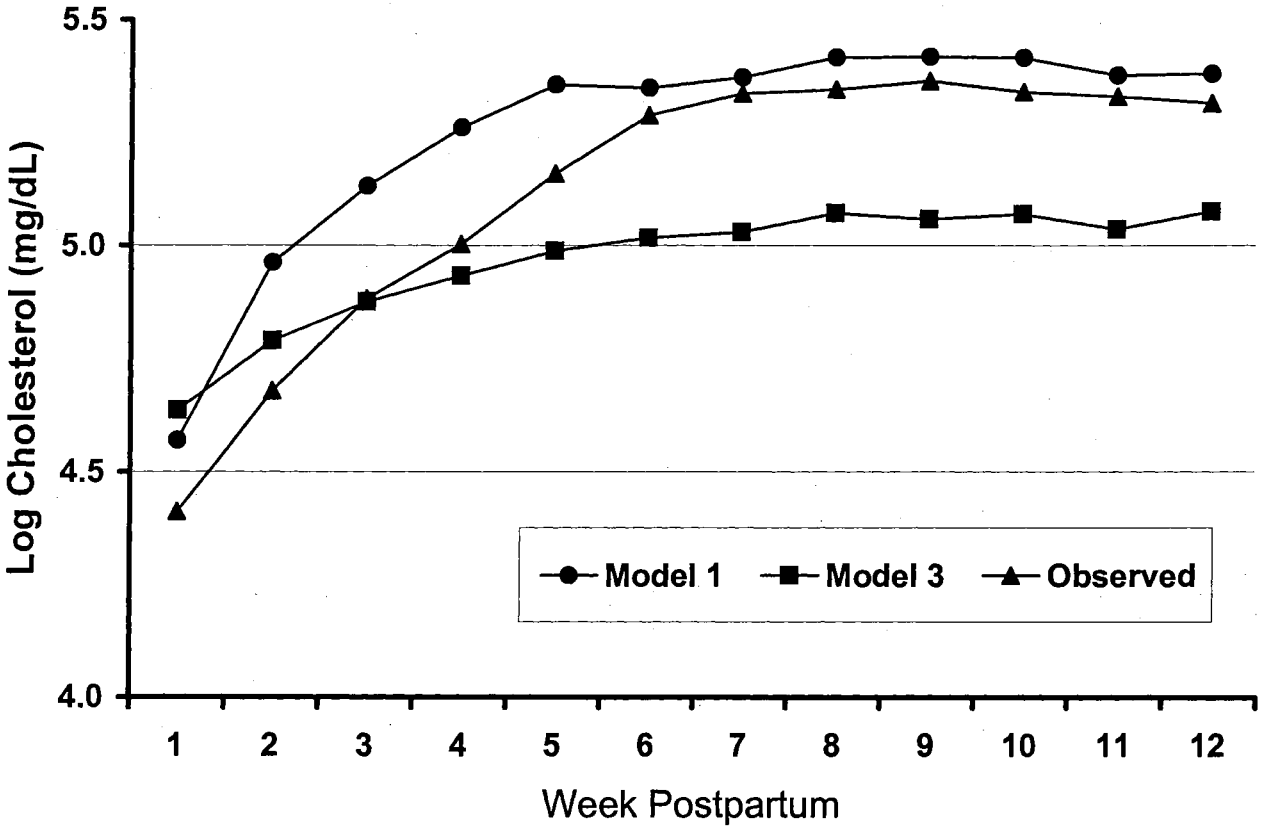

Figure 1. Predicted weekly means (n = 228) of log cholesterol with week postpartum using Models 1 (production variables) and 3 (plasma hormones and metabolites) compared with actual measured values. SD = Model 1 (0.26), Model 3 (0.13) and Observed (0.18).

