ABSTRACT

NORDBLADH, LOUISE I. Postpartum Changes in Hormones and Metabolites During Early Lactation in Summer and Winter Calving Holstein Cows. (Under the direction of Dr. Scott C. Whisnant)

Changes in metabolites and metabolic hormones during the first 12 weeks postpartum in both summer and winter were analyzed on a total of 18 lactating, Holstein cows (summer, n=11; winter, n=7). The summer trial covered the months of August through October. The winter trial began in November and lasted through early February. Maximum and minimum temperature and humidity values were recorded daily. Blood was sampled in serum, fluoride and heparin tubes from the coccygeal vein once a week beginning at day of calving for 12 weeks. Concentrations of progesterone, cortisol, thyroxine, leptin, NEFA, cholesterol and insulin were analyzed from serum samples. The metabolites glucose, PUN and β-HBA were analyzed with plasma collected from fluoride and heparin containing tubes. No significant seasonal differences were found between summer and winter calving groups for P_4, T_4, glucose, insulin, PUN and NEFA. However, leptin ($P < 0.01$), cholesterol (CHL) ($P < 0.0001$) and β-HBA ($P < 0.0001$) had significant seasonal differences between the two calving groups. The mild heat stress during the early part of the summer trial may not have been severe enough to detect significant changes in serum T_4 or the animals had become acclimated to these temperatures. Summer calving cows may have experienced a drop in feed intake at calving (not measured) enough to increase their plasma ketone bodies (β-HBA), but not enough to dramatically reduce circulating levels of glucose and insulin.

POSTPARTUM CHANGES IN HORMONES AND METABOLITES DURING EARLY LACTATION IN SUMMER AND WINTER CALVING HOLSTEIN COWS

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master's of Science

DEPARTMENT OF ANIMAL SCIENCE

Raleigh

2003

APPROVED BY:

Chair of Advisory Committee

BIOGRAPHY

Louise I. Nordbladh was born on September 28, 1979 in Vaxjo, Sweden to the parents Leif and Margareta Nordbladh. Louise was the youngest of three children, preceded by brothers Andre and Marcus. At nine months of age, Leif moved the family to the state of Connecticut. Louise's fondest memories of her childhood include seven years of horseback riding and competing in horse shows. At age 13, the Nordbladh family made another big move to High Point, North Carolina, the furniture capital of the world. Louise attended High Point Central High School, with the future plans of going to North Carolina State University and eventually applying to veterinary school.

Upon acceptance into N.C. State's class of 2001, Louise majored in Animal Science. Although going on to apply to several vet schools, Louise began to realize the stiff competition for entrance. Graduate school was a possibility and became a reality upon learning she had not been accepted into vet school. Louise decided to enter the Animal Science graduate program at N.C. State under her advisor, Dr. Scott Whisnant, a reproductive endocrinologist. The focus of her research project was to study postpartum hormonal and metabolic changes in Holstein cows and compare potential seasonal differences between summer and winter calving groups. Completing her masters of science degree in two years, Louise has been admitted into the cytotechnology graduate certificate program at the University of North Carolina at Chapel Hill to become a certified cytotechnician analyzing human cell specimens for diagnosis of disease and cancer.

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INTRODUCTION

The effects of heat stress on cattle production, specifically reproductive efficiency have been reported as early as the 5th century B.C. by Hippocrates in *On Airs, Waters and Places*. Modern reports of depressed fertility during summer in cattle were reported in 1940 by Erb and colleagues (Hansen and Aréchiga, 1999). Fertility has been shown to be compromised due to the vulnerability of the early developing embryo to increased temperatures. Other effects include reduced estrus behavior, changes in follicular dynamics, disruption of gonadotropin secretions, hypophagia and potentially reduced milk yields under severe heat stress conditions.

Due to the genetic selection for higher producing dairy cows, milk yield has substantially increased since the 1950's. In order to sustain higher milk yields, these genetically superior dairy cows must naturally be provided with greater quantities of high-quality feed. This generates increased metabolic heat from the processes of ruminal microbial fermentation. Elevated ambient temperatures during hot summer months impose an additional heat load. The subsequent effects of elevated environmental temperatures seen in dairy cattle depend on the duration and degree of heat stress, individual ability to cope with the stress and implementation of cooling systems to alleviate these effects.

The transition period is likely the most stressful period in the dairy cow's annual cycle, both physiologically and metabolically. The drastic reproductive changes that occur at parturition as well as the high metabolic demands required to initiate lactation impose great stress on these animals. Of particular interest for the current research is the postpartum, early lactation Holstein cow. Comparing seasonal effects between summer

and winter calving groups and the analysis of 10 different hormones and metabolites was the focus of this research project.

REVIEW OF LITERATURE

Effects of Heat Stress

It has been well documented that heat stress has detrimental effects on reproductive performance and milk production in cows. High environmental temperatures have been linked to reduced milk yields in dairy cows in their subsequent lactation cycles (Collier et al., 1982). It has been estimated that up to 60% of cattle worldwide are affected by heat stress, a major contributor to reduced fertility, especially in the lactating dairy cow (Wolfenson et al., 2000). Signs of heat stress include increased rectal temperatures and respiration rates (Yousef and Johnson, 1966). The cow compensates by reducing feed intake and increasing water consumption to prevent excessive metabolic heat production. Reduced fertility of the cow may be caused by direct effect on embryonic development or disruption of maternal endocrine functioning (Thatcher, 1974). One of the parameters used to determine severity of heat stress is the temperature-humidity index (THI) that is an average measure of temperature and humidity over a 24-hour period (Ingraham et al., 1974). A THI value of less than 70 is considered to be within the cow's thermoneutral zone (Ingraham et al., 1976). Temperatures above 80˚F may result in significant reductions in feed intake, while temperatures above 90˚F may decrease milk yield (McGuire et al., 1991). Under heat stress conditions, the reduction in feed intake and total nutrient absorption may be an additional factor leading to a decline in milk production (McGuire et al., 1989).

Lactation

The periparturient cow or transition cow may be most adversely affected by heat stress, with increases seen in respiration rates and rectal, vaginal and skin temperatures during summer versus winter months. Heat stress did not significantly alter heart rate (Huhnke and Monty, 1976). There are significant hormonal and metabolic changes that occur within a 4 week period as the cow makes the transition from a state of tissue deposition during pregnancy, to a state of tissue mobilization for lactation (Stockdale and Roche, 2002). The transition period is generally about 3 weeks prepartum to 3 weeks postpartum, a time when the incidence of metabolic disease is greatest (Drackley, 1999). One study reported that late lactation, heat stressed cows (32˚C) had a 69% decline in milk production (Yousef and Johnson, 1966). Pregnant cows exposed to no shade treatment had depressed milk yields in the subsequent lactation versus cows assigned to shade. This was directly related to decreased calf weights that seem to significantly alter the cow's postpartum milk yield (Collier et al., 1982). Benson and Morris (1971) reported that pregnant rats exposed to elevated temperatures late in gestation had pups of lower birth weights and reduced milk production postpartum. They also observed a decrease in food consumption, body weight and increased water intake. The bovine fetus has increased energy demands during the last month of gestation as it grows rapidly, gaining the majority of its weight. The mammary gland is simultaneously preparing for the ensuing lactation cycle (Bell et al., 1995; Bell and Bauman, 1997). Thus, the cow's priority is to provide nutrients for the calf, not for her own tissue. The combined physiological and environmental stresses imposed on the transition cow make it

especially important to reduce preventable stressors and optimize feed intake prior to parturition.

Feed Intake

Beginning about 3 weeks prepartum, the dairy cow reduces her feed intake. This seems to be a normal adaptation response seen in both ruminant and monogastric species during the periparturient period (Ingvartsen and Andersen, 2000). Dry matter intake can decline 30% with a more significant decline the final two days before parturition (Bertics et al., 1992; Grummer, 1995). Other studies have reported comparable results with a 20% decline in feed intake the last 10 days of gestation (Hartwell et al., 2000). Cows with the greatest depression in feed intake the last 2 to 3 days prepartum are typically the slowest to regain appetite postpartum (Grummer, 1995). Postpartum dairy cows need to increase their feed intake about 4 to 6 fold in order to meet the energy demands of lactation (Roche et al., 2000). During the transition period the dairy cow has two sources of energy, dietary intake and mobilized energy reserves mainly from adipose tissue. Most transition cows experience some degree of negative energy balance during early lactation since the amount of energy required by the cow for maintenance and milk production exceed the supply of nutrients from dietary sources alone. Early lactation is considered to be the first 5 weeks of the lactation cycle. Therefore, there is a need for adipose tissue to be mobilized to provide energy for the cow's bodily functions (Goff and Horst, 1997). Dairy cows can lose up to 60% of their adipose tissue reserves during early lactation (Tamminga et al., 1997). During the first 3 weeks of lactation, dry matter intake increases between about 1.5 to 2.5 kilograms per week (Bertics et al., 1992). It is

therefore crucial that feed intake is maximized or the decline in feeding be minimized. It has been recommended that producers begin increasing dry matter availability about 3 weeks prepartum in attempts to lessen the severity of negative energy balance. Since there is currently little knowledge on what dietary components and amounts to feed the transition cow, producers should minimize competition and not limit feed availability. Social interactions among the herdmates should be monitored as competition for feeding may drive down intake more significantly in subordinate cows, calling for separate feeding areas (Stockdale and Roche, 2002). Some studies have shown that increasing prepartum feed intake resulted in increased milk yields postpartum, but these results have not always been consistent (Stockdale, 2001). Other than feeding management, environmental factors may also impose stress on the transition cow. Temperature is a major factor that can influence feeding behavior, as intake is not only reduced prepartum but the rate of increase postpartum may be slowed (Grant and Albright, 1995). Baile and Forbes (1974) reported that increasing temperatures may have a direct effect on the appetite control center within the hypothalamus. Hypophagia has been linked to increased incidence of metabolic diseases such as hepatic lipidosis and ketosis in transition cows. During late gestation and into early lactation, the dip in feed intake coincides with changes in metabolism, reproductive status and adipose tissue. The mechanisms that control voluntary feed intake are complex. There are a number of hormones that are potentially direct or indirect regulators of feed intake. The amount of fat, as well as reproductive hormones, stress hormones, leptin and insulin are thought to be important regulators of feed intake in the transition cow (Ingvartsen and Andersen, 2000). Nutritional management beginning 3 weeks prior to and following parturition is

critical to improving reproductive efficiency. In order to achieve normal resumption of ovarian cyclicity, including corpora lutea development and progesterone secretion in the high-producing dairy cow, it is crucial that feed intake during the early postpartum period be maximized (Roche et al., 2000).

Reproduction

The potential effects of heat stress on fertility include decreased conception rates, increased embryonic death, decreased estrus behavior and disruption of normal gonadotropin and estradiol secretions (Ronchi et al., 2001). Studies have demonstrated that heat stress caused lower GnRH pulse frequency with a subsequent decrease in LH, smaller diameter of the dominant follicle and a longer interval to first estrus (Roche et al., 2000; Wolfenson et al., 2000). It has been postulated that the most direct effect of hyperthermia is the alteration of cellular functioning, including suppression of the dominant follicle so that the second wave dominant follicle could arise earlier than normally. At the cellular level, heat stress disrupts theca and granulosa cell capabilities for steroidogenesis (i.e. depressed aromatase activity), endometrial secretions and decreased progesterone production by luteal cells. In addition, oocyte and embryonic growth and functioning are impaired. Therefore, it is thought that it is not the bovine follicular wave pattern that is disrupted by heat stress, but the follicular dynamics (Wolfenson et al., 2000).

The effects of heat stress on fertility have been demonstrated in other species as well. In heat stressed ewes, fertilized ova upon reaching the uterus, were significantly damaged when examined compared to ova in non-heat stressed ewes (Ulberg and

Burfening, 1967). Studies conducted with pregnant ewes and ovariectomized cows exposed to acute heat stress conditions revealed decreased uterine blood flow (Brown and Harrison, 1981; Roman-Ponce et al., 1978). This can be associated with a decreased conception rate and slowed fetal growth in late gestation (Collier et al., 1982). Even a subtle increase in the pregnant cow's body temperature due to high ambient temperatures can have severe consequences to the developing embryo. Ulberg and Burfening (1967) reported that as bovine rectal temperatures increased by 1°C, embryo survival rates dropped from 61 to 45% between days 35 to 42 post-insemination. Dunlap and Vincent (1971) reported that 100% of beef heifers exposed to high temperatures of 32.2°C for 72 hours immediately following breeding lost their pregnancies while 48% of heifers exposed to 21.1°C maintained their pregnancies.

