ENVIRONMENTAL AND GENETIC EFFECTS ON PROLACTIN PHYSIOLOGY AND IMMUNE STATUS OF HOLSTEIN HEIFER CALVES

By

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To my family for their endless support

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"*Dictionary is the only place that success comes before work. Hard work is the price we must pay for success. I think you can accomplish anything if you're willing to pay the price.*" ~ Vince Lombardi

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropin
BW	body weight
CRF	corticotrophin releasing factor
CL	corpus luteum
CP	crude protein
DM	dry matter
DMI	dry matter intake
DPBS	Dulbecco's phosphate buffered saline
HT	heat stress
IGF	insulin-like growth factor
JAK2	janus kinase
LDPP	long day photoperiod
mRNA	messenger ribonucleic acid
PBS	phosphate buffered saline
PRL	prolactin
PRLR	prolactin receptor
PRLRKO	prolactin receptor knock out
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SCN	suprachiasmatic nucleus
SDPP	short day photoperiod
SOCS	supressor of cytokine signaling
TMR	total mixed ration

Abstract of Thesis Presented to the Graduate School
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Experiments with Holstein dairy calves were conducted to further elucidate the impact of environmental effects on slick calves compared with wild-type calves. Furthermore, the effect of heat stress and photoperiod on immune status was evaluated to investigate the role of PRL signaling and effects on protein expression.

In the first study, the effect of heat stress on prolactin-signaling gene expression and protein abundance and immune status of Holstein calves expressing either the *slick hair* gene or normal hair coats was evaluated. Calves went through one week of acclimation prior to three weeks of either heat or thermoneutral conditions, two weeks of acclimation, and then three weeks of heat or thermoneutral exposure. During heat stress calves had greater prolactin concentrations in plasma, but no differences in immune response or prolactin receptor were identified. However, calves expressing the *slick hair* gene had lower neutrophil phagocytosis, oxidative burst, greater prolactin receptor protein, and circulating blood prolactin concentrations. These data suggest that prolactin mediated responses are influenced by genotype but not heat stress in dairy calves.

The second study evaluated the effect of photoperiod on prolactin-signaling gene expression and prolactin receptor protein abundance and immune status of Holstein calves

expressing either the *slick hair* gene or normal hair coats. Calves went through two weeks of acclimation then exposed to three weeks of either long day or short day photoperiod, two weeks of acclimation, and then three weeks of long day or short day photoperiod conditions. Under long day exposure calves had greater prolactin concentration in plasma, but no differences in immune data, relative to short days. Slick calves tended to have lower circulating concentrations of prolactin independent of photoperiod. Calves exposed to LDPP had lower mRNA expression of prolactin-receptor in lymphocytes as determined by realtime quantitative RT-PCR.

CHAPTER 1
INTRODUCTION

Environmental factors such as high ambient temperatures exert significant effect on animal health and performance (Hahn, 1999). Indeed, heat stress results in decreased milk production, efficiency, reproduction, feed intake, and growth in dairy cattle (Hahn, 1985). Exposure to high temperatures disturbs the animal's physiological balance, particularly thermal and hormonal regulation and water balance, which result in lower productivity (Johnson, 1980). One hormone, prolactin (PRL), has been identified as an acute stress hormone and changes in PRL affect immune function. Because PRL increases under heat stress conditions (Collier et al, 1982), PRL may act as a link between heat stress and the immune system.

Prolactin regulates physiological functions via actions on cellular processes including proliferation, differentiation, cell survival and immune function modulation (Yu-Lee, 2002; Reber, 1993). When considering the adaptive arm of the immune system, lymphocytes are critical because they respond to infectious agents through the elaboration of antibodies, cytokines and via specific T-cell immunity (Detilleux, 1994). Furthermore, lymphocytes in cattle express PRL receptor (PRL-R) mRNA (Schuler et al., 1997) and PRL-R expression is inversely related to circulating concentrations of PRL (Auchtung et al., 2003). For example, Do Amaral et al. (2009) demonstrated heat stressed cattle had greater circulating PRL, but lower lymphocyte proliferation and PRL-R mRNA expression in lymphocytes compared with cooled cows.

Yet other environmental cues, such as photoperiod, are used by many species to time seasonal events associated with reproduction, growth and lactation (Nelson and Demas, 1996; Dahl et al., 2000). Likewise, manipulating the light exposure to animals is linked to other physiological changes, for example immune response (Nelson and Demas, 1996; Dowell, 2001; Bilbo et al 2002a; Bilbo 2002b). Short day photoperiod (SDPP) increases natural killer cell's

spontaneous blastogenesis in lymphocytes of Siberian hamsters (Yellon et al., 1998a). Photoperiod management of dairy cattle, specifically SDPP, improves cellular immune function in cattle relative to long-day photoperiod (LDPP) during the dry period (Auchtung, 2004). Photoperiod consistently alters PRL release across species, and because PRL regulates physiological functions via actions on cellular processes including proliferation, differentiation, cell survival and immune function modulation, it is a strong candidate to mediate photoperiod-induced effects (Yu-Lee, 2002; Reber, 1993). However, the relationship of between PRL signaling and immune status is not fully characterized in the bovine.

When considering the adaptive immune response, lymphocytes are critical because they respond to infectious agents through the elaboration of antibodies, cytokines and through specific T-cell immunity (Detilleux, 1994). Furthermore, lymphocytes in cattle express PRL-R mRNA (Schuler et al., 1997) and PRL-R expression is inversely related to circulating concentrations of PRL (Auchtung et al., 2003). However, the effect of circulating PRL on PRL-R protein expression is unknown. Thus, the first objective was to determine the relationship among PRL, PRL-R mRNA and PRL-R protein in calves under LDPP and SDPP conditions. In addition, confirmation was sought of the relationship of PRL signaling to that of immune status in calves that previously observed in older cattle (Auchtung et al., 2003).

Previous studies indicate that Senepol cattle, a *Bos taurus* breed, have heat tolerance comparable to Brahman cattle, a *Bos indicus* breed (Hammond et al, 1996). Moreover, certain progeny of Senepol background express a short, sleek hair coat, termed “slick hair” (Olson et al., 2003). In a study to evaluate the thermoregulatory ability of slick-haired Holstein cows under acute heat stress compared to wild-type cattle, *slick hair* cows had lower vaginal temperatures, respiration and sweating rates relative to their wild type counterparts (Dikemen et al., 2008).

That outcome suggests that aspects of heat tolerance associated with *slick* expression can be upgraded into cattle of Holstein background.

The application of the *slick hair* gene for use in subtropical climates could prove to be a method of reducing heat stress symptoms in dairy production systems, yet the impact of the slick gene on immune function is unknown. Moreover, the *slick hair* gene has been mapped to chromosome 20 near the PRL-R gene (Mariasegaram et al., 2007), which is involved in hair cycling (Ouhtit et al., 1993, Nixon et al., 2002) as well as modulation of immune and inflammatory response (Yu-lee, 2002). Therefore, the second objective was to test the hypothesis that slick animals have altered PRL signaling and immune status relative to wild-type calves, and that slick calves would have improved immune status under heat stress.

This thesis literature review (Chapter 2) will summarize: 1) photoperiod and how daylength is linked to immunity, 2) heat stress in mammals 3) prolactin actions and signal transduction and 4) the *slick hair* gene. Chapter 3 consists of two experiments that describe the effect of heat stress and photoperiod, consecutively, on PRL signaling and immune function in slick-haired and wild type Holstein calves. Moreover, Experiment 1 describes potential advantages of the expression of the *slick hair* gene in cattle under heat stress.