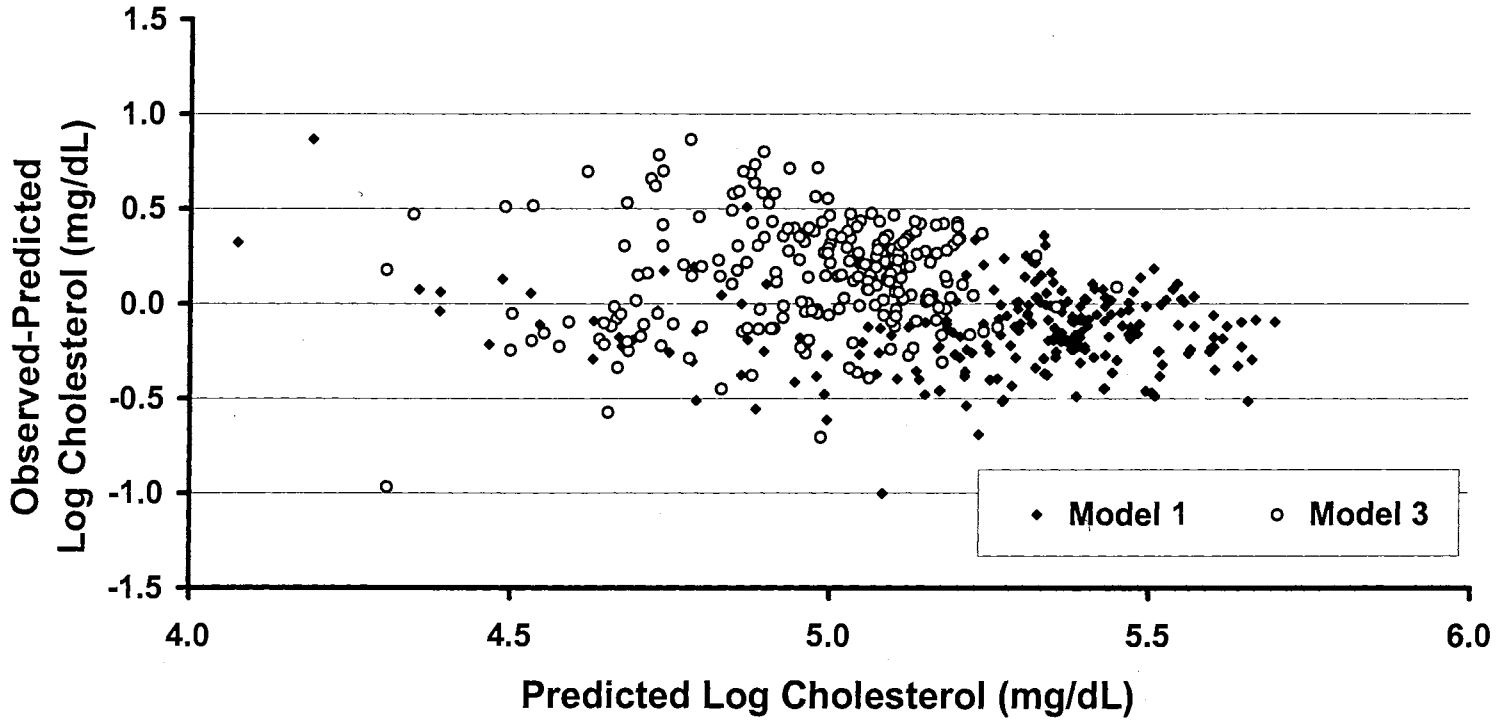

Figure 2 . Plot of residuals of observed -predicted log cholesterol versus predicted log cholesterol values using regression coefficients of Model 1 (production variables) and Model 3 (plasma hormones and metabolites).