During the hotter months, a lactating dairy cow will experience mild to severe heat stress, however this is dependent on how intense the heat is, her level of milk production and whether cooling systems are implemented in the barn (Wolfenson et al., 2000). The implementation of cooling systems in barns to lessen the severity of heat stress during the summer has been shown to be beneficial in some studies. One study in Arizona compared seasonal differences in fertility by exposing cows to evaporative cooled shades, water foggers under shade or shade alone. The results indicated that the evaporative cooled group had the least seasonal differences in conception rate and this was the most effective in ameliorating the effects of heat stress on reproduction (Ray et al., 1992). However, some argue that cooling systems in barns have not been able to significantly improve fertility during the summer. There was still significant variation between summer and winter pregnancy rates, despite a facility equipped with shade, fans

and sprinklers (Hansen and Aréchiga, 1999). It seems that in order to eliminate the effects of heat stress on cows, cooling systems must be used frequently and intensively, combining sprinkler and ventilation systems. This may improve fertility so that both summer and winter groups are similar in their conception rates, however this is a costly financial investment for the producer (Wolfenson et al., 1988).

Hormones During Early Lactation

Thyroid Hormone

The thyroid hormones, thyroxine and triiodothyronine are calorigenic, metabolic regulators. Thyroid hormones are important for maintenance of pregnancy and for a normal ensuing lactation cycle. It was found that thyroid hormone is a necessary hormone in the development of mouse mammary tissue. Hypothyroid female mice had abnormal ductal development (Vonderhaar and Greco, 1979). In the dairy cow, plasma T_4 levels gradually rose prepartum, followed by a rapid decrease around the time of parturition. There is a gradual rise following calving with prepartum levels of T_4 significantly higher compared to postpartum concentrations (Kunz et al., 1985; Goff and Horst, 1997). Levels of T_3 and T_4 were measured in Estonian cows during different stages of lactation. During early lactation, plasma thyroid hormone concentrations were lower and progressively increased as lactation continued. These significantly lower levels of plasma thyroid hormone could suggest that this is a mechanism to reduce metabolic rate and heat production during the onset of lactogenesis (Tiirats, 1997). Reist and colleagues (2003) reported that in multiparous dairy cows, higher levels of T_3 and T_4

may be beneficial as they could act as metabolic signals associated with earlier resumption of postpartum ovarian cyclicity.

Cortisol

The major stress hormone produced by the adrenal glands in the ruminant is the glucocorticoid, cortisol. During the transition period, cortisol is elevated prior to, during and following parturition, signifying an increased release of the trophic hormone, adrenocorticotropin (ACTH) (Hunter et al., 1970). For many years glucocorticoids have been used for their immunosuppressive effects. Because cortisol peaks at the time of parturition, the dairy cow's immune system is compromised. Cows are more likely to develop clinical mastitis in the first month of lactation than at any other time (Goff and Horst, 1997). It was reported that cows that developed milk fever had greater secretions of cortisol at parturition than cows that did not experience hypocalcemia (Goff et al., 1989). These increased concentrations of cortisol may be further depressing the immune system that is already suppressed to some degree in the periparturient cow. Another indicator of depressed immunity during early lactation was the finding that neutrophils have impaired functioning during this period (Goff and Horst, 1997).

Insulin

The pancreatic hormone insulin is involved in the delivery of glucose to cells for energy and is a potential regulator of feed intake. Levels of insulin are normally greatly elevated during the last 3 weeks of gestation in cows. Some studies have seen a decrease in insulin prepartum with levels steeply rising at calving up to about 16 mU/L. Levels of

insulin following calving then dropped back down to about 5 mU/L and gradually rose up to 10 mU/L after 100 days into lactation (Kunz et al., 1985; Grum et al., 1996). Plasma insulin levels have also been reported to be low (300 pg/ml) up until day 14 of lactation, when levels began increasing (Smith et al., 1997). Taylor and colleagues (2003) saw a postpartum decline in plasma insulin up until week 4 in primiparous cows, followed by a gradual increase until the end of the study at week 20. With the onset of lactation, plasma insulin levels are depressed, while triacylglyceride accumulation in the liver is increased from mobilized adipose tissue to support the demands of lactation (Smith et al., 1996; Ingvartsen and Andersen, 2000). Chronic injections of insulin in low doses has been shown to induce hypophagia in rats and sheep (Ingvartsen and Andersen, 2000). It is an important regulator of feed intake and body weight, acting endogenously on the ventromedial nucleus of the hypothalamus. Therefore, it is possible that some hormone(s) are suppressing insulin during early lactation in order to stimulate feeding in the cow (Ingvartsen and Andersen, 2000).

Progesterone

Progesterone is the product of the corpus luteum and is delivered to the uterus via circulation. It is the essential steroid hormone for maintenance of pregnancy. From day 32 to 256 of gestation, plasma progesterone concentrations remained fairly constant, with a significant reduction just prior to parturition (Short et al., 1958). Plasma progesterone levels in the dairy cow increase steadily until about day 250 of gestation, when concentrations peak between 7 and 8 ng/ml. From there on until day of calving, levels drop to between 3 to 4 ng/ml while plasma estrogens are concomitantly increasing. The

last day prepartum, progesterone drops down to almost undetectable levels. Both progesterone and estrogen levels remain low from calving until about 3 weeks postpartum. Prostaglandin metabolites decreased to basal concentrations after parturition. The postpartum increase in progesterone, determined by a concentration greater than 1 ng/ml, was not detected until about day 23 postpartum (Eley et al., 1981). Another study on primiparous, first lactation dairy cows had the postpartum increase in progesterone occur at a mean of 30 +/- 4.1 days (Taylor et al., 2003).

Leptin

Leptin is a protein hormone produced and secreted primarily by white adipocytes. It was discovered in 1994 and has been shown to be a potential regulator (inhibitor) of feed intake and could influence the hypothalamo-pituitary-gonadal axis. Effects of leptin on the immune system such as enhanced cytokine production have also been documented. Our present knowledge of leptin's function comes thus far from studying primarily primates and rodents, with little focus on ruminant species. Leptin may play a role as a signal to the CNS indicating energy status of the animal, with its most important function believed to be that of conservation of energy during times of nutrient deprivation (Block et al., 2001). Evidence of leptin having direct effects on the reproductive tract have come from the discovery of receptor mRNA found in the anterior pituitary, hypothalamus and within the ovary of several species (Spicer, 2001). Block and colleagues (2001) discovered that maternal circulating leptin in the periparturient dairy cow was the greatest in late pregnancy with a postpartum drop of about 50%. Leptin levels began to decline between weeks 1 and 2 prepartum from an average of

5.8 to 5.5 ng/ml. Levels reached their lowest concentration in early lactation with a negative energy balance and a reduction in synthesis by adipocytes part of the cause. At 1 week, 3 weeks and 8 weeks into lactation, average leptin was 3.0, 3.2 and 2.9 ng/ml, respectively. Despite an improved energy balance by week 8 postpartum, plasma leptin levels continued to be depressed.

Plasma leptin levels are positively correlated to amount of body fat, glucose and insulin and negatively correlated to NEFA levels. The reason for reduced leptin during this time could be that as lactation progresses and body condition has not improved, mobilization of adipose tissue has led to considerable depletion of the cow's supply of white adipocytes, the source of leptin synthesis (Block et al., 2001; Kadokawa et al., 2000). In another study, Soliman and colleagues (2002) showed that leptin concentrations did not differ significantly over a 50-day period in both pregnant and non-pregnant cows. Over the periparturient period, serum leptin levels remained relatively constant, averaging between 5.9 to 9.2 ng/ml. This finding is in conflict with the two previous studies and may be due to differing diets and/or immunoassay techniques. Chilliard and colleagues (2001) reported that the length of photoperiod altered circulating concentrations of leptin in the ewe. Longer daylength increased both mRNA in adipose tissue as well as plasma leptin concentrations. Another study reported that plasma leptin was increased in ovariectomized, estradiol-implanted cows from January up until the summer solstice. It was also noted that slight changes in a mature cow's body weight or body condition did not significantly change the level of plasma leptin (Garcia et al., 2002). There is growing interest in the role of leptin especially in the high-producing, early lactation dairy cow, as drastic changes in energy balance and endocrine physiology

combined with immunosuppression increase the incidence of reproductive and metabolic disorders.

Metabolites During Early Lactation

Glucose

During late pregnancy and for approaching lactogenesis, the dairy cow must increase its production of glucose. The ruminant in general relies predominantly on hepatic gluconeogenesis for its glucose supply. The demands of the uterus and mammary gland for glucose, in turn suppress utilization of glucose by adipose and muscle tissues (Bell and Bauman, 1997). Glucose tends to have an acute dip in concentration around the time of approaching parturition, signifying the increased demands of the fetus and mammary tissue (Vazquez-Anon et al., 1994; Grum et al., 1996; Reist et al., 2000; Stockdale and Roche, 2002). This is consistent with the drop in feed intake associated with parturition, decreasing the supply of nutrients such as glucose to the cow. On day 1 prior to parturition, glucose concentrations peaked sharply reaching levels close to 90 mg/dl which could be an indication of endocrine changes that promote gluconeogenesis. Up until about 10 days postpartum, glucose levels significantly drop down to about 63 mg/dl, after which levels gradually begin to rise again (Vazquez-Anon et al., 1994). Other studies have shown that glucose levels remained low through day 14 of lactation and then levels increased as lactation progressed (Smith et al., 1996). Herbein and colleagues (1985) demonstrated that plasma glucose levels increase progressively with lactation. It has also been shown that plasma glucose may be depressed up to 3 weeks postpartum (Kunz et al., 1985). Reist and colleagues (2000) reported that mean plasma

glucose was slightly above 54 mg/dl and dropped down to 36 mg/dl at parturition. There was a slow and gradual increase in concentration by week 6 of lactation. There seems to be an inverse relationship between glucose and NEFA and β-HBA. Glucose is depressed during times of elevated NEFA and β-HBA, which is typical of the periparturient period (Ingvartsen and Andersen, 2000).

Non-Esterified Fatty Acids (NEFA)

The drop in feed intake that can begin at 3 weeks prepartum causes a deficiency of nutrient supply from diet alone for the cow's own maintenance, her rapidly developing fetus and lactogenesis. As compensation for a state of negative energy balance, triacylglycerides from adipose tissue are mobilized causing a rise in plasma NEFA concentrations and lipid accumulation in liver hepatocytes. It is common to see elevated NEFA and low blood glucose during the periparturient period as there is a shortage of energy and a need for energy partitioning or mobilization. This may occur prior to or at the time of parturition, usually simultaneous with the cow's drop in feed intake (Bertics et al., 1992). Although every dairy cow experiences some degree of negative energy balance, which varies depending on how the individual cow copes with stress and lipid mobilization, not every cow experiences high lipid accumulation in the liver. High accumulation of liver triacyglycerides has been associated with impaired immune function and adverse effects on fertility, with increased days open and lower pregnancy rates. Several studies have reported a significant peak in NEFA concentrations at the time of parturition (Kunz et al., 1985; Bertics et al., 1992; Vazquez-Anon et al., 1994; Grummer, 1995; Grum et al., 1996; Smith et al., 1997; Reist et al., 2000).

Kunz and colleagues (1985) reported that plasma NEFA levels rose from 4 mg/dl at about 25 days prepartum, peaked at 11 mg/dl on day of calving, and then returned to prepartum concentrations. The fatty infiltration into the liver is most notable in early lactation and is one of the primary consequences of the negative energy balance (Bertics and Grummer, 1999). Lipid accumulation may not occur until plasma NEFA concentrations have increased at parturition (Vasquez-Anon et al., 1994). Ingvartsen and Andersen (2000) found that NEFA concentrations began rising 2 to 3 weeks prior to calving, where levels peaked either at parturition or during the first week of lactation. These results agree with Grummer (1995) who found that NEFA levels gradually began rising about 10 days prepartum, prior to the drop in feed intake. NEFA levels rose rapidly at calving and dropped to normal levels postpartum. Other studies have demonstrated a peak in NEFA concentrations in early lactation occurring about day 7 postpartum, with levels returning to normal at 21 days postpartum (Smith et al., 1997). Hartwell and colleagues (2000) saw NEFA concentrations rise significantly at calving, and by day 56 into lactation, levels dropped to precalving concentrations. At 10 days prepartum, Vazquez-Anon and colleagues (1994) found plasma NEFA levels to be 300 μM, peaking at calving up to about 1100 μM and thereafter, declining by day 20 postpartum to about 400 μM.

One of the main factors that may be contributing to reduced fertility in the postpartum transition cow is the degree of negative energy balance (NEB), a direct result of depressed feed intake at parturition. The more severe the NEB, the greater the amount of NEFA mobilization from adipose tissue and the subsequent accumulation of triacylglycerides in liver hepatocytes. Pregnancy rates were 30% lower for cows with TG

values exceeding 50 mg/g, combined with longer intervals between parturition and first ovulation (Jorritsma et al., 2000).