CHAPTER 2
REVIEW OF LITERATURE

Introduction

A first step to understanding the physiological aspect of individual animals, and in turn populations of animals, is to understand the relationship they have with their environment. Manipulation of the environment is one of the oldest strategies used to elicit a physiological change or response. Changing the environment surrounding the animal can be as simple as changing the lighting scheme in animal housing, as in the case of photoperiod. However, environmental factors such as heat stress have been proven to be expensive and thus difficult to control in dairy cattle production systems. Moreover, the introduction of genes from a more heat tolerant bovine breed has recently been introduced into Holstein cattle, termed the *slick hair* gene, is an alternative method of increasing or maintaining productivity of dairy cattle under high ambient temperatures. The objective of this review is to examine the effect of heat stress and photoperiodic manipulation on animal physiology focusing on lactation and immune function. In addition, the effect of genetic variability on immune function among bovine breeds was also explored. An overview of PRL and PRL-R expression on immune function will also be introduced as a mechanistic foundation for chronic animal responses to heat and light.

Environmental Influence

Most research focusing on photoperiodic alterations to elicit physiological responses use consistent daylengths. Long day photoperiod (LDPP) consists of 16 hours of light and 8 hours of darkness, whereas SDPP is associated with 8 hours of light and 16 hours of darkness. Photoreceptors, located in the retina of the eye, receive and transduce light signals. Light signals inhibit melatonin synthesis in the pineal gland, which is catalyzed by the action of N-acetyltransferase, the rate-limiting enzyme of melatonin synthesis (Morgan, 2000). In darkness

the inhibition of melatonin synthesis is removed and melatonin is secreted from the pineal gland. Melatonin acts through its G-coupled receptor, which is most abundant in the suprachiasmatic nucleus (SCN) and the pars tuberalis of the hypothalamus (Pevet, 2003). This pattern of information that melatonin secretory variation sends to the SCN is what drives the circadian rhythm of mammals.

The melatonin signal secreted under nocturnal conditions is critical to synchronize the circadian rhythm, outwardly expressed through the "clock" genes (Pevet, 2003). In order to generate and sustain this endogenous rhythmicity, a transcription/translation feedback loop of clock gene expression and protein accumulation occurs (Tournier et al., 2003). This feedback works in such a way that clock genes form heterodimers that drive transcription of *Period* and *Cryptochrome* genes. To counteract, Period and Cryptochrome genes form heterodimers that repress the *Clock* gene transcription (Kume et al., 1999). Although the clock genes form heterodimers, each gene is regulated individually by photoperiodic manipulation. Melatonin is thought to play only a minor role in SCN function, other than providing information on the photoperiod cycle. The thought still exists that melatonin may play a more significant in peripheral clocks (Stehle et al., 2003).

Factors such as high ambient temperatures exert significant effect on animal health and performance (Hahn, 1999). Heat stress results in decreases in milk production, feed efficiency, reproduction, feed intake and overall growth in dairy cattle (Hahn, 1985). Exposure to high temperatures disturbs the physiological balance of animals, particularly thermal and hormonal regulation and water balance, which result in lower productivity (Johnson, 1980).

Genetics

During times of immunosuppression, significant genetic variability occurs within the Holstein breed with regard to innate immune variables including neutrophil chemotaxis and

neutrophil function (Detilleux, 1994). In addition, Brown Swiss cows are less sensitive to hyperthermic conditions relative to Holstein cows on the basis of rectal temperature in a hot environment (Johnson 1965; Correa-Calderon et al. 2004). However, when comparing the adaptive immune status of these two breeds the Brown Swiss cows are less tolerant of chronic heat stress relative to Holstein cows (Lacetera et al. 2006).

Lymphocytes isolated from heifers of three different beef breeds demonstrated a decrease in proliferative activity at 42^{o}C *in vitro* conditions (Elvinger et al., 1991). In contrast, when cells were isolated from Holstein cattle and placed in either heat stress (42^{o}C) or thermoneutral (38.5^{o}C) conditions no difference in proliferation was observed. Using the apoptotic response of lymphocytes as a measure of thermo-tolerance, differences are observed between cattle of even greater genetic diversity, with Brahman and Senepol being more tolerant than Holstein and Angus (Paula-Lopes et al. 2003). These observations support the concept that genetic variation exists in stimulated immune responses among cattle breeds and lines.

Previous studies indicate that Senepol cattle, a *Bos taurus* breed, have heat tolerance comparable to Brahman cattle, a *Bos indicus* breed (Hammond et al., 1996). Moreover, certain progeny of Senepol background express a short, sleek hair coat, termed slick hair (Olson et al., 2003). In a study to evaluate the thermoregulatory ability of slick-haired Holstein cows under acute heat stress compared to wild-type cattle, slick-haired cows had lower vaginal temperatures, respiration and sweating rates relative to their wild type counterparts (Dikmen et al., 2008). The outcome suggests that aspects of heat tolerance associated with slick expression can be upgraded into cattle of Holstein background. The *slick hair* gene has been mapped to chromosome 20 near the PRL-R gene (Mariasegaram et al., 2007), which is involved in hair cycling (Ouhtit et al 1993, Nixon et al., 2002) as well as modulation of immune and inflammatory response (Yu-lee, 2002).

The application of the *slick hair* gene for use in subtropical climates could prove to be a method of reducing heat stress symptoms in dairy production symptoms, yet the impact of the slick gene on immune function is unknown.

Prolactin Physiology

Prolactin

Considered as an endocrine hormone and an autocrine/paracrine growth factor (Kelly et al., 1991), PRL is secreted from lactotrophs of the pars distalis. Secretion of PRL is inhibited by dopamine (Ben-Johnathan and Hnasko, 2001), which is produced in tuberoinfundibular neurons of the hypothalamus, acting on receptors located in lactotrophs of the pituitary. Although it is often considered the primary lactogenic hormone (Ostrom, 1990), PRL is also integral to mammary gland development (Akers, 1985; Tucker, 1994).

Prolactin Receptor

Prolactin binding receptors, part of the cytokine receptor family, are single-transmembrane proteins in structure and their subunits have no intrinsic tyrosine kinase activity (Goupille et al., 2000). Most mammals have two isoforms of the PRL-R that are created from alternative splicing of a single gene, diverging at the intracellular domain (Schuler et al., 1997). The following section covers the PRL-R signaling pathways along with receptor expression in different tissues and at various physiological states.

Signaling Pathways

When PRL binds to its receptor the receptor undergoes dimerization causing a conformational shift. This shift leads to the phosphorylation and activation of the protein kinase Janus kinase 2 (JAK2) pathway and the receptor (Lebrun et al., 1994). In turn, protein tyrosine phosphotase, phosphatidylinositol 3-kinase and STAT-5 interact with the interaction sites exposed by the phosphorylation of the receptor (Goupille et al., 2000). It appears that signal

transduction is primarily accomplished through the JAK/STAT pathway in cells of the immune system (Lebrun et al., 1995).

The next step to be accomplished is to turn off the PRL signaling. Because PRL is in the cytokine family it is logical that proteins capable of suppressing cytokine signaling create a negative feedback loop to end the signaling (Pezet et al., 1999). Indeed, these suppressors of cytokine signaling (SOCS) bind to the JAK pathways and depress tyrosine kinase activity, which in turn decreases STAT phosphorylation. However, one of the forms of SOCS, labeled SOCS-2, is thought to resensitize cells to PRL and restore PRL signaling by interacting with SOCS-1 (Pezet et al., 1999). Thus, some forms of SOCS do stop PRL signaling, however different forms of SOCS can play different roles by restoring PRL signaling.