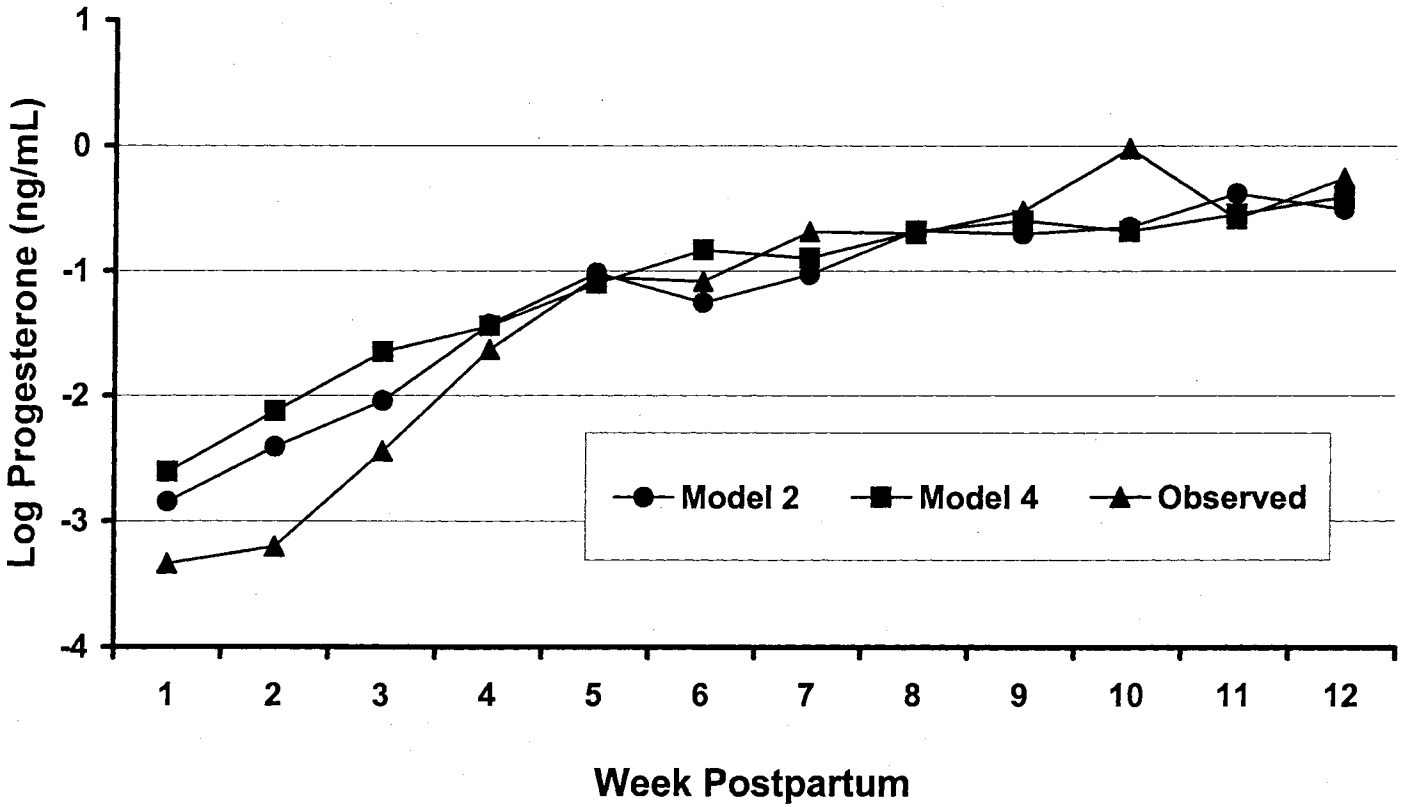

Figure 3. Predicted weekly means (n = 228) of log progesterone with week postpartum using Models 2 (production variables) and 4 (plasma hormones and metabolites) compared with actual measured values. SD = Model 2 (0.79), Model 4 (0.69) and Observed (1.12).