β-Hydroxybutyrate (β-HBA)

During early lactation, β-HBA is a ketone derived from an inability of the TCA cycle to metabolize to completion fatty acids that have been mobilized from adipose tissue at increasing rates. Oxalaoacetate is the intermediary substrate necessary for the processes of gluconeogenesis and the TCA cycle. When there becomes a short supply of this intermediary, the liver is limited in the amount of fatty acids (NEFA) that can be oxidized to completion. The little amount of oxaloacetate that is available to the cow must be utilized for gluconeogenesis and the cow inevitably takes on increasing amounts of ketone bodies (Stockdale and Roche, 2002). This typically occurs when glucose reserves are depleted from either a voluntary drop in feed intake or implementation of feed restrictions. Elevated ketone bodies arise from increased synthesis of acetyl-coenzyme-A which is converted to acetoacetate, the precursor for the reduced form β-HBA. Elevated plasma levels of β-HBA are indicative of a negative energy balance, the result of a previous drop in feed intake combined with exceedingly large energy demands (Reist et al., 2000). Ketone bodies show up in blood, milk and urine (Goff and Horst, 1997). Postpartum changes in β-HBA are opposite to those of glucose, as are the changes associated with plasma NEFA concentrations (Ingvartsen and Andersen, 2000).

β-HBA tends to increase postpartum, especially with the onset of lactation (Smith et al., 1997). β-HBA levels are generally constant during late gestation at about 8.5 mg/dl. Beginning at day 5 prepartum, levels dropped to a nadir of 7 mg/dl, followed

by a sharp rise in concentrations at day 1 prepartum, reaching a plateau at about 10 mg/dl up until 30 days into milk (Vasquez-Anon et al., 1994). Some have reported that pre-calving β-HBA levels are about 9 mg/dl, significantly rising to approximately 14 mg/dl on day of calving, with a continued rise up to 25 mg/dl on day 20 postpartum. Once the cows reached about 50 days into lactation, β-HBA leveled out at 13 mg/dl (Kunz et al., 1985). Another study showed an increase in β-HBA levels up until about week 5 postpartum, signifying a change to fatty acid metabolism when glucose supplies were low (Taylor et al., 2003).

Cholesterol

Serum cholesterol has been shown to differ significantly as the various stages of lactation progress. In one study during early lactation, serum cholesterol in Holstein cows was lowest during the first month of lactation, followed by a rise and reaching a maximum concentration at month 5 of lactation. Thereafter, levels of serum cholesterol dropped back down in late lactation (Arave et al., 1975). The transition to the dry period and pregnancy may lower circulating cholesterol levels due to increased nutrient demands of the fetus, combined with an increased synthesis of the hormone progesterone (Arave et al., 1975). There is a close association between glucose and cholesterol. Glucose has been shown to promote cholesterol uptake and thus steroidogenesis in bovine ovarian cells. Therefore, glucose availability may be a determining factor in the amount of steroid hormone precursor utilized by the ovary (Reist et al., 2003). Moss and colleagues (2002) reported that there was a close relationship between these metabolites in cows having poor fertility. These cows (both primiparous and multiparous) tended to

have low serum cholesterol, low glucose, high β-HBA and shorter calving to first service interval.

Plasma Urea Nitrogen (PUN)

It has been shown that feeding diets high in dietary protein improved milk yield in early lactation but possibly at the expense of optimal reproductive performance. Between 17 and 19% crude protein (CP) is considered to be high, whereas about 12% CP is low in lactating dairy cows. Within the rumen, microbial fermentation of protein can lead to ammonia that escapes and is detoxified in the form of urea by the liver. Before being excreted through the urine, urea is circulated throughout the body and is permeable to all tissues (Butler, 1998). Most studies evaluating effects of PUN on uterine environment and embryonic development, as a result of feeding high CP, have demonstrated significant changes. Ferguson and colleagues (1988) fed dairy cows a diet high in rumen degradable protein that resulted in PUN concentrations greater than 20 mg/dl on the day of AI, and reported a significant decline in conception rates. This has been confirmed by other studies in which PUN exceeding 19 mg/dl was inversely related to fertility (Butler et al., 1996). Effects of high PUN on ovarian activity have been conflicting and thus far, no consistent data have been provided linking high CP to follicular development (Butler, 1998). Embryonic development has been reported to be both adversely affected by high PUN and seemingly immune to its effects. In lactating cows, Blanchard and colleagues (1990) reported that embryos experienced poor growth. However, in a study using superovulated, nonlactating cows, a high CP diet of about 27% versus 12% demonstrated no differences in the number of preovulatory and ovulatory follicles. The number and

quality of embryos collected were not adversely affected by feeding high CP (Garcia-Bojalil et al., 1994). One consideration is that between these two studies, nonlactating and lactating cows have a differing energy status. A lactating cow in a more severe negative energy balance while being fed high CP may be more susceptible to reduced fertility. It is possible that the interaction between high CP and negative energy status of the lactating cow contributes to compromised ovarian activity and follicular dynamics (Butler, 1999). Another significant observation is the effect of elevated PUN on the bovine uterine environment. Elrod and colleagues (1993) reported that feeding high amounts of protein altered uterine environment by preventing the normal rise in pH following estrus. This study also demonstrated an inverse relationship between uterine pH and concentration of PUN. A study conducted with primiparous, first lactation cows showed plasma urea concentrations at week 3 postpartum to be about 95 mg/dl. By weeks 5 and 8 levels increased from approximately 104 mg/dl to 113 mg/dl (Taylor et al., 2003).

Heat Stress Effects on Hormones

Thyroid Hormone

One mechanism the dairy cow uses to adjust to heat stress involves decreased metabolic rate and thyroid activity. Other general changes that occur under heat stress conditions are increased water consumption, reduced feed intake, increased body temperature and reduced milk production. Length and degree of heat stress may alter the extent of depression in thyroid activity. A significant reduction in T_4 concentration was demonstrated after 10 days of heat stress exposure. A high negative correlation was seen

between rectal temperatures and amount of T_3 excretion in milk. As temperature increased, there was less excretion of thyroid hormones through the milk, signifying decreased hormonal synthesis (Premachandra et al., 1958; Magdub et al., 1982). Under an acute heat stress environment, Holstein cows had a significant decline in thyroid activity after the first 60 hours of heat exposure. The dairy cow compensates for the additional heat load by decreasing its own bodily heat production, thus reducing thyroxine utilization. As rectal temperature increased 1.5°C, thyroid hormone concentrations and metabolic heat generation were depressed (Yousef et al., 1967). Chronic heat stress has been shown to depress not only T_4 and T_3 synthesis (monodeiodination), but also milk yield. Even exposure for acute periods under moderately high temperatures resulted in a 6.2% depression in T_4 synthesis (Johnson and Vanjonack, 1976). However, it has not always been the case that inducing acute heat stress conditions has altered plasma concentrations of T_4. McGuire and colleagues (1991) saw no difference in plasma concentrations of T_4 after 8 days in control versus acutely heat stressed cows. In two groups of pregnant cows approaching parturition, T_4 concentrations declined under both shade and no shade treatments. However, the no shade group had lower T_4 levels versus the shade group. This more exaggerated decline in maternal T_4 levels could influence mammary tissue development, postpartum milk yield and fetal growth (Collier et al., 1982).

Cortisol

Numerous stressors can cause increased glucocorticoid secretion in acute situations. Under acute heat stress conditions, the initial shock causes a rise in cortisol

secretion, followed by an adaptation phase under chronic conditions in which cortisol levels are reduced to normal physiological levels. Being a calorigenic hormone, the cow will compensate by reducing its endogenous heat production to bring the body into thermal equilibrium with its environment. Cows responded to an acute moderate thermal stress of 35°C after 20 minutes of exposure by increased cortisol levels from 30 to 37 μg/L. The hormone levels remained elevated following 12 hours after initiation of exposure, thereafter declining to normal levels after days 1 and 2. As chronic thermal stress set in, cortisol levels continued to decline as adjustment was made to the environment (Christison and Johnson, 1972). Following a 1 hour acute exposure to 40°C, nonlactating dairy cows had a 38% increase in glucocorticoid concentration. After 2 hours of exposure, levels increased 62% and by hour 4, levels increased to 120%. Thereafter, glucocorticoid concentrations dropped to normal levels and remained constant.

With acute exposure to heat stress, cortisol levels increase concomitantly with rising rectal temperatures. Under conditions of chronic thermal stress, cortisol levels will fall to normal physiological levels while rectal temperatures continue to rise. Therefore, cortisol levels decrease when heat stress is prolonged, a mechanism that could be assisting the cow during the adaptation phase to reduce overall metabolic heat production (Alvarez and Johnson, 1973; Johnson and Vanjonack, 1976). Other studies have shown that cows exposed to chronic heat stress during hot summer months displayed depressed cortisol levels (Abilay et al., 1975; Ronchi et al., 2001). A normal response to stressful conditions is activation of the hypothalamic-pituitary-adrenal axis (Christison and Johnson, 1972). Both psychological and physical stressors likely cause the release of the

hypothalamic corticotropin releasing factor which increases ACTH (Collins and Weiner, 1968). During the periparturient period, stressors are above what the cow normally experiences, eliciting an increased release of ACTH (Hunter et al., 1970). Cortisol is also increased as part of the endocrine control of parturition. This could result in more metabolic problems when combined with heat stress.

Insulin

Limited studies have reflected light on the effects of heat stress on insulin concentrations in the lactating cow. Itoh and colleagues (1998) demonstrated that insulin levels were elevated in lactating, thermally stressed cows. They also observed a depression in milk yield in this group versus the thermally neutral cows.

Progesterone

Studies conducted on heat stressed cows to determine changes in progesterone have been conflicting, however the disruption of ovarian steroid production in heat stressed animals has been well documented. Pubertal Holstein dairy heifers experiencing 95 days under high ambient temperatures (32°C) had a significant decrease in plasma progesterone levels compared to the control group under thermal comfort (Ronchi et al., 2001). In one study, reproductive changes were monitored in postpartum Holstein cows that had differing prepartum environments during the month of June (shade and no shade). Progesterone levels on day of calving did not significantly differ between the two groups. It took all cows an average of 12 days postcalving for the progesterone levels to increase above 1 ng/ml (Lewis et al., 1984). Collier and colleagues (1982) found that in

pregnant cows exposed to no shade, progesterone levels were higher until the day of parturition. No differences were found between the two treatment groups of shade versus no shade on the day of parturition. This could indicate that cows exposed to elevated temperatures could have increased progesterone secretion or a reduction in progesterone metabolism. However, the major source of progesterone is different in late gestation (placenta) than in the estrous cycle (corpus luteum). Wise and colleagues (1988) subjected two groups of lactating dairy cows to indoor cooling and outdoor, no shade conditions. Plasma progesterone concentrations were similar between the groups. The changes sometimes seen in progesterone concentrations in heat stressed cows could also be due to the vasoconstriction of ovarian blood flow. This was demonstrated in rabbits that were heat stressed. They had a 20-30% decrease in ovarian, oviductal and cervical blood flow (Lublin and Wolfenson, 1996). Lactating cows exposed to chronic heat stress tended to have suppressed progesterone levels, typical of a long summer season. Acute heat stress such as that in a controlled chamber has been shown to increase plasma progesterone (Howell et al., 1994). Heat stress effects have also been demonstrated with in vitro studies on theca and granulosa cells luteinized for 9 days in the summer or winter. Plasma progesterone was significantly lower in the summer, indicating that not only developing follicles may be adversely affected by chronic heat stress, but also the normal functioning of the CL (Wolfenson et al., 2002). There have been conflicting reports on heat stress effects on progesterone production. The variable results could be due to a number of factors, including acute versus chronic thermal stress, length and severity of hyperthermia, adrenal output of progesterone, stage of lactation, controlled

climatic chambers versus field exposure, photoperiod, multiparous versus primiparous cow, rate of liver metabolism and quality of feed (Jonsson et al., 1997).

Heat Stress Effects on Metabolites

Glucose

It has been suggested that heat stress may suppress a rise in plasma glucose, another indicator of why milk production may be compromised in summer, lactating dairy cows. This was shown in one study in which heat stressed lactating cows had depressed glucose levels compared to their non-thermally stressed herdmates (Itoh et al., 1998). Kappel and colleagues (1984) found that in heat stressed Holstein cows, plasma glucose rose prior to calving, dropping to nadir levels between days 11 and 25 postpartum. By day 25 postpartum, plasma glucose concentrations began rising. The normal range for plasma glucose was found to be about 62 +/- 8 mg/dl. Jonsson et al. (1997) compared summer and winter calving groups, finding that the average plasma glucose levels tended to be higher in the winter group at 63 mg/dl versus 58 mg/dl in the summer group. Cows with lower plasma glucose tended to have increased days until their first postpartum ovulation. The relationship between energy status and reproductive efficiency, particularly resumption of ovarian cyclicity, has been noted in previous studies (Canfield and Butler, 1991; Jolly et al., 1995).