Variation of Prolactin Receptor Expression

Depending on the numbers of PRL-R in a given tissue it is speculated that PRL-R regulation by PRL is concentration dependent (Djiane et al., 1979). For example, in fetal rats levels of PRL-R messenger RNA (mRNA) and receptor protein expression increase during late developmental stages, which explains predicts diverse biological actions in fetal and neonatal development (Royster et al., 1995). The expression of PRL-R in rodents is suppressed during pregnancy, but increases throughout lactation in the mammary gland (Jahn et al., 1991; Mizoguchi et al., 1997). Interestingly, expression of PRL-R in the rat corpora lutea is increased in the presence of PRL and acts opposite of the PRL-R in other tissues of the rat by increasing during pregnancy, but declines after parturition (Telleria et al., 1997).

Photoperiod-Driven Prolactin Secretion

Prolactin secretion is consistently and highly influenced by photoperiod treatment in cattle and other species (Dahl et al., 2000). Whereas wavelength of light does not appear to affect PRL secretion in cattle (Leining et al., 1979), continous lighting can cause cattle to become less

affected (Buchanan et al., 1992). According to Sweeney et al., (1999) there is a variation in responsiveness of the PRL axis to changes in melatonin depending on the season. This can be observed not only in the ewe (Sweeney et al., 1997), but the mare as well (Fitzgerald et al., 2000). This is most prevalent in the spring when the PRL axis is sensitive to the inhibitory feedback signal of melatonin and is most refractory at other times of the year.

Regulation of PRL secretion is thought to be driven by change in the duration of melatonin elevations in response to darkness, resulting from photoperiod manipulation (Lincoln et al., 2003). The pars tuberalis of the pituitary gland has melatonin receptors, but according to the studies on mammalian species to date, the lactotrophs of the pars distalis do not have melatonin receptors. In sheep, once melatonin is bound to its receptor, tuberalin transmits a signal from the pars tuberalis to the pars distalis, which in turn acts on the lactotrophs to secrete PRL (Hazelrigg, et al., 1996; Morgan, 2000). It is unknown if the described mechanistic pathways hold true for cattle.

Following hypophyseal stalk transection, beef calves continue to respond to seasonal changes (Cho et al., 1998). However, photoperiodic changes in PRL in cattle are not controlled by changes in dopamine or 5-hydroxytryptamine concentrations (Zinn et al., 1991). Indeed, PRL concentrations from calves that were pinealectomized did not change and responded to photoperiod (Stanisiewski et al. (1988a), despite the observation that pinealectomy did alter melatonin concentrations (Stanisiewski et al., 1988b). On the contrary, feeding melatonin to prepubertal heifers depressed PRL concentrations (Sanchez-Barcelo et al., 1991). An *in vitro* study conducted by Hanew (et al., 1980) on pituitary cells discovered that melatonin enhanced PRL secretion, whereas others reported no effect of melatonin (Padmanabhan et al., 1979). This

current research leads one to believe that cattle differ from seasonal breeders with regard to photoperiod driven PRL secretion.

Photoperiod and Lactation

Increasing milk production is a common goal between researchers and producers alike. Exposure of Holstein cattle to LDPP increases milk yield compared with cows on natural photoperiod or 12 hours of light, making photoperiod manipulation an attractive management approach for lactating cattle (Peters et. al., 1978; Stanisiewski et al, 1988b; Evans and Hacker, 1989). As expected, dry matter intake also increases under LDPP, but this increase follows rather than leads to increase in milk yield (Peters et al., 1981; Dahl et al., 2000). Milk composition is not altered by photoperiod manipulation (Dahl et. al., 1997), despite the greater yield.

The phenomenon of increased milk production when exposed to LDPP has not been fully explained; however it is likely that several hormones play a role. It has been speculated that insulin-like growth factor-I (IGF-I) is one of the key players in this relationship (Dahl et. al., 1997; Dahl et. al., 2000). Growth hormone (GH) has also been recognized as a promoter of milk production in lactating cows (Bauman and Vernon, 1993), yet GH is not influenced by photoperiodic manipulation in cattle (Peters and Tucker, 1978). Moreover, PRL is yet another candidate due to its relationship with photoperiod and lactogenesis, although Plaut et. al., (1987) reported that exogenous PRL administration did not increase milk production during lactation. This evidence supports the theory that IGF-1 mediates the increased milk production in response to LDPP. It is understood that there is a lactational effect associated with photoperiod manipulation and hormone fluctuation. However, a more in-depth investigation is needed to understand how environmental changes can affect other areas of dairy cattle physiology, such as the immune state of the animal.

Immune Status

Survey of Immune Measures

Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow. Leukocytes are found throughout the body, including the blood and lymphatic system. These white cells have different tasks in the immune system and respond very differently to foreign antigens in the blood stream. Therefore, measuring the immune response in mammals has proven to be difficult. Some of the more commonly used methods to be investigated include lymphocyte proliferation, neutrophil phagocytosis and neutrophil chemotaxis.

Lymphocyte proliferation through stimulation by the use of phytomitogens, such as concanavalin A, is a widely used measure of competence of the adaptive immune system (Sloane et al., 1978). The use of concanavalin A causes a stimulation of primarily T cells. Use of radio-labeled thymidine incorporated into DNA allows for stimulated cells to be measured (Lichtman et al., 1983). This general method has been validated for use in dairy calves as described by Kelley (et al., 1980). In that study, blood was collected from calves under heat and cold stress conditions and used the lymphocyte proliferation by mitogen stimulation to determine if animals were immunosuppressed. Furthermore, neutrophil phagocytosis and chemotaxis are methods used to measure innate immunity in cattle.

A central aspect to the innate immune system is the recruitment and activation of neutrophils at infected sites to assist in the elimination of pathogens. Like most cells of the immune system, neutrophils are not limited to one particular area; in fact they are mobile cells that travel throughout the body (Lee et al., 2003). When an infection is detected cells respond by secreting cytokines with chemoattractant abilities termed chemokines. A laboratory procedure

used to measure the neutrophils ability to migrate to infected sights in response to a chemoattractant is referred to as neutrophil chemotaxis.

The mobility of the neutrophils in animals of different ages and stress conditions has not been well characterized, however a method to estimate neutrophil mobility has been validated. In a study conducted by Zwahlan and Roth (1990) neutrophils isolated from adult and neonatal calves were investigated for their ability to migrate in response to various chemoattractants using a microwell filter assay. The researchers documented an excessive response to the stimulus in neonatal calves, which presents a major functional difference when compared to other species. The authors additionally suggested that this could also have an effect on other neutrophil functions.

After the neutrophil has migrated to the site of infection, its primary responsibility is to rid the body of foreign particles. The neutrophil accomplishes this by recognizing the antigen, engulfing it, and eliminating the pathogen and cell debris. This white blood cell is equipped with specialized receptors to recognize foreign particles. The complex process includes the internalization of the receptor, which initiates an assortment of specialized mechanisms that degrade the antigen of interest, resulting in the killing and disposal of the engulfed particles (Lee et. al., 2003).

A study conducted in dairy cattle measured the phagocytic ability of the animals pre- and post-partum. The phagocytosis activity was measured using a flow cytometer and revealed that the activity was most robust two weeks post parturition; however, there was a sharp decrease directly after parturition (Saad et al., 1989). One possible interpretation is that the stress caused by parturition negatively impacted the phagocytic ability of the neutrophil.

Heat Stress affect on Immunity and Endocrine Action

Cattle under heat stress conditions have demonstrated adverse responses in immunity. For example, Soper et al., (1978) observed an improvement in lymphocyte proliferation during heat stress periods relative to cooler months of the year in mature cows. Yet, slight to moderate heat stress did not affect lymphocyte proliferation in mature cows (Lacetera et al., 2002). Through a reevaluation of how immune status reacts under hot summer conditions, Lacetera et al. (2005) reported a decrease in immune function. This suggests that heat stress, depending on the intensity, elicits an endocrine response, which can have adverse affects on the overall immunity of the animal.