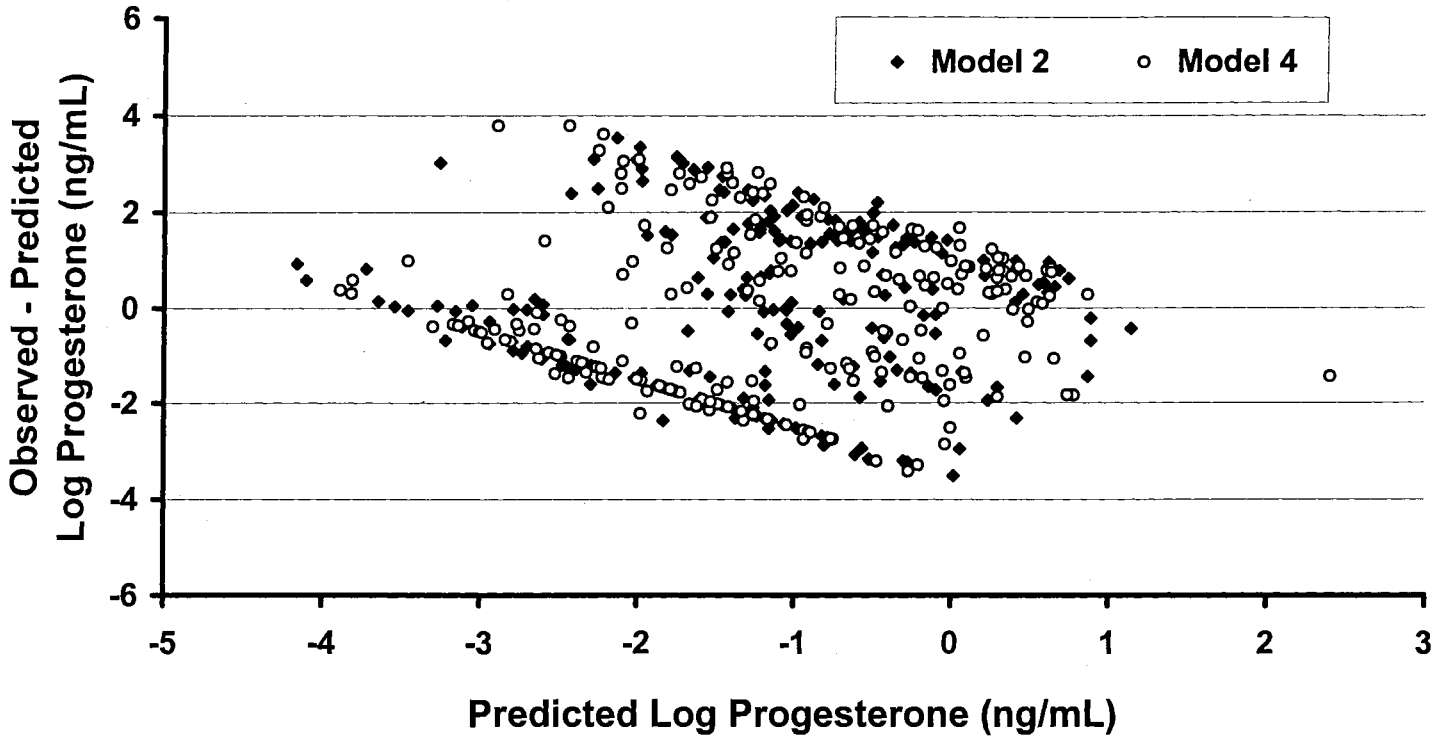

Figure 4 . Plot of residuals of observed -predicted log progesterone versus predicted log progesterone values using model coefficients of Model 2 (production variables) and Model 4 (plasma hormones and metabolites).