The major contributors to lower glucose levels in summer, heat stressed cows may be attributed to reduced feed intake, changes in metabolic hormone secretion rates and a reduced ability to efficiently absorb dietary nutrients. The consequences of hypoglycemia in the early postpartum period may be a reduction in milk yield.

Alternately, high producing dairy cows may eventually reach below normal plasma glucose concentrations due to the excessive energy demands of lactation (Jonsson et al., 1997).

Non-Esterified Fatty Acids (NEFA)

Pregnant dairy cows exposed to shade versus no shade conditions revealed elevated plasma NEFA concentrations found in the heat stressed group 80 days prepartum. This pattern was similar for both treatment groups, with a rapid rise in NEFA levels about 8 days prior to calving, with the heat stressed group levels remaining above that of the thermoneutral group. The rise in NEFA concentrations in thermally stressed cows may be due to an altered endocrine and metabolic state (Collier et al., 1982). A similar study analyzed postpartum changes in the dairy cow following the two treatments imposed during late gestation. There was a marked increase in prostaglandin metabolite seen in the cows that had been heat stressed prepartum. It was suggested that the increased production of PGFM postpartum was due to the previous increase in non-esterified fatty acid synthesis prepartum (Lewis et al., 1984).

Cholesterol

Little research has been done on seasonal differences in cholesterol or on changes in cholesterol during the transition period. Arave and colleagues (1975) reported that serum cholesterol levels in summer-calving Holstein cows were significantly higher than winter calving cows. The mean monthly serum cholesterol for cows ranged from their highest concentrations in June (191.7 mg/100 ml) to their lowest in January (165.7

mg/100 ml). The authors reported that heritability, age, sex and diet were all factors related to level of serum cholesterol.

Plasma Urea Nitrogen (PUN) and β-Hydroxybutyrate (β-HBA)

No studies relating seasonal differences in β-HBA or PUN were found.

Based on the review of the literature, an experiment was designed to examine seasonal differences in several hormones and metabolites that have been suggested to be important in the early postpartum dairy cow.

Materials and Methods

Animals and Data Collection:

Multiparous Holstein dairy cows were used for the summer (n=11) and winter trials (n=7) at North Carolina State University. The summer calving cows began calving in early August of 2002 and the 12-week trial lasted until the latter part of October. The winter calving cows began calving in early November of 2002 and the 12-week trial lasted until early February. Cows were milked twice daily and fed a corn silage based TMR containing 17.8% crude protein following parturition. Cows had access to shade and no fans or cooling systems were utilized. Blood was drawn at approximately 0900 starting on day of calving followed by once weekly collection from the coccygeal vein for 12 weeks. Sampling was conducted at approximately the same time of day each week. Blood from each cow was collected in three separate tubes—10 ml sodium heparin, 10 ml no additive and 6 ml sodium fluoride potassium oxalate. Heparin and fluoride tubes were placed in ice immediately after collection until centrifugation. Blood was centrifuged at 5 ˚C within 30 minutes of collection and serum and plasma separated into 5 ml plastic tubes stored at -20 ˚C. Resumption of cyclicity was determined by plasma progesterone concentrations greater than 1 ng/mL. Body condition scores were taken immediately following calving for all cows in both seasonal calving groups and were based on a 5 point scale. Daily maximum and minimum temperature and humidity indexes were recorded for the entire length of the study. Temperature-humidity index (THI) was calculated using the equation:

THI = T – (.55 - .55 RH) * (T – 58) where T is the temperature (°F) and RH is the relative humidity (Ingraham et al., 1974).

Radioimmunoassays:

The Cobra II Auto-Gamma counter from Packard Instrument Company was utilized for all hormone assay readings. Serum samples were assayed for progesterone using the Coat-A-Count kit from Diagnostic Products Corporation (TKPG, DPC), Los Angeles, CA, as validated for bovine serum (Whisnant and Burns, 2002). Progesterone assays were performed on three separate days. The intra assay coefficient of variation was 5.70%. Inter assay coefficient of variation for P_4 was 4.18%. The P_4 assay sensitivity was approximately 0.02 ng/ml.

Serum samples were assayed for cortisol using the Coat-A-Count kit from Diagnostic Products Corporation (TKC, DPC), Los Angeles, CA following the procedures stated by the manufacturer. The one exception being that 100 µl of bovine serum was utilized instead of the recommended 25 µl amount. The cortisol assay kit was validated for bovine serum by establishing that dilutions of bovine serum resulted in a curve that was parallel to the standards in human serum supplied with the kit. Cortisol assays were performed on three separate days. The intra assay coefficient of variation for cortisol was 4.0%. Inter assay coefficient of variation for cortisol was 2.61%. The cortisol assay sensitivity was approximately 0.2 µg/dL.

Serum samples were assayed for total T_4 using the Coat-A-Count kit from Diagnostic Products Corporation (TKT4, DPC), Los Angeles, CA. The T_4 assay was validated for bovine serum by testing serial dilutions of bovine serum from 25 to 250 µl for parallelism. Thyroid hormone assays were performed on three separate days. The intra assay coefficient of variation was 2.52%. Inter assay coefficient of variation for T_4 was 1.95%. The total T_4 assay sensitivity was approximately 0.25 µg/dL.

Insulin was measured from serum samples, following the protocol of the porcine insulin kit by Linco Research, Inc. (Cat. # PI-12K), St. Charles, MO with the exception of the use of bovine insulin (Sigma Chemicals) as the standard. The primary antibody had a cross reactivity of 90% with bovine insulin as determined by the company (Linco Research Porcine Insulin Assay Technical Bulletin). Insulin assays were performed on three separate days. The intra assay coefficient of variation was 48.92%. Inter assay coefficient of variation for insulin was 26.49%. The insulin assay sensitivity was approximately 2 μg/mL.

Plasma urea nitrogen and β-HBA were measured using heparinized samples, following the procedures given by the manufacturer, Sigma Diagnostics (Procedure No. 535 and Procedure No. 310-UV), St. Louis, MO. The PUN assay was performed on one day. The intra assay coefficient of variation could not be determined from a lack of standard duplications. The β-HBA assays were performed on thirteen separate days with an intra assay coefficient of variation of 1.20%. Inter assay coefficient of variation for β-HBA was 10.55%.

Serum samples were used for the NEFA assay, following the procedures from the NEFA C kit by Wako Chemicals USA, Inc. (ACS-ACOD Method), Richmond, VA. The NEFA assay was performed on one day, with an intra assay coefficient of variation of 2.63%.

Leptin was measured from serum samples, following the protocol from the multi-species RIA kit by Linco Research, Inc. (Cat. # XL-85K), St. Charles, MO as validated for bovine serum by Lloyd et al., 2002. The leptin assay was performed on one day, with

an intra assay coefficient of variation of 9.54%. The leptin assay sensitivity was approximately 1.0 ng/mL.

Fluoride-containing tubes were used for measuring glucose, following the protocol from Stanbio Laboratory (Procedure No. 1070), Boerne, TX. The plasma glucose assay was performed on one day, with an intra assay coefficient of variation of 2.72%.

Serum samples were used for the cholesterol assay following the procedures given by the manufacturer, Sigma Diagnostics (Procedure No. 352), St. Louis, MO. The cholesterol assay was performed on one day with an intra assay coefficient of variation of 4.08%. For all metabolite assays, concentrations were read using the Spectronic 1001 spectrophotometer from Bausch & Lomb.

Statistical Analysis:

Calculations for relative hormone and metabolite concentrations were based on mean weekly values. Statistical analysis was performed using the PROC MIXED procedure of SAS (8.2). Fixed effects included week and season while cow was a random effect. Week x season interactions were also analyzed. The Student-Newman-Keuls option was used as a means separation test when significance was indicated. Least squares means were used to determine significance between both seasonal groups at specific weeks postpartum. Asterisks in tables and graphs indicate statistical significance with a P value ≤ 0.05. Both intra and inter assay coefficient of variations were calculated for the appropriate assays.

RESULTS

Progesterone:

Although previous studies have reported conflicting results concerning the secretion rate of progesterone under heat stress conditions, our present results indicate a significant effect of week ($P < 0.0001$), but not season. Progesterone concentrations increased over time postpartum as more cows became cyclic. Effects of season and week x season interaction were not significant. For both summer and winter calving groups, serum progesterone was at nadir levels for the first 3 weeks postpartum. The winter group had an earlier rise in mean serum progesterone than the summer group, indicative of an earlier ovulation. The mean day of first rise in progesterone postpartum was 30.3 ± 7 for the winter group and 47.2 ± 6 days for the summer group. Table 1 includes mean serum P4 concentrations by week postpartum and season and significance. Between the two seasonal groups, week 7 was significantly different ($P = 0.04$). Figure 1 depicts the weekly cumulative number of summer versus winter postpartum cows with serum P4 concentrations greater than 1 ng/mL. Figure 2 depicts mean weekly serum P4 between postpartum summer and winter calving groups over the 12-week trial.

Thyroid Hormone (T4):

For the current study, serum T4 gradually increased following parturition and continued to do so as lactation progressed. Mean weekly T4 was similar for both summer and winter groups. Effect of week tended to be significant ($P = 0.10$). There was no significant effect of season or week x season interaction. Table 2 includes mean serum T4 concentrations by week postpartum and season and significance. There was no

significance found between the two seasonal groups during the 12-week period. Figure 3 depicts mean weekly serum T_4 between postpartum summer and winter calving groups over the 12-week trial. At week 4 for the summer group, there was a sharp increase in serum T_4 which was determined to be the result of an outlier.

Cortisol:

Our results indicated for both seasonal groups, a decrease in serum cortisol from time immediately following parturition to week 2 postpartum. The summer group had a greater mean cortisol value immediately following parturition and levels were suppressed until about week 8 of lactation. On average, cortisol for the winter group began increasing after week 3 and levels were not suppressed for the remainder of the trial. The interaction of week x season was significant ($P = 0.05$) with no significant main effect of week or season. Table 3 includes mean serum cortisol concentrations by week postpartum and season and significance. Differences between the two seasonal groups were significant at weeks 5 ($P = 0.04$), 7 ($P = 0.05$) and 11 ($P = 0.05$). Figure 4 depicts mean weekly serum cortisol between postpartum summer and winter calving groups over the 12-week trial.

β-Hydroxybutyrate (β-HBA):

Both seasonal groups showed an increase in mean plasma β-HBA levels within the first few weeks of lactation. The summer group's mean reached a plateau at week 4 and decreased thereafter. The winter group mean reached a plateau at week 3 and remained fairly constant for the remainder of the trial. Effect of season was significant

($P < 0.0001$), with summer calving cows having higher β-HBA than winter calving cows. The overall mean concentration for the summer group was 5.80 mg/dL ± 1.06 and 3.26 mg/dL ± 1.29 for the winter group. There was no significant effect of week or week x season interaction. Table 4 includes mean plasma β-HBA concentrations by week postpartum and season and significance. Differences between the two seasonal groups were significant at weeks 1 ($P = 0.02$), 3 ($P = 0.01$), 4 ($P = 0.02$) and 5 ($P = 0.02$). Figure 5 depicts mean weekly plasma β-HBA between postpartum summer and winter calving groups over the 12-week trial. There was a sharp increase at week 10 (winter) which was determined to be an outlier.

Plasma Urea Nitrogen (PUN):

Figure 6 depicts mean weekly plasma PUN between postpartum summer and winter calving groups over the 12-week trial. There was a significant effect of week ($P < 0.0001$). Both treatment groups had increasing plasma PUN following parturition up until about week 4. The trend was similar amongst the two groups. Effect of season tended to be significant ($P < 0.10$) with no significant week x season interaction. Table 5 includes mean plasma PUN concentrations by week postpartum and season and significance. Differences between the two seasonal groups were significant at weeks 2 ($P = 0.04$) and 11 ($P = 0.02$).

Insulin:

There was no significant effect of week, season or week x season interaction. Table 6 includes mean plasma insulin concentrations by week postpartum and season and

significance. Following parturition, the summer group tended to have a lower mean plasma insulin level of about 3μU/mL compared to the winter group's mean of about 6μU/mL. Figure 7 depicts mean weekly plasma insulin between postpartum summer and winter calving groups over the 12-week trial. There was no significance between the two seasonal groups during the 12-week period. At week 6 for the summer group there was a sharp increase in serum insulin which was determined to be the result of an outlier.

Leptin:

There was a significant effect of season ($P < 0.01$) between the two calving groups, with levels in summer calving cows being higher. There was no significant effect of week or week x season interaction. Table 7 includes mean serum leptin concentrations by week postpartum and season and significance. Figure 8 depicts mean weekly serum leptin between postpartum summer and winter calving groups over the 12-week trial.