The endocrine response begins at the hypothalamic-pituitary-adrenal (HPA) system, which controls stress responses. The hypothalamic sympathetic system causes release of catecholamines from the brain and the adrenal medulla. In turn, certain stressors have effects on important hormones such as PRL, thyroxine, and potentially other hormones (Sapolsky et al., 2001). Neurons that originate in the nucleus of the hyphothalamus secrete corticotrophin releasing factor (CRF) from terminals that end in the median eminence. Corticotropes in the anterior pituitary respond to the CRF by synthesizing and secreting adrenocorticoptropin hormone (ACTH; Elenkov et al., 1999). The ACTH released from the pituitary travels through the circulation to the adrenal cortex where it elicits secretion of glucocorticoids, typically in the form of cortisol in pigs and cattle.

The cortisol released has a negative feedback effect on catecholamine synthesis. Yet, catecholaminergic neurons in the adrenal cortex activate cortisol-producing cells, which in turn activate catecholamines (Munck et al., 1984). The ACTH is then released which in turn causes cortisol release. This chronic exposure to cortisol elevation leads to a depression of

catecholamines and thus an overall depression of the inflammatory response (Sapolsky et al., 2001).

The Link between Photoperiod and Immune Function

As seasons change it has been observed that this is reflected in shifts in immune status and disease (Cook et al., 2002). It is difficult to explain if this due to the direct impact of the environment, for example temperature and photoperiod, or is it simply the prevalence of pathogens at particular times of year (Dowell, 2001). More than likely this shift in morbidity reflects an effect of environment on both the host (i.e. immune function) and the pathogen load.

Researchers have studied the particular relationship of photoperiod and its effects on immune status. A popular model used to investigate the relationship between photoperiod and immunity is the rodent. When testing lymphocyte proliferation in the presence of the mitogen concanavalin A, it was observed that proliferation was enhanced in SDPP relative to LDPP in both deer mice (Demas and Nelson, 1998) and Siberian hamsters (Prendergast et al., 2002). After an exposure to SDPP for an extended period of time, in this case 40 weeks, the hamsters became less sensitive to the treatment and the enhanced immune function response was lost. The hamsters under SDPP treatment had greater leukocyte trafficking (Bilbo et al., 2002b) and natural killer cell performance (Yellon et al., 1999b) compared with LDPP animals. Yet, LDPP actually increased neutrophil function and activity (phagocytosis and oxidative burst) when compared with hamsters under SDPP (Yellon et al., 1999b). Still others record only increased lymphocyte proliferation in Syrian hamsters on SDPP vs. LDPP, observing no differences in innate immunity (Zhou et al., 2002). It is generally accepted that SDPP increases lymphocyte proliferation in rodents, however results in other species could differ, just as the innate immunity contrasts within the rodent model.

The underlying endocrine mechanisms that influence immunoregulation through SDPP are unclear. It is widely accepted that cortisol is not affected by photoperiod treatment (Demas and Nelson, 1996; Drazen et al., 2001), although focus has been put on melatonin and leptin. Some suggest that the melatonin rhythm mediates humoral immunity through SDPP (Yellon et. al., 1999a). In contrast, leptin has a direct effect on cells of the immune system. Leptin is produced by adipocytes with circulating amounts increasing with the overall amount of fat in the mammal (Ahern et al., 1997). Leptins' effect on the immune function is photoperiod dependent in Siberian hamsters, however, in a study by Drazen (et. al., 2001) it was observed that exogenous leptin could overcome the reduced immune function observed in the hamsters under SDPP. Yet there was no connection between leptin and immune function in mice (Bhat et al., 2003), suggesting a possible species-specific response.

It is apparent that the results do not support the theory of leptin or melatonin as a mediator of photoperiod on immune function. However, a study conducted by Auchtung et al., (2003) discovered that PRL is an appropriate candidate as a mediator of photoperiodic manipulation in cattle. The results indicate that with an increase in circulating PRL there was a decrease in immune status of dairy cattle. It has also been reported that there is a negative relationship between PRL and PRL-R (Auchtung et al., 2004; Amaral et al., 2009), however the influence of photoperiod on PRL-R protein abundance has not yet been investigated in cattle.

Summary

Environmental management is one of the oldest techniques used to increase health and productivity of production animals. However, in order to know what aspect of the environment has the greatest effect on animals one must understand the physiological effects on the animal. Although the exact mechanism responsible for physiological changes to the environment is unknown, PRL is highly influenced by environmental events and is a likely candidate. It is now

known that immune function can be manipulated by photoperiod, however how heat stress effects immune status is still controversial.

Prolactin has shown to have a negative relationship with PRL-R mRNA expression. However, the environmental effect on PRL-R protein has not yet been evaluated in immune cells, specifically lymphocytes. Furthermore, studies involving bovine expressing the *slick hair* gene have not yet explored the immune status and PRL signaling mechanisms specific to this genotype. The experiments in the following chapter were performed to address these questions.

CHAPTER 3
EFFECTS OF HEAT STRESS, PHOTOPERIOD AND GENETICS ON THE PROLACTIN PHYSIOLOGY AND IMMUNE STATUS OF HOLSTEIN HEIFER CALVES

Abstract

Environmental factors such as photoperiod and heat stress influence health and hormone secretion in cattle and many other species. Genetic background may also modulate immune status in cattle. The first objective of experiment 1 was to test the hypothesis that heat stress depressed immune function in cattle via an alteration of PRL physiology in response to elevated concentrations of PRL during high ambient temperatures. A second objective was to examine the affect of the *Slick Hair* gene on immune and PRL status in dairy calves. Calves defined as slick-haired possess a dominant gene of Senepol origin that when expressed produces a very short, sleek coat.

Slick (n=4) and wild-type (n=4) calves were kept in controlled-temperature chambers for a period of 9 weeks. Calves were exposed to heat stress and thermoneutral conditions with a 1-week pretreatment acclimation and 2 week acclimation period between temperature treatments in a 2x2 cross-over design. Dry matter intake (DMI), water intake and infrared (IR) skin temperature were measured daily. Jugular blood samples were collected weekly and evaluated for lymphocyte proliferation, neutrophil phagocytosis and neutrophil oxidative burst activity. Relative to thermoneutral conditions, heat stress increased AM (35.0 vs. 30.6 °C; $P < 0.001$) and PM skin temperatures (36.8 vs. 31.6 °C; $P < 0.001$). Calves under heat stress increased daily water consumption (29.2 vs. 17.8 L; $P < 0.04$) and decreased DMI as percentage of body weight (2.29 vs. 3.83%; $P < 0.001$) compared with the thermoneutral period. No difference in any immune variable was observed during heat stress, relative to thermoneutral conditions. However, neutrophils from wild type calves had greater phagocytic ($P < 0.01$) and oxidative burst ($P < 0.07$) activity compared with slick-haired calves. Lymphocyte proliferation from wild type

calves did not differ from slick animals regardless of thermal treatment ($P < 0.15$). However, circulating prolactin hormone was increaseed in heat stress animals and decreased in slick animals when compared to wild type. Moreover, prolactin receptor protein had greater abundance in slick animals in comparison to wild type. Results indicate that wild type calves had improved immune status compared to slick-haired calves regardless of environmental temperatures.