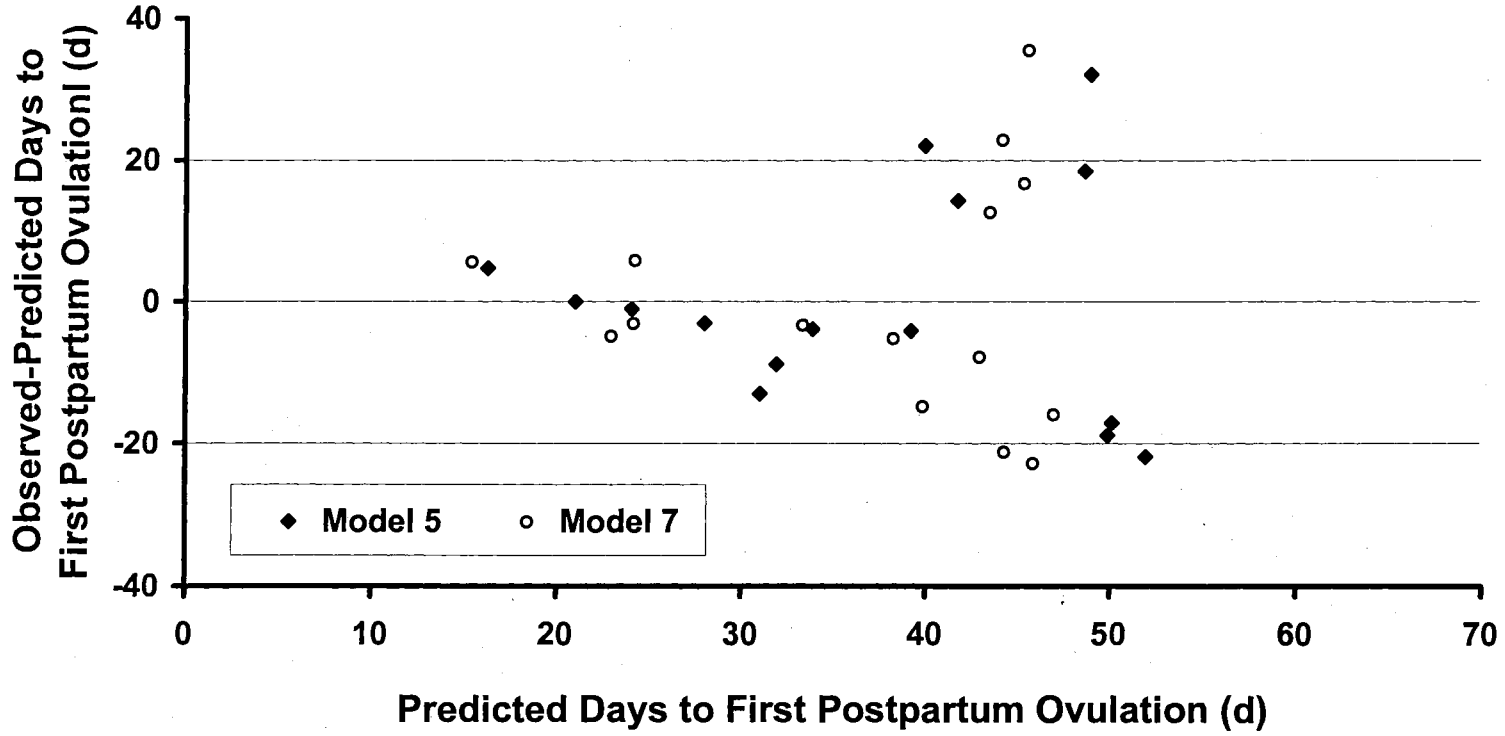

Figure 5 . Plot of residuals (n = 15) of observed -predicted days to first postpartum ovulation versus predicted days to first postpartum ovulation values using regression coefficients of Models 5 (production variables) and Model 7 (plasma hormone and metabolites) .