Non-Esterified Fatty Acids (NEFA):

There was a significant effect of week ($P < 0.0001$) between the two calving groups, but no significant effect of season or week x season interaction. The summer calving cows had higher NEFA levels the first 2 weeks postpartum with a gradual decrease in concentration as lactation progressed. The winter calving cows had lower levels following parturition with concentrations changing sporadically over time. By the end of the 12 weeks, the winter group had decreased NEFA levels compared to week 1. Table 8 includes mean plasma NEFA concentrations by week postpartum and season and significance. Differences between the two seasonal groups were significant at week 1

($P = 0.02$). Figure 9 depicts mean weekly plasma NEFA between postpartum summer and winter calving groups over the 12-week trial.

Glucose:

There was no significant effect of week, season or week x season interaction. Following parturition, the concentration of plasma glucose was close to 85 mg/dL for both seasonal groups. Thereafter, the summer group's mean dropped to about 73 mg/dL by week 2 while the winter group's mean dropped to about 68 mg/dL. On average, the winter calving group had an earlier increase in serum glucose beginning about week 2 postpartum. Figure 10 depicts the mean weekly values of plasma glucose between postpartum summer and winter calving groups over the 12-week trial. Table 9 includes mean plasma glucose concentrations by week postpartum and season and significance. There was no significance between the two seasonal groups during the 12-week period.

Cholesterol:

Effects of week and season were significant ($P < 0.0001$) with no significant week x season interaction. Figure 11 depicts the mean weekly values of serum cholesterol between postpartum summer and winter calving groups over the 12-week trial. Differences between the two seasonal groups were significant at weeks 3 ($P = 0.03$), 4 ($P = 0.02$), 5 ($P = 0.0003$), 7 ($P = 0.004$), 8 ($P = 0.002$), 10 ($P = 0.004$), 11 ($P = 0.0004$) and 12 ($P = 0.0002$). Table 10 includes mean serum cholesterol concentrations by week postpartum and season and significance. The present results show lower serum cholesterol levels in the early postpartum period, with both seasonal

groups showing a progressive increase in cholesterol as lactation advanced. Throughout the duration of the trial, the winter calving group had higher mean levels of cholesterol than the summer calving group.

Body Condition Scores:

Scores were based on a 5 point scale and were only taken once following calving. The mode was similar for both summer and winter calving groups. The summer group's mode was 2.75 and the winter group's mode was 2.25. The scores ranged from 2.5 to 3.5 for the summer group and 2.25 to 3.0 for the winter group. Table 11 includes the body condition scores for summer and winter calving cows taken immediately post-calving.

Prevalence of Infection:

Treatment for any infection was recorded for both seasonal groups throughout the 12 week postpartum period. Eight out of the eleven cows in the summer group were treated at some point in time. Five were treated for mastitis, two for retained fetal membranes and one for a foot abscess. Three out of the seven cows in the winter calving group were treated all for mastitis. Table 12 includes the prevalence of infection for the two seasonal calving groups.

Temperature-Humidity Index (THI):

THI is one method of determining the degree of heat stress. It is an average value of temperature and humidity combined, over a 24-hour period. The summer trial began in early August and ended in the latter part of October. The winter trial began in early

November and ended in early February. Over the 12-week period for both trials, THI values were calculated on a weekly basis. THI values for August were in the mild heat stress range averaging 75, while September values were borderline mild heat stress averaging 71. For the remainder of the summer trial, cows were not considered to be experiencing heat stress from the sole standpoint of the THI values. The winter trial THI values averaged about 45, far below the range of heat stress values.

Table 1. Mean serum progesterone concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	P4 (ng/mL)	*P* - value
1	S	0.56±0.62	0.56
1	W	0.02±0.74	
2	S	0.83±0.59	0.47
2	W	0.14±0.74	
3	S	0.51±0.59	0.63
3	W	0.05±0.74	
4	S	0.25±0.59	0.19
4	W	1.48±0.74	
5	S	0.99±0.59	0.07
5	W	2.68±0.74	
6	S	1.93±0.59	0.61
6	W	2.41±0.74	
7	S	2.77±0.59	0.04*
7	W	0.77±0.74	
8	S	3.32±0.59	0.31
8	W	2.36±0.74	
9	S	3.27±0.59	0.97
9	W	3.23±0.74	
10	S	2.39±0.65	0.75
10	W	2.04±0.88	
11	S	1.41±0.98	0.80
11	W	1.07±0.98	

*$P \leq 0.05$

Table 2. Mean serum T4 concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	T4 (µg/dL)	*P* - value
1	S	2.04±0.26	0.47
1	W	2.33±0.32	
2	S	2.17±0.27	0.31
2	W	1.75±0.32	
3	S	2.32±0.26	0.48
3	W	2.61±0.32	
4	S	2.84±0.26	0.12
4	W	2.20±0.32	
5	S	2.76±0.26	0.59
5	W	2.54±0.32	
6	S	2.33±0.26	0.42
6	W	2.66±0.32	
7	S	2.83±0.26	0.37
7	W	2.46±0.32	
8	S	2.48±0.26	0.89
8	W	2.53±0.32	
9	S	3.06±0.26	0.13
9	W	2.44±0.32	
10	S	2.87±0.26	0.44
10	W	2.56±0.32	
11	S	3.19±0.27	0.19
11	W	2.65±0.32	
12	S	2.63±0.35	0.57
12	W	2.89±0.32	

Table 3. Mean serum cortisol concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	Cortisol (µg/dL)	*P* - value
1	S	4.27±0.63	0.09
1	W	2.53±0.79	
2	S	1.16±0.66	0.26
2	W	2.33±0.79	
3	S	1.89±0.63	0.59
3	W	1.33±0.79	
4	S	1.70±0.63	0.76
4	W	2.01±0.79	
5	S	1.63±0.63	0.04*
5	W	3.78±0.79	
6	S	1.09±0.63	0.30
6	W	2.15±0.79	
7	S	1.59±0.63	0.05*
7	W	3.61±0.79	
8	S	1.59±0.63	0.29
8	W	2.68±0.79	
9	S	2.18±0.63	0.63
9	W	1.68±0.79	
10	S	2.13±0.63	0.13
10	W	3.65±0.79	
11	S	2.92±0.63	0.05*
11	W	0.93±0.79	
12	S	1.45±0.86	0.37
12	W	2.51±0.79	

* = $P \leq 0.05$

Table 4. Mean plasma β-HBA concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	β-HBA (mg/dL)	*P* - value
1	S	6.49±1.03	0.02*
1	W	2.60±1.29	
2	S	6.68±1.03	0.12
2	W	4.12±1.29	
3	S	7.61±1.03	0.01*
3	W	3.33±1.29	
4	S	7.42±1.03	0.02*
4	W	3.45±1.29	
5	S	7.38±1.03	0.02*
5	W	3.46±1.29	
6	S	5.39±1.03	0.08
6	W	2.53±1.29	
7	S	5.32±1.03	0.09
7	W	2.48±1.29	
8	S	5.51±1.03	0.16
8	W	3.19±1.29	
9	S	4.57±1.03	0.49
9	W	3.43±1.29	
10	S	4.13±1.03	0.81
10	W	4.53±1.29	
11	S	4.37±1.08	0.59
11	W	3.46±1.29	
12	S	4.77±1.39	0.24
12	W	2.53±1.29	

* = $P \leq 0.05$

Table 5. Mean plasma PUN concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	PUN (mg/dL)	*P* - value
1	S	13.29±1.74	0.92
1	W	13.02±2.08	
2	S	15.62±1.74	0.04*
2	W	21.35±2.08	
3	S	18.79±1.74	0.36
3	W	21.26±2.08	
4	S	21.27±1.74	0.60
4	W	22.67±2.08	
5	S	17.63±1.74	0.50
5	W	19.48±2.08	
6	S	22.18±1.74	0.19
6	W	18.65±2.08	
7	S	18.64±1.74	0.06
7	W	23.78±2.08	
8	S	22.60±1.74	0.22
8	W	19.29±2.08	
9	S	21.93±1.74	0.67
9	W	20.79±2.08	
10	S	21.97±1.74	0.91
10	W	21.67±2.08	
11	S	19.92±1.83	0.02*
11	W	26.21±2.08	
12	S	21.42±2.08	0.40
12	W	23.88±2.08	

* = $P \leq 0.05$

Table 6. Mean serum insulin concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	Insulin (µU/mL)	*P* - value
1	S	3.36±2.39	0.48
1	W	6.05±3.0	
2	S	2.59±2.39	0.29
2	W	6.65±3.0	
3	S	6.77±2.39	0.78
3	W	5.70±3.0	
4	S	8.64±2.39	0.77
4	W	9.78±3.0	
5	S	8.53±2.39	0.91
5	W	8.08±3.0	
6	S	14.92±2.39	0.15
6	W	9.37±3.0	
7	S	9.0±2.39	0.76
7	W	7.83±3.0	
8	S	10.29±2.39	0.46
8	W	7.45±3.0	
9	S	9.36±2.39	0.63
9	W	7.49±3.0	
10	S	11.29±2.39	0.25
10	W	6.88±3.0	
11	S	7.92±2.51	0.55
11	W	10.29±3.0	
12	S	11.05±3.24	0.97
12	W	10.86±3.0	

Table 7. Mean serum leptin concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	Leptin (ng/mL)	*P* - value
1	S	4.93±0.82	0.13
1	W	2.91±1.03	
2	S	4.86±0.82	0.45
2	W	3.85±1.03	
3	S	4.67±0.82	0.63
3	W	4.04±1.03	
4	S	4.47±0.82	0.60
4	W	3.77±1.03	
5	S	4.84±0.82	0.60
5	W	4.15±1.03	
6	S	4.93±0.82	0.37
6	W	3.74±1.03	
7	S	5.12±0.82	0.34
7	W	3.85±1.03	
8	S	5.05±0.82	0.84
8	W	4.79±1.03	
9	S	5.81±0.82	0.29
9	W	4.42±1.03	
10	S	5.47±0.82	0.41
10	W	4.39±1.03	
11	S	5.22±0.86	0.53
11	W	4.38±1.03	
12	S	6.52±1.12	0.16
12	W	4.39±1.03	

Table 8. Mean serum NEFA concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	NEFA (mEq/L)	*P* - value
1	S	0.67±0.06	0.02*
1	W	0.44±0.07	
2	S	0.64±0.06	0.34
2	W	0.55±0.07	
3	S	0.51±0.06	0.77
3	W	0.49±0.07	
4	S	0.52±0.06	0.13
4	W	0.37±0.07	
5	S	0.42±0.06	0.62
5	W	0.47±0.07	
6	S	0.37±0.06	0.34
6	W	0.28±0.07	
7	S	0.28±0.06	0.25
7	W	0.39±0.07	
8	S	0.28±0.06	0.08
8	W	0.44±0.07	
9	S	0.24±0.06	0.40
9	W	0.32±0.07	
10	S	0.20±0.06	0.14
10	W	0.34±0.07	
11	S	0.24±0.06	0.67
11	W	0.20±0.07	
12	S	0.23±0.08	0.93
12	W	0.24±0.07	

* = $P \leq 0.05$

Table 9. Mean plasma glucose concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	Glucose (mg/dL)	*P* - value
1	S	84.32±4.79	0.96
1	W	83.90±6.0	
2	S	72.11±4.79	0.62
2	W	68.28±6.0	
3	S	64.41±4.79	0.38
3	W	71.19±6.0	
4	S	74.65±4.79	0.80
4	W	76.63±6.0	
5	S	65.56±4.79	0.50
5	W	70.70±6.0	
6	S	75.12±4.79	0.78
6	W	77.24±6.0	
7	S	65.95±4.79	0.16
7	W	76.76±6.0	
8	S	71.65±4.79	0.74
8	W	74.21±6.0	
9	S	71.96±4.79	0.95
9	W	72.40±6.0	
10	S	74.96±4.79	0.80
10	W	73.00±6.0	
11	S	73.31±5.02	0.13
11	W	61.26±6.0	
12	S	76.55±6.48	0.51
12	W	70.70±6.0	

Table 10. Mean serum cholesterol concentrations in Holstein cows by week postpartum and season (summer and winter) and *P* values

Week	Season	CHL (mg/dL)	*P* - value
1	S	56.18±10.72	0.38
1	W	71.33±13.44	
2	S	75.78±10.72	0.37
2	W	91.07±13.44	
3	S	90.27±10.72	0.03*
3	W	128.79±13.44	
4	S	106.61±10.72	0.02*
4	W	145.54±13.44	
5	S	116.41±10.72	0.0003*
5	W	179.24±13.44	
6	S	159.52±10.72	0.15
6	W	184.60±13.44	
7	S	159.52±10.72	0.004*
7	W	209.04±13.44	
8	S	159.16±10.72	0.002*
8	W	213.73±13.44	
9	S	163.14±10.72	0.07
9	W	194.98±13.44	
10	S	172.94±10.72	0.004*
10	W	222.77±13.44	
11	S	189.85±11.24	0.0004*
11	W	253.01±13.44	
12	S	192.45±14.51	0.0002*
12	W	266.52±13.44	

* = $P \leq 0.05$

Table 11. Body condition scores for summer and winter calving cows taken immediately post-calving.