The objective of Experiment 2 was to test the hypothesis that photoperiod alters immune function in cattle via change PRL physiology. A second objective was to examine the effect of the *Slick Hair* gene on immune status and PRL physiology. Slick (n=4) and wild-type (n=4) calves were kept in controlled-temperature chambers for a period of 10 weeks. Calves were exposed to long day (16L:8D) and short day (8L:16D) conditions with a 2 week pretreatment acclimation and 2 week acclimation period between photoperiod treatments in a 2x2 cross-over design. Dry matter intake (DMI) and water intake were measured daily. Jugular blood samples were collected weekly and evaluated for lymphocyte proliferation, neutrophil phagocytosis, neutrophil chemotaxis, neutrophil function and PRL. Isolated white blood cells were analyzed for PRL-R mRNA and protein. Prolactin concentrations in cattle under LDPP were higher than those of cattle under SDPP (35.8 vs. 22.3 ng/ml; SEM = 3.54 ng/ml d; $P = 0.03$). When considering the mRNA, lower expression of PRL-R was observed in calves exposed to LDPP when compared with SDPP (5.60 vs. 6.49 dCT; SEM = 1.4 dCT; $P = 0.04$). However, lymphocyte mRNA from wild type and slick calves did not differ regardless of photoperiod treatment ($P < 0.18$). Results confirm the down-regulation of PRL-R mRNA by circulating PRL due to photoperiod management.

Introduction

Environmental factors such as high ambient temperatures exert significant effect on animal health and performance (Hahn, 1999). Indeed, heat stress results in decreased milk production, efficiency, reproduction, feed intake, and growth in dairy cattle (Hahn, 1985). Exposure to high temperatures disturbs the animal's physiological balance, particularly thermal and hormonal regulation and water balance, which result in lower productivity (Johnson, 1980). Prolactin has been identified as an acute stress hormone and the effect of changing PRL concentrations on immune function has been investigated. Because PRL increases under heat stress conditions (Collier et al, 1982), and considering the possible relationship between PRL and immune status, initiating further investigation was of interest.

Manipulating the light exposure of animals is also linked to physiological changes, of interest immune response (Nelson and Demas, 1996; Dowell, 2001; Bilbo et al 2002a; Bilbo 2002b). Short day photoperiod increases natural killer cell's spontaneous blastogenesis in lymphocytes of Siberian hamsters (Yellon et al., 1998a). Photoperiod management of dairy cattle, specifically SDPP, improves cellular immune function in cattle relative to LDPP (Auchtung, 2004). Photoperiod consistently alters PRL release across species, and because PRL regulates physiological functions via actions on cellular processes including proliferation, differentiation, cell survival and immune function modulation, it is a strong candidate to mediate photoperiod-induced effects (Yu-Lee, 2002; Reber, 1993). However, the relationship between PRL signaling and immune status is not fully characterized in the bovine.

When considering the adaptive arm of the immune system, lymphocytes are critical because they respond to infectious agents through the elaboration of antibodies, cytokines and via specific T-cell immunity (Detilleux, 1994). Furthermore, lymphocytes in cattle express PRL-R mRNA (Schuler et al., 1997) and PRL-R expression is inversely related to circulating

concentrations of PRL (Auchtung et al., 2003). For example, do Amaral et al. (2009) demonstrated heat stressed cattle had greater circulating PRL, but lower lymphocyte proliferation and PRL-R mRNA expression in lymphocytes compared with cooled cows. However, the effect of circulating PRL on PRL-R protein expression is unknown. Thus, the first objective was to determine the relationship among PRL, PRL-R mRNA and PRL-R protein in calves under either heat stress or thermoneutral conditions (as observed in experiment 1) or LDPP and SDPP conditions(as demonstrated in experiment 2). Confirmation was also sought of the relationship of PRL to immune status in calves that had previously observed in older cattle (Auchtung et al., 2003).

Previous studies indicate that Senepol cattle, a *Bos taurus* breed, have heat tolerance comparable to Brahman cattle, a *Bos indicus* breed (Hammond et al, 1996). Moreover, certain progeny of Senepol background express a short, sleek hair coat, termed "slick" hair (Olson et al., 2003). In a study to evaluate the thermoregulatory ability of slick-haired Holstein cows under acute heat stress compared to wild-type cattle, slick-haired cows had lower vaginal temperatures, respiration and sweating rates relative to their wild type counterparts (Dikemen et al., 2008). That outcome suggests that aspects of heat tolerance associated with slick expression can be upgraded into cattle of Holstein background.

The application of the *slick hair* gene for use in subtropical climates could prove to be a method of reducing heat stress symptoms in dairy production systems; however the effect of photoperiod manipulation and the impact of the slick gene on immune function are unknown. Moreover, the *slick hair* gene has been mapped to chromosome 20 near the PRL-R gene (Mariasegaram et al ., 2007), which is involved in hair cycling (Ouhtit et al 1993, Nixon et al., 2002) as well as modulation of immune and inflammatory response (Yu-lee, 2002). Therefore, a

second objective was to test the hypothesis that slick animals have altered PRL physiology and immune status relative to wild-type calves, and slick-haired calves would have improved immune status under heat stress.

Materials and Methods

Animals, Treatments, and Sampling

In experiment 1, calves were housed in temperature-controlled environmental chambers located at the University of Florida (Gainesville, FL) for 63 d commencing in September, 2008. After a week of pretreatment acclimation (12L:12D; 20 ^{o}C), heifers were randomly assigned to either heat stress (HT; 36.7 ^{o}C) or thermoneutral conditions (20 ^{o}C). Lighting was provided by fluorescent lights at approximately 530 $\pm$ 10 lux at eye level (~1 M above the floor) of the heifers. Heifers in on trial (n=8) were maintained on their initial assigned controlled temperature regimen for 3 weeks and then switched to the opposite thermal condition and maintained at that temperature for the duration of the experiment. Average age of the calves at the start of the experiment was 98 $\pm$ 14 days and average starting weight was 128 $\pm$ 15 kg.

In experiment 2, calves were housed in temperature-controlled environmental chambers located at the University of Florida (Gainesville, FL) for 70 d commencing in February 2009. After two weeks of pretreatment acclimation (12L:12D; 20 ^{o}C), heifers were randomly assigned to either LDPP (16L:8D; n=4) or SDPP (8L:16D; n=4) exposure. Lighting was provided by fluorescent lights at approximately 530 $\pm$ 10 lx at eye level of the heifers. Heifers were maintained on their assigned temperature controlled regimen for 3 weeks and then switched to the opposite lighting schedule and maintained at that photoperiod for the duration of the experiment. At the start of the experiment, average age of the calves was 112 $\pm$ 14 d and average BW was 130 $\pm$ 23 kg.

In both experiments, calves expressing the *slick hair gene* (n=4) were paired with wild-type calves (n=4) of similar age and BW. Heifers were individually fed a total mixed ration formulated according to the guidelines of the National Research Council (2003). Dry matter intake was recorded and adjustments were made daily. Water was accessible to the heifers at all times.

Prolactin Assays

Blood was collected on a weekly basis into sterile Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ) containing sodium heparin from the jugular vein of calves restrained individually in their pens. Collection occurred between 0800 and 1000 each day of sampling. Samples were immediately placed on ice after collection. Plasma for hormone determination was obtained from whole blood after centrifugation (1850 x g, 30 min, 4^{o}C) and stored at -20^{o}C until assayed for PRL. Plasma PRL concentrations were determined by radioimmunoassay described by Miller et al. (1999).