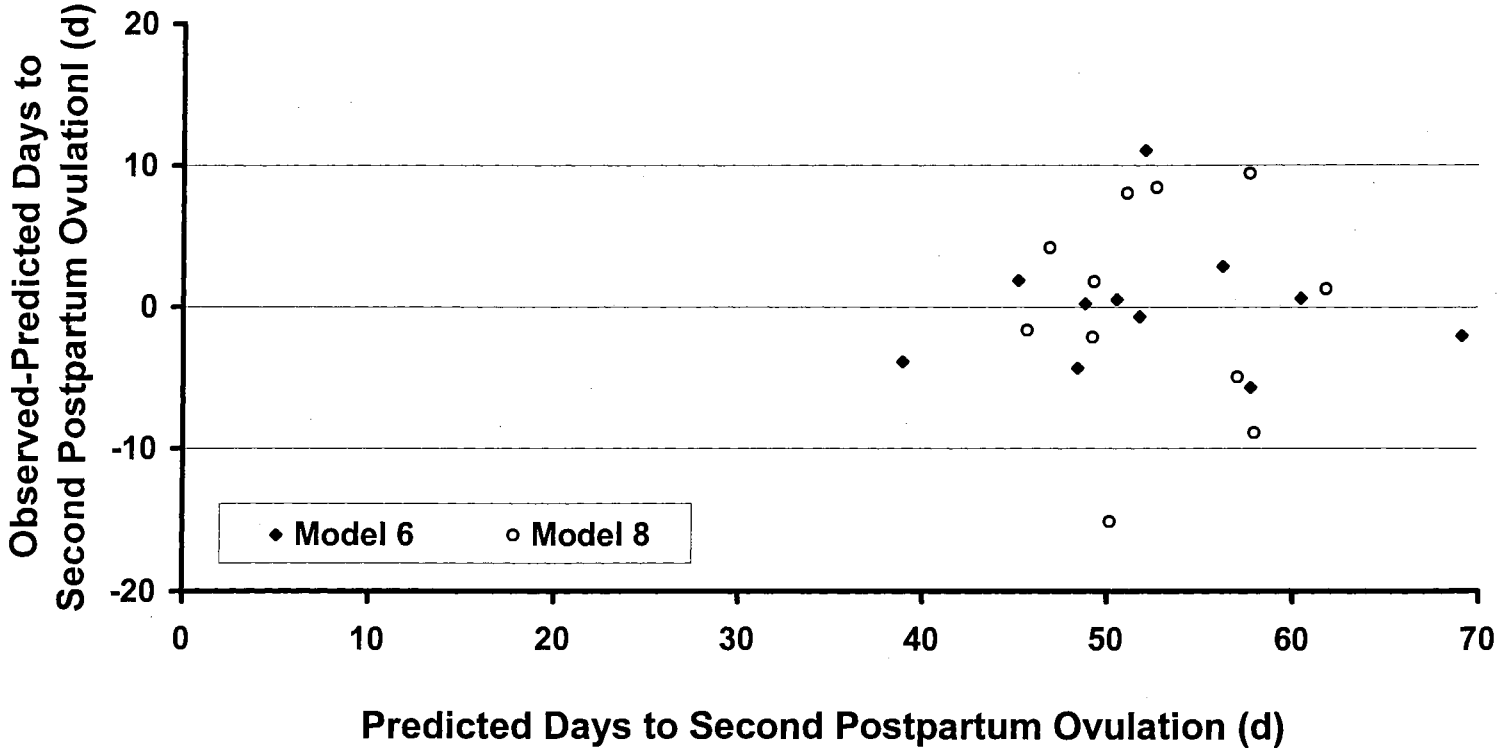

Figure 6 . Plot of residuals (n = 11) of observed -predicted days to second postpartum ovulation versus predicted days to second postpartum ovulation values using regression coefficients of Models 6 (production variables) and Model 8 (plasma hormones and metabolites).

VITA

Cristina C. Francisco

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Doctor of Philosophy

Thesis: **DIRECTLY FED PROPIONIBACTERIA: EFFECTS ON ENERGY BALANCE, METABOLITES, HORMONES AND REPRODUCTION IN EARLY POSTPARTUM DAIRY COWS**

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