Summer	Winter
Range: 2.5 – 3.5	Range: 2.25 – 3.0
Mode: 2.75	Mode: 2.25

Table 12. Prevalence of infection in summer vs. winter calving cows.

	Summer (n=11)	Winter (n=7)
Prevalence	73 %	43 %
Mastitis	5	3
Retained Fetal Membranes	2	0
Foot Abscess	1	0

Figure 1. Weekly cumulative number of summer vs. winter postpartum cows with serum P4 > 1 ng/ml.

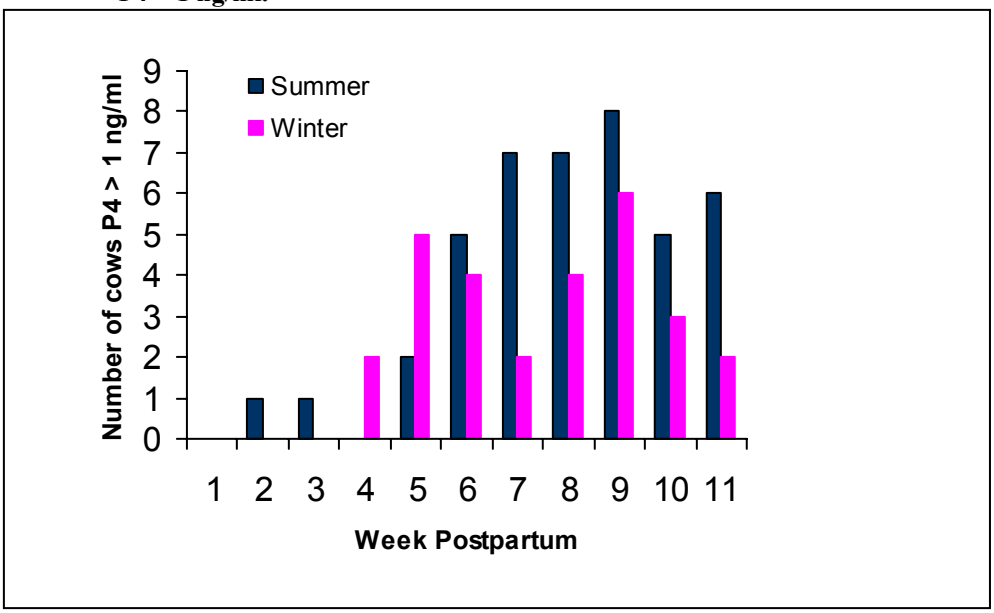
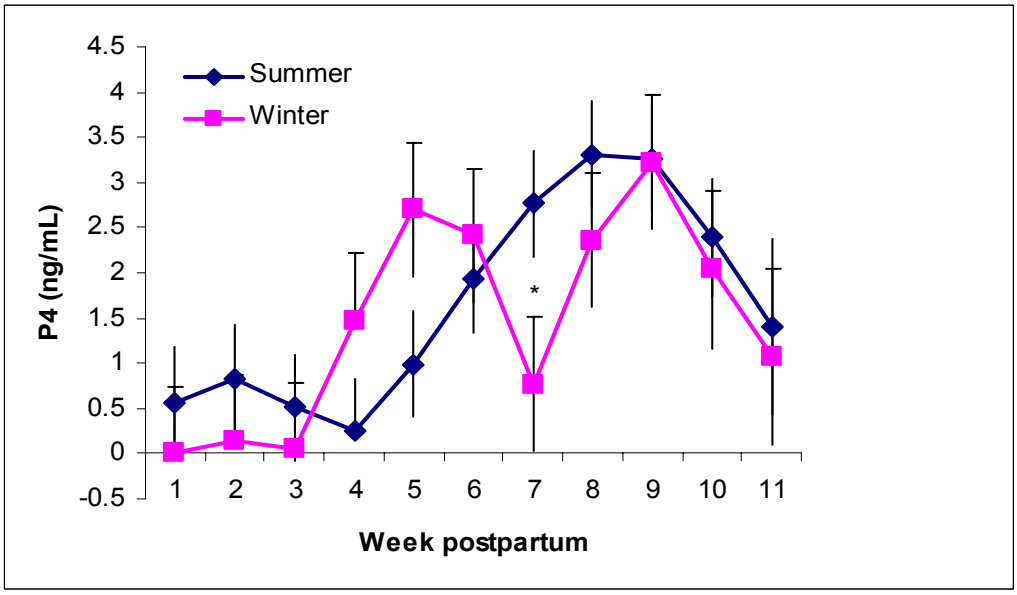


Figure 2. Mean weekly serum P4 between postpartum summer and winter calving groups.



*** = $P \leq 0.05$**

Figure 3. Mean weekly serum T4 between postpartum summer and winter calving groups.

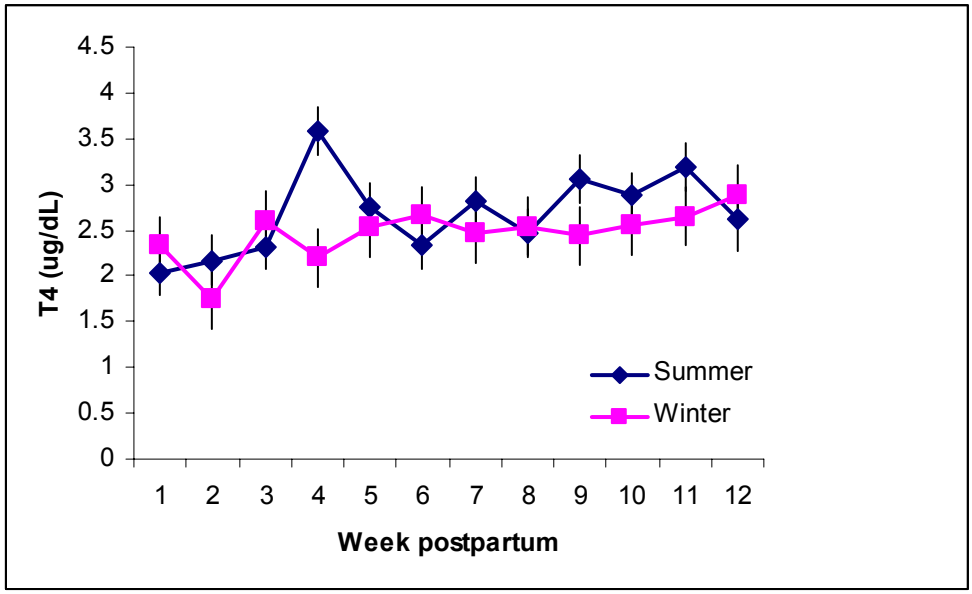
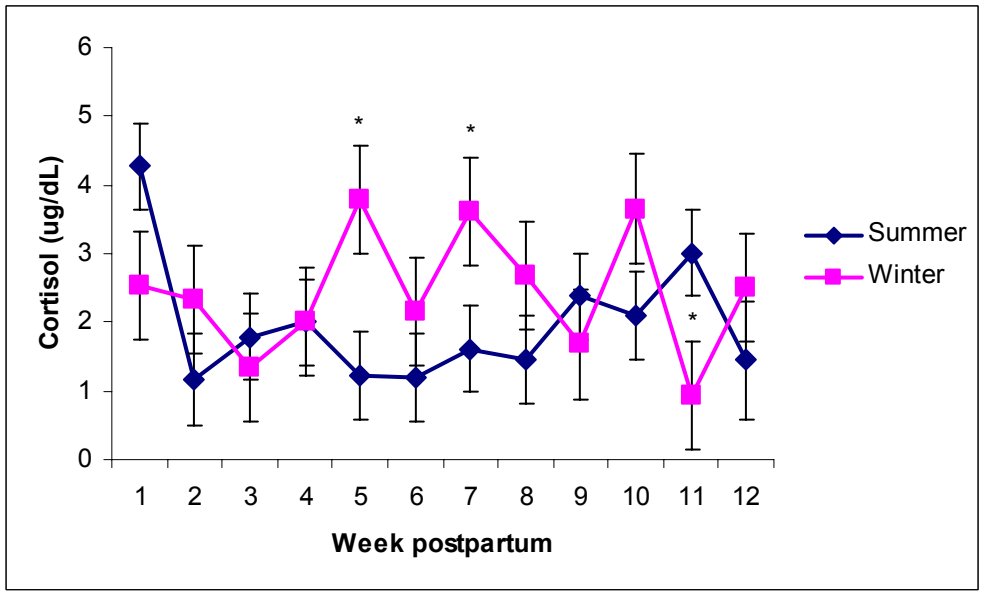
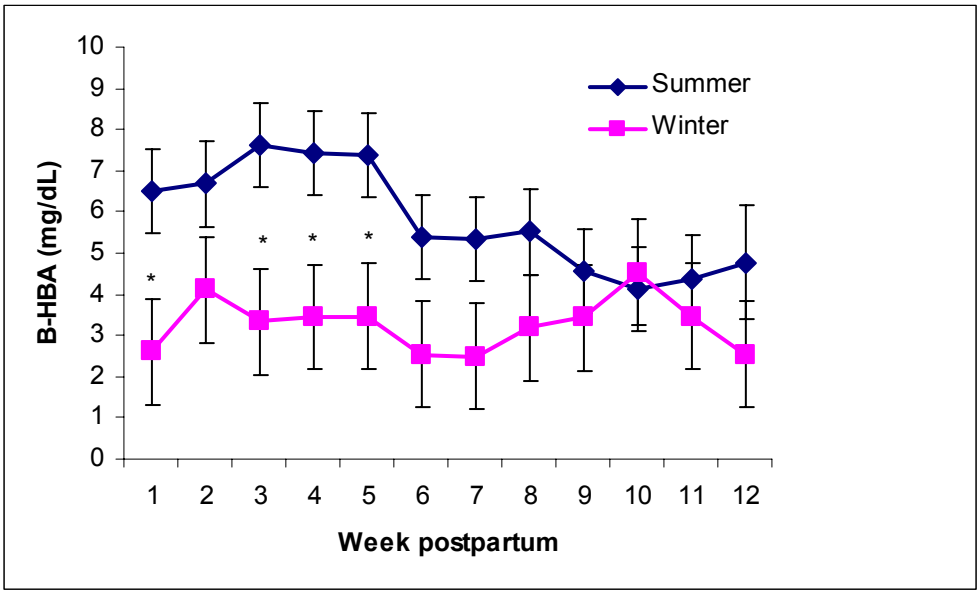


Figure 4. Mean weekly serum cortisol between postpartum summer and winter calving groups.



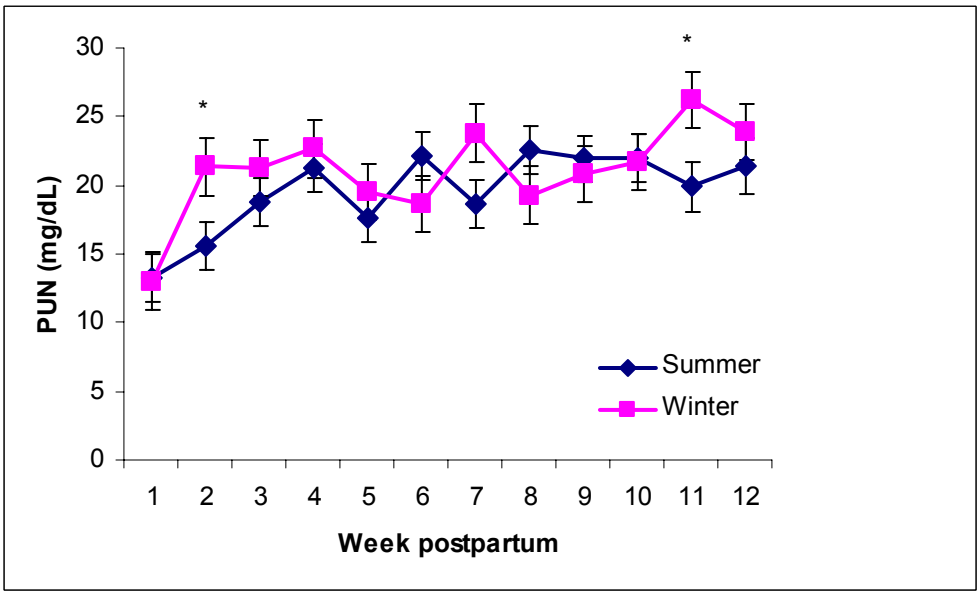
* = $P \leq 0.05$

Figure 5. Mean weekly plasma β-HBA between postpartum summer and winter calving groups.