Lymphocyte Isolation

Blood was collected once weekly for lymphocyte isolation to examine mRNA expression, protein abundance and proliferation. Bovine peripheral blood mononuclear cells (PBMCs) were used as the source of lymphocyte mRNA for real-time PCR. Bovine PBMCs were isolated from blood samples collected on sodium heparin by density gradient centrifugation through Fico/Lite-LymphoH (density: 1.077; Atlanta Biologicals, Lawrenceville, GA). The PBMCs were washed twice in Tissue Culture Media 199 (TCM; Fisher Scientific, M199 Powder media) and resuspended in Dulbecco's Phosphate Buffered Saline (DPBS, Sigma). For the lymphocyte proliferation assay, the cell concentration was adjusted to 3 x 10^6 cells/ml using TCM 199 supplemented with 5% horse serum (Atlanta Biologicals, Lawrenceville, GA) and 200

U/mL penicillin (MP Biomedicals, Irvine, California). Lymphocytes stored for RNA were washed once in TCM-199 to reduce RNA degradation and then frozen.

Lymphocyte Proliferation Assay

Diluted bovine lymphocytes (3×10^6 cell/ml; 100 ul) were added to 6 wells of a 96-well flat-bottom sterile plate. The mitogen concanavalin A (ConA, Sigma) was used to stimulate T cells and added in triplicate at 20 ug/mL, 3 wells were used as controls. Cells were then incubated for 48 h at 37 ^{o}C in 5% CO_2. After incubation, 0.2 μCi of ^{3}[H]-Thymidine (MP Biomedicals) diluted 10 fold in DPBS, was added to each well and incubated. Approximately 24 h later, cells were collected using a cell harvester and aspirating each well 5 times with 0.9% saline, and flushed 10 times with double deionized water to lyse the cells. Filters containing cell residue after lysis were placed in scintillation vials and 1mL of CytoScint scintillation liquid (Fischer Scientific) was added and read in a β-counter.

Neutrophil Function (Experiment 1)

Blood (6 mL) for neutrophil isolation was collected using Vacutainer tubes containing acid citrate dextrose. Within 2 hours of collection 100 μl from each sample was added to three separate sub-samples (negative control, positive control, and *E. coli* treatment). The 500 μM dihydrorhodamine (DHR) stock was diluted 10 fold into 1X PBS and 10 μl of the working DHR solution was added to each sample. After loading the samples they were incubated for 10 min at 37^{o}C under constant rotation to load the DHR into the cells. After incubation PMA was added to one of the sub-samples to create a positive control and create a measure for oxidative burst activity. Propidium Iodide (PI) labeled *E. coli* was added to the third sub-sample at a bacterium to neutrophil ratio of 40:1. Tubes were removed at the appropriate times and placed on ice to stop phagocytosis and oxidative burst activity. The samples were processed for flow cytometry using the automated Q-Prep Epics immunology workstation set on the 35-second cycle (Coulter

Counter). After processing, cold distilled water and 0.4% trypan blue was added to each tube. All tubes were vortexed and kept on ice until read using the three color fluorescence, light-duty sorting facsort flow cytometer (BD Biosciences, San Jose, CA).

Neutrophil Function (Experiment 2)

Blood (6 mL) for neutrophil isolation was collected using Vacutainer tubes containing acid citrate dextrose. Within 2 hours of collection 100 µl from each sample was added to two separate sample tubes (negative control and E. coli treatment). Then, 40 µl of pHrodo E. coli BioParticles from a Phagocytosis kit (Invitrogen, Carlsbad, CA) was added to the whole blood and incubated for 2 hours at 37^{o}C under constant rotation. After incubation the red blood cells were lysed by the addition of cold water. This step was repeated until a neutrophil pellet was obtained. Tubes were removed immediately and placed on ice to stop phagocytosis and oxidative burst activity.All tubes were vortexed and kept on ice until reading using the three color fluorescence, light-duty sorting facsort flow cytometer.

Real-Time PCR

Total RNA was extracted from lymphocytes using Tri reagent (Sigma, St. Louis, MO) and stored at -80^{o}C until further processing. RNA was further processed through the use of Purelink RNA minikit (Invitrogen, Carlsbad, CA). Additionally, 2X DNase was added to each sample to decrease genomic contamination. Total RNA was reverse transcribed to complementary DNA (cDNA) using high capacity reverse transcription (RT) kit (Applied Biosystems, Foster City, CA). Real-Time PCR was performed on the cDNA using primers designed for PRL-R using the Primer Express software and GAPDH RNA was amplified as the endogenous reference. Sequences of the PRL-R forward and reverse primer were 5'-GAACCTCAGGCCCATCCCT-3' and 3'-CTCTTCGACCTCTTAGGCCT-5', respectively. Sequences of the GAPDH primer forward and reverse primer were 5'-

ACCCAGAAGACTGTGGATGG-3' and 3'-GTGAGGGTTGCACAGACAAC-5', respectively. Detection was performed using an ABI 7300 Sequence Detector (Applied Biosystems). Amplification mixes (45 µl) contained 5 µl of cDNA, 40 µl (GAPDH or PRL-R) and 2X SyberGreen PCR master mix. Reactions were run in triplicate, with PRL-R and GAPDH run in separate wells on each plate. Eight dilutions of cDNA obtained from corpora luteal tissue was used to obtain the relative standard curve for calculation. The formula used to calculate the input amounts of both PRL-R and GAPDH was

$$(C_T\text{-}b)/m=\log \text{ input amount}$$

where C_T is the threshold cycle; b is the y-intercept of standard curve line; and m is the slope of the standard curve line. The log input amount was then converted to input amount by the formula 10^(log input amount) and the input amounts were normalized to the GAPDH (endogenous control) values. The final values are reported as expression values relative to a calibrator cDNA within animal and as change in cycle threshold.

Western Blotting

Western blotting of the PRL-R protein was accomplished using mAB U5 (Pierce, Rockford, IL) that binds to the different PRL-R forms due to the common extracellular epitope. Lymphocytes were re-suspended in RIPA buffer (Pierce) at the concentration of 10^6 cells. Samples were boiled for 5 minutes in denaturing loading buffer. Based on the estimated molecular weight of both short (34 kDA) and long (65 kDA) forms of PRL receptor the denatured proteins were separated in a 10% SDS/polyacrylamide gel together with a protein ladder (Benchmark™ Pre-stained Protein Ladder, Invitrogen). The proteins were blotted on 0.45-um-pore-size polyvinylidene fluoride membranes (Immobilon-P™ Transfer Membranes, Millipore, Billerica, MA). Protein transfer was determined by staining the membrane with Ponceaus red staining. Once the membranes were de-stained they were blocked in a 5% non-fat

dried milk powder at 21 ^{o}C for 1 hour. Following blocking, the immobilized antigens of interest were marked with 1ug/ml U5 antibody applied at 4 ^{o}C overnight and the related horseradish-peroxidase linked secondary antibody (0.2 ug/ml) incubated at 21 ^{o}C for 1 hour. Binding sites were detected by the enhanced chemiluminescent method (ECL Western blotting detection reagents and analysis system, Amersham Biosciences) and the signals were captured on KODAK film with exposure times of 1, 3, and 5 min. The membranes were re-probed for the detection of β-actin as a housekeeping gene control using the anti-actin rabbit monoclonal antibody (Cell Signaling Technology).

Statistical Analysis

Repeated measure data (DMI, water intake, surface temperatures, PRL, neutrophil chemotaxis, lymphocyte proliferation, neutrophil phagocytosis and oxidative burst, and gene expression) were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). For the gene expression, the samples taken during the acclimation periods were considered as the baseline and all data were expressed relative to the baseline value. The model included the fixed effects of treatment, time, genotype, and treatment × time interaction, and the random effect of cow. Data were tested to determine the structure of best fit, namely AR (1), ARH (1), CS, or CSH, as indicated by a lower Schwartz Bayesian information criterion value (Littell et al., 1996).

Experiment 1 Results

Surface Temperature, Chamber Temperature, and Relative Humidity

Chamber Temperature Humidity Index (THI) was kept between 61-64 during the acclimation and thermoneutral periods and between 79-82 THI during the heat stress period. Surface temperatures were taken daily morning (35.0 vs. 30.6 ^{o}C; $P = 0.001$) and afternoon (36.8 vs. 31.6 ^{o}C; $P < 0.001$). In addition, cows under heat stress conditions had decreased DMI (2.29

vs. 3.83%; $P < 0.001$) as percent of body weight (Figure 3-1a). Daily water consumption increased in cattle under heat stress (29.2 vs. 17.8L/d; $P < 0.04$; Figure 3-1b).

Prolactin Concentrations

Calves exposed to HT had increased PRL concentrations (15.76 vs. 7.38 ng/ml; SEM = 2.80 ng/ml d; $P = 0.03$) compared with a thermoneutral environment (Figure 3-2). There was no difference in the relative response of circulating PRL between slick-hair and wild-type calves under heat stress conditions. However, calves expressing the *slick hair* gene had lower overall PRL concentrations (6.01 vs. 17.13 ng/ml; SEM = 3.12 ng/ml; $P = 0.02$) when compared with wild-type calves (Figure 3-2).

PRL-R mRNA and Protein Abundance

Real time RTPCR was used to quantify PRL-R mRNA transcription. There were no significant differences in PRL-R mRNA between slick-hair and wild type calves (5.97 vs. 6.08 dCT; SEM = 1.41 dCT; $P = 0.78$) or between heat stress and thermoneutral conditions (6.07 vs. 5.98 dCT; SEM = 1.50 ng/ml; $P = 0.85$).

Both forms of the PRL-R protein are present in WBCs, however the relative abundance of the long form of the PRL-R is greater than that of the short form. Relative to thermoneutral conditions, exposure of calves to heat stress did not drastically influence the relative abundance of PRL-R protein. However, slick-hair calves had greater protein concentrations compared with that of wild-type (Figure 3-4).

Lymphocyte Proliferation

Lymphocytes isolated from slick hair and wild-type calves (164.67 vs. 223.21%; SEM = 34.54 %; $P = 0.25$) stimulated with ConA, did not differ regardless of thermal conditions as presented in percent of baseline (167.85 vs. 220.03%; SEM = 35.42 %; $P = 0.31$). There was a tendency to observe differences in responses in slick-hair calves (175.13 vs. 154.21%; SEM =

51.26 %) and within wild-type calves (160.58 vs. 285.84%; SEM = 50.25 %) under thermoneutral and heat stress conditions in the genotype by treatment interaction ($P = 0.15$).

Neutrophil Phagocytosis and Oxidative Burst

Neutrophil function, measured by phagocytosis (52.7 vs. 51.1%; SEM = 3.08; $P< 0.73$) and oxidative burst (59.2 vs. 61.4%; SEM = 3.41; $P< 0.65$), did not differ between thermal treatments. However, calves expressing the *slick hair* gene did have a suppression of the percent of neutrophils undergoing phagocytosis (39.9 vs. 64.0%; SEM = 3.56; $P< 0.001$; Figure 3-3a) and oxidative burst (50.93 vs. 69.6%; SEM = 3.56; $P< 0.01$) relative to wild type calves (Figure 3-3b).

Experiment 2 Results

Dry Matter and Water Intake

Calves under LDPP conditions had no change in DMI (3.85 vs. 3.65%; $P< 0.90$) as percent of body weight when compared with SDPP. Daily water consumption was not altered by photoperiodic changes (20.4 vs. 19.1L/d; $P< 0.51$).

Prolactin Concentrations

As expected, exposure of calves to LDPP increased PRL concentrations compared with SDPP conditions (35.8 vs. 22.3 ng/mL; SEM = 3.54 ng/mL d; $P = 0.03$;). Calves expressing the *slick-hair* gene had a tendency to have lower PRL concentrations when compared with wild-type calves (Figure 3-5; 23.5 vs. 34.7 ng/mL; SEM = 3.92 ng/mL; $P = 0.09$).

PRL-R mRNA and Protein Abundance

With regard to PRL-R mRNA expression, no differences were observed between slick-hair and wild type calves (6.19 vs. 5.97 dCT; SEM = 1.41 dCT; $P = 0.80$). However, lower expression of PRL-R was apparent in calves exposed to LDPP when compared to exposure to SDPP (5.60 vs. 6.49 dCT; SEM = 1.4 dCT; $P = 0.04$; Figure 3-6). Calves exposed to SDPP did

not differ in concentrations of PRL-R protein expressed in lymphocytes. However, slick hair calves had greater protein concentrations compared with wild-type calves. Both forms of the PRL-R are present in leukocytes however the long form of the PRL-R is more highly expressed in the lymphocyte (Figure 3-7).

Lymphocyte Proliferation

Stimulation with ConA was similar in lymphocytes isolated from slick hair and wild type calves regardless of LDPP (234 vs. 299.9 %; SEM = 88.54 %; $P = 0.60$) or SDPP (207.74 vs. 326.38%; SEM = 86.9 %; $P = 0.36$) treatment, respectively. When genotype was considered, no difference was observed within slick hair calves (265.28 vs. 203.11%; SEM = 125.32 %) and within wild-type calves (150.19 vs. 249.64%; SEM = 123.09 %) under LDPP and SDPP conditions in the genotype by treatment interaction ($P = 0.18$).

Neutrophil Function

Neutrophil function, measured by phagocytosis (88.76 vs. 93.41%; SEM = 2.52; $P< 0.21$), did not differ between LDPP and SDPP treatments, respectively. Calves expressing the *slick-hair* gene did not differ significantly in the percent of neutrophils undergoing phagocytosis (88.95 vs. 93.49%; SEM = 2.55%; $P< 0.25$) relative to wild type calves.

Discussion

Environmental factors such as high ambient temperatures exert significant effect on animal health and performance (Hahn, 1999). Calves under high ambient temperatures experienced a decrease in DMI consuming 3% of their body weight under thermoneutral conditions and dropping to 2% under heat stress, which is consistent with previous studies (Hahn, 1985). As expected and consistent with earlier work, an increase in water intake was observed in animals subjected to high ambient temperatures in the present study (Johnson, 1980). An increase of circulating PRL was observed under heat stress conditions and is consistent with previous findings (Collier et al., 1982; Amaral et al., 2009). Collectively this model of heat stress yields consistent effects on metabolic and endocrine measures and was thus appropriate to test the hypothesis regarding PRL and immune function of heat stressed calves.

Whereas an increase in circulating PRL was expected under heat stress conditions, PRL was also higher in wild-type when compared with slick animals. This supports the work by Hammond et al. (1996) wherein Romosinuano cattle, a heat-tolerant *Bos taurus* breed comparable to Senepol cattle, had lower PRL levels relative to Angus cattle. This is of interest in the current study as PRL is not only an acute stress hormone but can also act as an immune modulator (Yu-Lee, 2002; Reber, 1993). Previous studies established that circulating PRL and immune status are inversely related in mature and young dairy cattle (Auchtung et al., 2003; Auchtung and Dahl, 2004; Amaral et al., 2010). Thus, this inverted relationship of immune status and PRL may be extended to tropically adapted *Bos taurus* lines of cattle as well.

Unlike heat stress, the impact of photoperiod is well documented across species and is perhaps the most consistent response to an environmental variable (Dahl et al., 2000). Thus, the observed increase in circulating PRL was expected under LDPP conditions relative to SDPP exposure as demonstrated in Experiment 2. However, PRL was also higher in wild-type when

compared with *slick* animals. This supports the observations in Experiment 1 wherein slick calves had lower PRL relative to wild-type calves. Because the PRL genotype effect was independent of photoperiod and temperature, with wild-type calves having higher PRL relative to slick calves, it appears that the slick genotype confers a homeostatic decrease in PRL release compared with wild-type animals.