*** = $P \leq 0.05$**

Figure 6. Mean weekly plasma PUN between postpartum summer and winter calving groups.



*** = $P \leq 0.05$**

Figure 7. Mean weekly serum insulin between postpartum summer and winter calving groups.

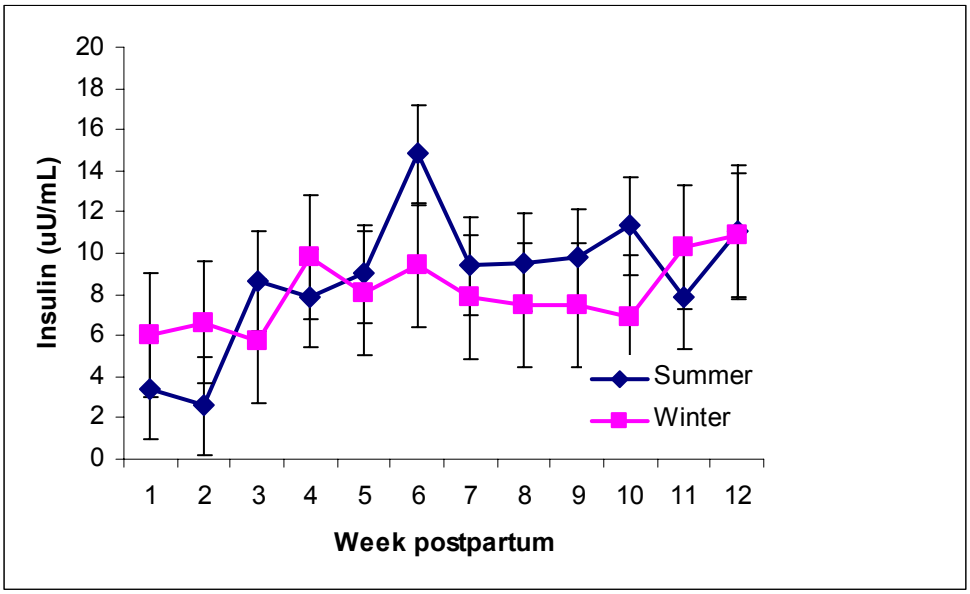


Figure 8. Mean weekly serum leptin between postpartum summer and winter calving groups.

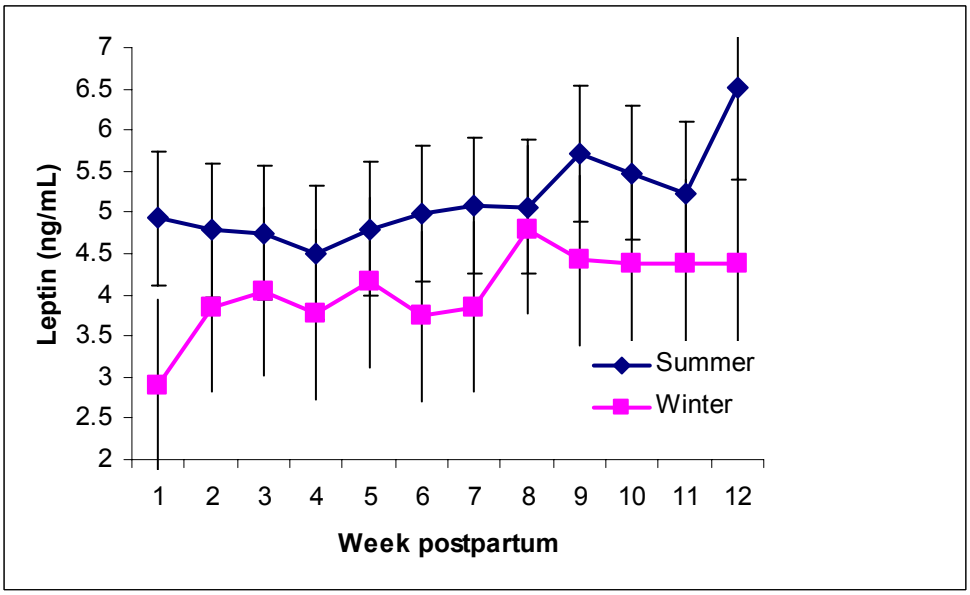
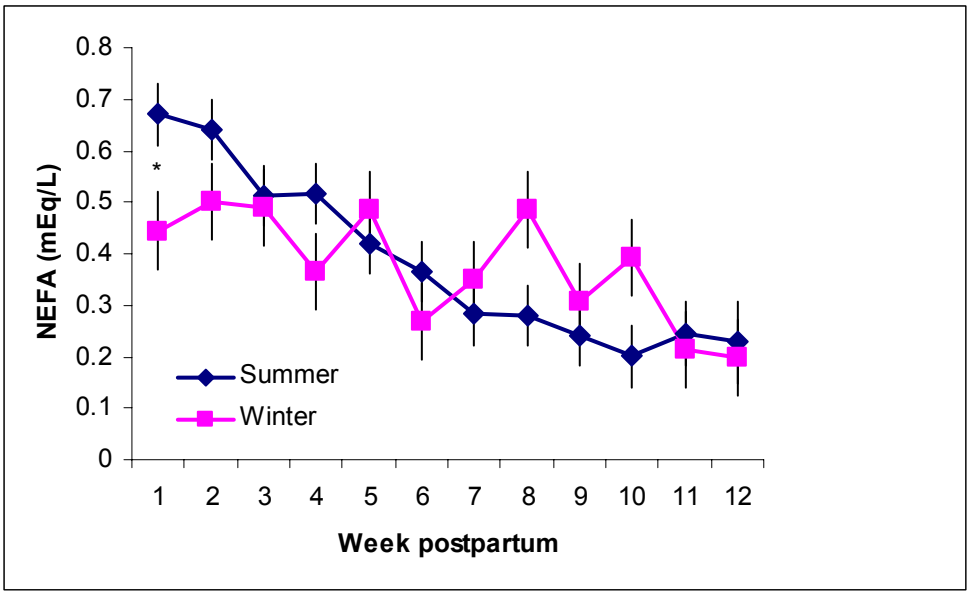


Figure 9. Mean weekly serum NEFA between postpartum summer and winter calving groups.



*** = $P \leq 0.05$**

Figure 10. Mean weekly plasma glucose between postpartum summer and winter calving groups.

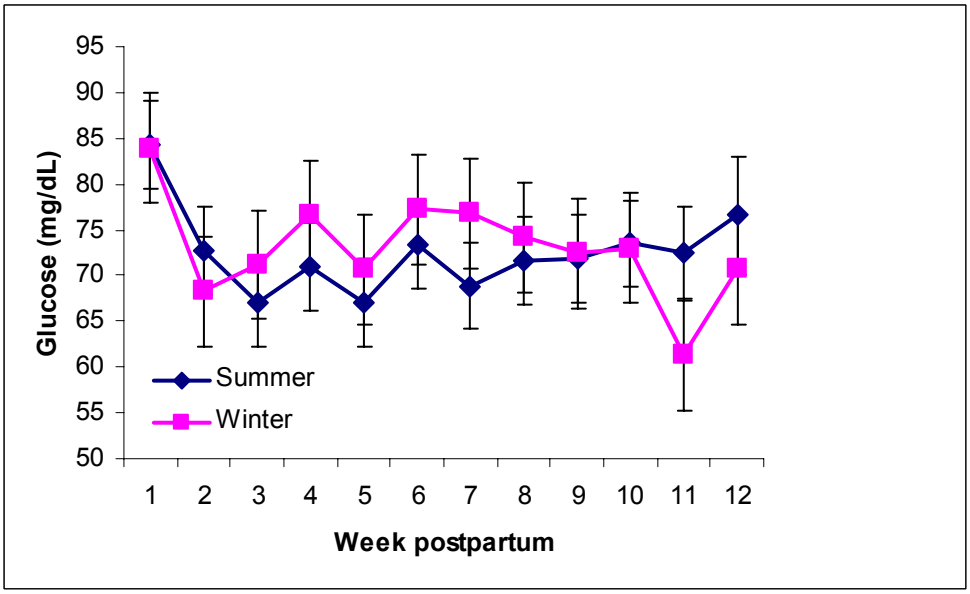
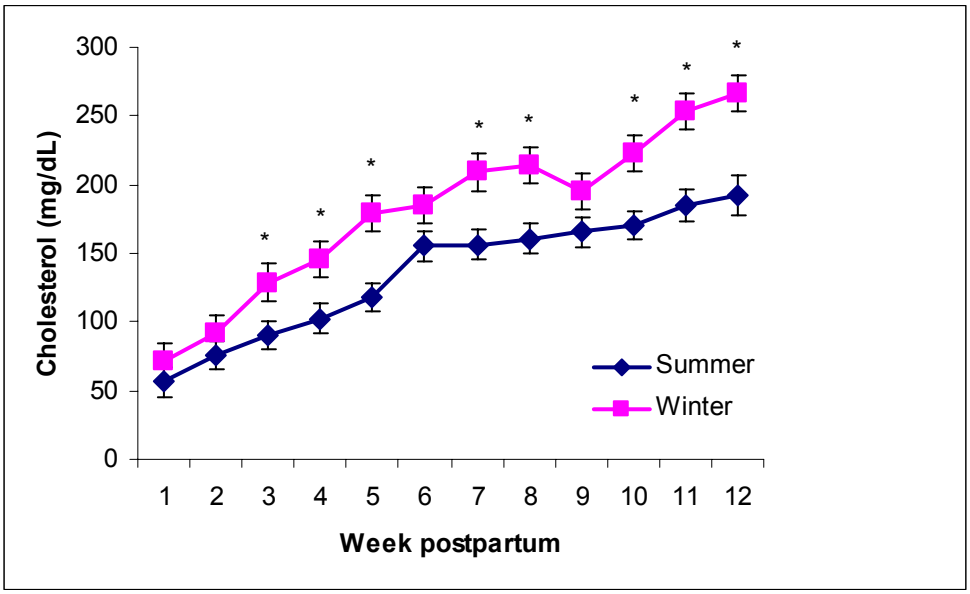


Figure 11. Mean weekly serum cholesterol between postpartum summer and winter calving groups.



*** = $P \leq 0.05$**

DISCUSSION

Progesterone:

Postpartum changes in serum P4 are evident over time as the cow prepares for another estrous cycle. By measuring serum P4 levels, time of first ovulation can be determined. Serum progesterone levels are nearly undetectable until about day 30 postpartum (Taylor et al., 2003). Levels exceeding 1 ng/mL are considered to indicate resumption of ovulation, with it not being uncommon for the first estrous cycle to be shorter in length than normal. The present results show a significant effect of week, indicating a secretory pattern that changes accordingly with resumption of ovarian cyclicity and subsequent estrous cycles. Between the two seasonal groups, the winter calving group had an earlier rise in serum P4 compared to the summer calving group. Previous heat stress studies have reported conflicting results on the pattern of

progesterone secretion. Wise and colleagues (1988) found that lactating dairy cows subjected to either shade or no shade treatment had similar P4 concentrations. Howell and colleagues (1994) reported that lactating dairy cows experiencing chronic heat stress which would be typical of a long summer had decreased P4 concentrations, in contrast to increased P4 levels in acutely heat stressed cows. The THI values for August and September (summer) of the current study were considered mild and borderline heat stress values. Although our results show no overall significant effect of season, there was a significant difference between the two groups at week 7 and there tended to be a difference at week 5. Also, the average day to first ovulation differed by a mean of 17 days with the summer calving group having a later rise in serum P4. The effects of a chronic heat stress from several previous months of summer may have caused a prolonged interval to first ovulation in this group of cows.

Thyroid Hormone (T4):

Thyroid hormone synthesis has been shown to increase steadily as early lactation progresses. During gestation, thyroid hormones may play a role in maintenance of pregnancy and during lactogenesis. It may be an important hormone for normal ductal development of the mammary gland (Vonderhaar and Greco, 1979). Additionally, higher levels of thyroid hormones may act as a metabolic signal to the cow to begin resumption of ovarian activity (Reist et al., 2003). Being metabolic regulators as well as calorigenic hormones, several heat stress studies have shown that under both acute and chronic heat stress conditions, T3 and T4 synthesis were reduced and subsequently, less was found in milk or plasma (Johnson and Vanjonack, 1976; Magdub et al., 1982). The dairy cow

compensates for the additional environmental heat load by reducing synthesis and secretion of these calorigenic hormones. Other studies have had conflicting results with no differences found in the amount of thyroid hormone synthesis with control versus acutely heat stressed cows (McGuire et al., 1991). The severity and duration of the heat stress may account for some of the differences amongst these studies. The present study shows no significant effect of season between the two calving groups. The mild heat stress that the summer group experienced may not have been severe enough to reduce serum T4 compared with the winter group. Since the summer trial began in late summer, following several months of hot temperatures, this group may have already become acclimated to their environments by the time our trial began. Thus, there was no significant difference seen between summer and winter serum T4 levels.

Cortisol:

Cortisol is the major glucocorticoid produced by the adrenal cortex in response to stressful stimuli and also plays a role in glucose regulation. Several previous studies have shown that at the time of parturition, cortisol peaks, followed by a sharp drop in concentrations in early lactation (Hunter et al., 1970; Goff and Horst, 1997). Over time, cortisol synthesis will be increased. Acute heat stress has been shown to cause an increase in cortisol production, as the cow becomes stressed and experiences the 'shock' phase. Chronic exposure to high temperatures causes cortisol synthesis to decrease as the cow adapts to its environment, often times decreasing to levels at or below the normal, physiological level (Christison and Johnson, 1972; Alvarez and Johnson, 1973; Johnson and Vanjonack, 1976). The current study showed that immediately following parturition

(within 24 hours), serum cortisol concentrations were elevated and by week 2 or 3, levels had decreased. Three out of the 12 weeks showed significant differences between the two calving groups. The summer group's mean weekly serum cortisol tended to be lower than the winter group up until week 8. Thereafter, the summer group's mean serum cortisol concentration began to increase. This would have coincided with cooling temperatures in late September and October. With no overall significant effect of season, the summer calving cows had already been exposed to several months of hot summer weather which would be considered chronic heat stress. As in the findings from previous studies with differences in cortisol secretion patterns between acute and chronic heat stress, our results are in agreement since the summer group tended to have lower mean concentrations during the time of mild heat stress. This could indicate that during chronic exposure to a stressor, such as heat, ACTH secretion would be reduced from the pituitary gland to limit synthesis of cortisol which is a calorigenic hormone. This is a mechanism the cow uses to compensate for the environmental heat load and is an attempt to minimize any metabolic heat generation. Thus, over time the cortisol level is reduced to nadir levels. Once temperatures begin to drop, the negative feedback loop of the hypothalamo-pituitary-adrenal axis is weakened so that cortisol synthesis and secretion increases to detectable levels.

β-Hydroxybutyrate (β-HBA):

During the drop in feed intake around the time of parturition, glucose reserves are depleted thus resulting in increased ketone levels. With increased energy demands for both maintenance functions and lactogenesis, the ketone β-HBA tends to increase

following day of calving until about 30 days postpartum. Thereafter, levels begin to decline as the cow recovers from hypophagia and a negative energy balance (Vasquez-Anon et al., 1994). The results of the present study's summer mean weekly plasma β-HBA are in agreement with the results of Vasquez-Anon and colleagues. The ketone level gradually increased following day of calving up until week 3. Levels reached a plateau and began decreasing after week 5 postpartum. The winter group's mean weekly plasma β-HBA had a slight increase following parturition up until week 2 postpartum; levels thereafter remained relatively constant. The significant differences between the two seasonal groups in their levels of plasma β-HBA indicate that the summer group may have experienced a greater drop in periparturient feed intake. However, this conclusion cannot be confirmed since feed intake was not measured in the current study. An increased concentration of β-HBA is a sign of negative energy balance (Reist et al., 2000). Therefore, the summer group may have been in a greater negative energy balance than the winter calving group in early lactation. Previous studies have not shed light on whether or not ketone body production is altered under heat stress conditions. It seems that the level of β-HBA is directly related to feed intake and is inversely related to glucose availability (Ingvartsen and Andersen, 2000).

Plasma Urea Nitrogen (PUN):

Taylor and colleagues (2003) found that serum PUN tends to gradually increase postpartum and reach a plateau. The current results are in agreement with Taylor's study in that there was a significant effect of week as the level of plasma PUN for both seasonal groups increased considerably following parturition, thereafter reaching a plateau at

about week 4. Both summer and winter groups had a mean PUN concentration of about 13 mg/dL at calving with summer not exceeding 20 mg/dL and winter not exceeding 25 mg/dL during the first 12 weeks postpartum. Weeks 2 and 11 were significantly different between the two calving groups mean PUN. Overall, our study indicated a significant effect of week with a progressive increase in plasma PUN following parturition. There was limited change in concentration after week 4. By week 4, the summer group's mean plasma PUN was about 20 mg/dL and by week 2, the winter group's mean plasma PUN exceeded 20 mg/dL. The cows in this study were being fed a TMR with 17.8% CP. Previous studies have shown that feeding transition period cows between 17 and 19% CP may be a contributor to reduced fertility postpartum. Specifically, diets that resulted in PUN concentrations of greater than 19 mg/dL were inversely related to fertility (Ferguson et al., 1988; Butler et al., 1996). However, our study did not evaluate postpartum reproductive parameters such as first service conception rate, as we were focused on analyzing seasonal differences. Previous studies have not shown whether PUN is altered under heat stress conditions.

Insulin:

Previous studies have shown that insulin is depressed in the early postpartum period as liver triacylglycerides are accumulating in the liver to provide enough energy for lactogenesis (Smith et al., 1996; Ingvartsen and Andersen, 2000). Very little has been previously reported on the effects of heat stress on insulin production in the lactating cow. Itoh and colleagues (1998) reported that thermally stressed, lactating cows had elevated levels of insulin and decreased milk yields compared to the thermal neutral

group. Heat stress studies can vary greatly depending on whether controlled, climatic chambers or natural, environmental surroundings are utilized for the heat source. Other factors to consider are whether the heat stress is imposed acutely or whether the animals have had time to adjust. Although our results collectively did not have a significant effect of week, season or interaction, on average, the summer group's mean insulin tended to be lower the first 2 weeks postpartum compared to the winter group. This coincides with the summer group's tendency to have lower mean weekly plasma glucose concentrations in the early postpartum period, as well as more elevated NEFA and β-HBA concentrations at this time.

Leptin:

During early lactation, cows that are in a more severe negative energy balance have a greater mobilization rate of NEFAs from adipose tissue. As a result, adipocytes are depleted and leptin synthesis is reduced (Block et al., 2001). However, it has also been found that leptin secretion was altered by photoperiod. In sheep studies, longer daylength was found to increase leptin mRNA in adipose tissue as well as plasma leptin. As daylength increases, the availability of food is also increased in a grazing environment. Therefore, a longer photoperiod could aid the animal in adapting to its environment through interactions between leptin, insulin, glucocorticoids and brain hormones (Chilliard et al., 2001). In another photoperiod study, Garcia and colleagues (2002) found that in ovariectomized, estradiol-implanted cows, plasma leptin was increased from January up until the summer solstice. It has been reported that slight changes in cow body weight or body condition does not significantly alter circulating

plasma leptin (Chilliard et al., 2001). For the current study, there was a significant seasonal effect. The summer group on average tended to have a greater amount of leptin for the entire 12-week trial compared to the winter group. At calving, the summer group averaged about 5 ng/mL and the winter group averaged about 3 ng/mL. The winter group increased to an average of about 4 ng/mL by week 2 and levels remained fairly constant for the remainder of the 12 weeks. Likewise, the summer group's leptin concentrations did not deviate significantly over time, although by week 12, levels had risen to an average of about 6.5 ng/mL. Although we only recorded body condition scores one time following calving, our results suggest that the summer calving group did not experience a significant drop in body condition, enough to deplete their adipose reserves and circulating leptin. Longer daylength during the late summer compared to the winter could therefore explain the summer group's tendency to have greater concentrations of serum leptin postpartum.

Non-Esterified Fatty Acids (NEFA):

Bertics and colleagues (1992) reported that as a result of decreased feed intake around the time of parturition, an important metabolic response in the cow is to mobilize triacyglycerides from adipose tissue to the liver to provide enough energy for the rapid fetal growth in late gestation, lactogenesis and maintenance functions. Many studies have shown that due to the increased mobilization of TG's, NEFA levels are increased during the periparturient period (Kunz et al., 1985; Bertics et al., 1992; Vazquez-Anon et al., 1994; Grummer, 1995; Grum et al., 1996; Smith et al., 1997; Reist et al., 2000). Elevated NEFA concentrations around the time of early lactation are inversely related to

glucose and insulin concentrations. The current results indicate that effect of week was significant. Both seasonal groups had a general trend of gradual decline in NEFA concentration from time of calving to week 12 postpartum. At parturition, summer NEFA levels were on average greater than the winter group. Week 1 NEFA levels for summer averaged about 0.7 mEq/L and winter averaged about 0.45 mEq/L. For the summer group, elevated serum NEFA concentrations would seemingly agree with a more elevated plasma β-HBA concentration as well as lower circulating insulin the first 2 weeks postpartum. Plasma glucose for the summer group, although not significantly different from the winter group, took longer to increase postpartum and the weekly means tended to be lower in early lactation. Overall, there was no significant seasonal difference in NEFA concentrations. However, week 1 postpartum was significantly different between the two seasonal groups and there tended to be a significant week x season interaction.

Glucose:

Due to the high demands of energy for the processes of lactation and maintenance, glucose has been shown to be depressed in several studies in the early postpartum period, thereafter levels beginning to increase. This coincides with the cow's decrease in feed intake at parturition and a progressive recovery in appetite and energy status (Vazquez-Anon, 1994). Our results are consistent with this earlier study reported by Vazquez-Anon. For the present study, at parturition there was an acute increase in plasma glucose for gluconeogenesis, presumably due to the energy demands of the mammary gland. Both seasonal groups had an average of about 85 mg/dL at calving. By

week 2, the summer group's average had dropped to about 73 mg/dL and the winter group's average had dropped to about 69 mg/dL. Although there was no significant effect of week or season, the summer group's mean weekly concentrations tended to be lower than winter. This may imply that it took longer for the summer calving group to recover from a negative energy balance if their degree of hypophagia at parturition was more pronounced due to both stressors of calving and heat. This agrees with the summer group having greater levels of plasma β-HBA and NEFA, as well as a tendency to have lower insulin the first 2 weeks postpartum.

Cholesterol:

Previous studies have shown that postpartum serum cholesterol is at its lowest concentration in the first month of lactation, reaching its maximum concentration at about 5 months and thereafter decreasing into late lactation. Both the increased nutrient demands of the rapidly growing fetus from the previous pregnancy and the utilization of cholesterol for steroidogenesis could be contributing factors to a low level in early lactation (Arave et al., 1975). Although the present results are in agreement with this previous finding that cholesterol levels tend to be lower in early lactation, Arave and colleagues (1975) also reported that summer calving cows had higher levels of cholesterol compared to winter calving cows. Our findings are opposite to the seasonal differences reported by this author. Both seasonal groups had their lowest levels of cholesterol following parturition. There was both a significant effect of week and season. Levels for both calving groups gradually increased over time with the winter group tending to have greater weekly averages compared to the summer. Reist and colleagues

(2003) proposed that cholesterol and glucose are closely related metabolites. Glucose has been shown to promote the uptake of cholesterol for steroidogenesis. Although levels of glucose for the current study were not significantly different between summer and winter groups, the summer group did tend to have lower mean weekly concentrations which coincides with this group's lower mean weekly cholesterol concentrations. Additionally, Moss and colleagues (2002) reported that cows with poor fertility had similar relationships between low glucose, low cholesterol, high β-HBA and requiring more than two inseminations in the previous lactation cycle. In the present study, the summer calving group had a delayed rise in serum P4 which could be attributed, in part, to their lower serum cholesterol, the precursor for all steroid hormones.

CONCLUSIONS

The goals of a dairy producer are to optimize both milk yields and conception rates in the herd. Heat stress can be a barrier to both these goals, compromising fertility and under severe conditions, reducing milk yields. Typically, scientific research is conducted on a specific area of study, such as reproductive physiology or nutrition. The dairy cow is a complex organism comprised of all these integrated systems that we often times isolate into disciplines. Thus, in attempting to understand the responses of a complex animal, such as the dairy cow to heat stress, we cannot limit our focus to just one discipline.

In the current study, we examined a number of hormones and metabolites in two seasonal groups of postpartum Holstein cows. While we did not take the direct route of examining aspects of fertility such as postpartum conception rate as it related to heat

stress, there were significant changes found between a chronic, mildly thermally stressed postpartum cow versus a non-thermally stressed postpartum cow. The hormones progesterone, cortisol and leptin and the metabolites β-HBA and cholesterol had altered patterns of secretion between the two calving groups. Lower cholesterol in the summer calving group as well as mild heat stress in the first two months of the summer trial were potential contributors to the delayed rise in postpartum progesterone. These results indicate the important relationship between nutrition during the periparturient period and postpartum reproductive health. Understanding the effects and responses of chronic heat stress in cattle under natural, environmental conditions is key in attempting to alleviate and minimize the associated problems.

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