Neither heat stress nor genotype affected lymphocyte proliferation in Experiment 1. There is conflicting evidence with regard to lymphocyte function under heat stress in cattle. For example, Soper et al. (1978) observed an improvement in lymphocyte proliferation during periods of heat stress relative to cooler months of the year in mature cows. In contrast, other studies suggest that heat stress impairs lymphocyte proliferation (Elvinger et al., 1991; Kamawanja et al., 1994). Consistent with Experiment 1, slight to moderate heat stress did not affect lymphocyte proliferation in mature cows (Lacetera et al., 2002). However, unlike the adaptive immune response, the innate immune response is recognized as the first line of defense as it is usually the first to arrive at the site of affection (Parkins et al., 2001). These immune responses, especially under heat stress seem to differ among cattle breeds.

During times of immunosuppression, for example under heat stress, significant genetic variability occurs within the Holstein breed in regard to innate immune parameters including neutrophil chemotaxis and neutrophil function (Detilleux, 1994). Indeed, Brown Swiss cows are less sensitive to hyperthermic conditions relative to Holstein cows on the basis of rectal temperature in a hot environment (Johnson 1965; Correa-Calderon et al., 2004). However, when comparing the adaptive immune status of these two breeds the Brown Swiss cows are less tolerant of chronic heat stress relative to Holstein cows (Lacetera et al., 2006). Using the apoptotic response of lymphocytes as a measure of thermotolerance, differences are observed

between cattle of even greater genetic diversity, with Brahman and Senepol being more tolerant than Holstein and Angus (Paula-Lopes et al., 2003). These observations support the concept that genetic variation exists in stimulated immune responses among cattle breeds and lines.

Mechanistically it was hypothesized that PRL signaling as a mediator of heat stress responses to immune status in cattle. Indeed, circulating PRL concentrations differed between wild-type and slick Holstein heifer calves, in that wild-type calves had greater PRL concentrations relative to slick calves under thermoneutral and heat stress conditions. Similar increases in PRL have been observed during heat stress in bull calves and mature dairy cows (Tucker et al., 1990; Amaral et al., 2009).

Of interest, the *slick hair* gene has been identified as being mapped to chromosome 20 near the PRL-R complex. Further, previous work demonstrates an inverse relationship between PRL and PRL-R mRNA under temperature and light treatments that affected circulating PRL. Long day photoperiod and heat stress both increased PRL and that was followed by a decrease in expression of PRL-R in various tissues (Auchtung et al., 2003; Amaral et al. 2009). However, no difference was observed in PRL-R mRNA analyzed in lymphocytes isolated from slick and wild-type calves in Experiment 1, regardless of the temperature treatment. One possibility is that young calves are less responsive than mature cows, yet LDPP induced PRL increments suppress PRL-R in calves (Auchtung et al., 2003). Because both a long- and short-form of PRL-R is expressed, another explanation could be the PRL is influencing the ratio of long to short PRL-R mRNA expression. Because total PRL-R mRNA was measured rather than separating the forms in Experiment 1, testing of that hypothesis requires further study.

Consistent with Experiment 1, Experiment 2 observations confirm the work of Schuler et al., (1993) that lymphocytes express PRL-R protein and extends that study by showing a

substantial effect of genotype on PRL-R abundance in slick relative to wild-type calves. Yet there was no effect of photoperiod on PRL-R protein abundance in Experiment 2. The differential impact of genotype versus environment on the response at the level of mRNA and protein expression may reflect differences in mRNA processing between slick and wild-type calves. Indeed, given the location of the slick mutation relative to PRL-R, and the involvement of PRL-R in hair cycling (Ouhit et al. 2003), slick animals may compensate for lower sensitivity to PRL feedback at the level of mRNA processing with greater sensitivity at the protein expression stage to maintain similar homeostatic regulation of signal transduction.

Despite a lack of effect on PRL-R mRNA, a genotype effect was observed on PRL-R protein expression in both experiments. Specifically, slick haired calves expressed more PRL-R protein than wild-type. Because slick calves had lower circulating PRL concentrations in both experiments, the difference observed in PRL-R mRNA expression in lymphocytes from slick and wild-type calves extend previous findings that circulating PRL is inversely related to PRL-R mRNA expression in lymphocytes and other tissues (Amaral et al., 2009, Auchtung et al., 2003). The *slick hair* gene has been identified as being mapped to chromosome 20 near the PRL-R complex. Considering the aforementioned inverse relationship between PRL and PRL-R mRNA, it was expected that the mRNA expression would be greater in slick animals due to lower circulating PRL, yet there was no difference observed due to genotype. It is of interest to consider how genotype might affect protein expression independent of an influence on PRL-R mRNA expression. A possible explanation for the lack of response in slick animals could be related to a reduced sensitivity to PRL as a negative feedback regulator, although that possibility requires further study.

With regard to immune status in Experiment 1, the change in PRL concentrations and PRL-R abundance did not elicit a lymphocyte proliferation response, but genotype did influence the percentage of neutrophils undergoing oxidative burst and phagocytosis. Relative to the wild-type calves, slick calves had lesser immune responses in vitro, which suggests a less sensitive immune surveillance system or potentially a less robust immune system overall. The lower immune response despite greater PRL-R protein abundance does not support the hypothesis of a direct effect of PRL signaling on immune function, but must be considered in the context genotype. That is, there was no effect of heat stress on immune measures in either line of calves, thus, PRL signaling mechanisms may not contribute to the differences observed in slick calves relative to wild-type animals.

In Experiment 2, lack of any effect of photoperiod on lymphocyte proliferation or neutrophil action is puzzling given previous reports in cattle and other species. For example, there are reports of an enhancement of lymphocyte proliferation under SDPP relative to LDPP in deer mice (Demas and Nelson, 1998), Siberian hamsters (Prendergast et al., 2002) and dairy cattle (Auchtung et al., 2003). Moreover, Zhou et al. (2002) found only increased lymphocyte proliferation in hamsters under SDPP vs. LDPP, but recorded no differences in innate immune responses. However, Yellon et al. (1999b) observed that relative to SDPP, neutrophil phagocytosis and oxidative burst activity were increased by LDPP. Previous studies indicate an effect of elevated PRL concentrations on neutrophil phagocytosis and oxidative burst in mature dairy cows (Amaral et al., 2010). Thus, a possible explanation for the lack of immune responses in the present studies are an age related effect with lower responsiveness in younger versus older cows, but this hypothesis would require more rigorous testing to confirm.

Conclusion

In conclusion, slick calves had lower circulating PRL concentrations; the response of slick calves with elevated PRL-R protein abundance supports the concept of a negative relationship between PRL and PRL-R. Relative to the wild-type calves, in Experiment 1, slick calves had lesser immune responses in vitro, which suggests a less sensitive immune surveillance system or potentially a less robust immune system overall. However, the lower immune response despite greater PRL-R protein abundance does not support the hypothesis of a direct effect of PRL signaling on immune function. Further research is recommended to identify possible mechanisms affecting PRLR expression between the two genotypes.

The results of Experiment 2 confirm the inverse relationship between PRL and daylength. In addition, the results provide evidence of a substantial difference between wild-type and slick genotype calves with regard to circulating PRL and PRL-R protein abundance in lymphocytes. The lack of effect of photoperiod on immune measures is in contrast to earlier work and requires additional study to evaluate differences in responsiveness due to age or physiological state.

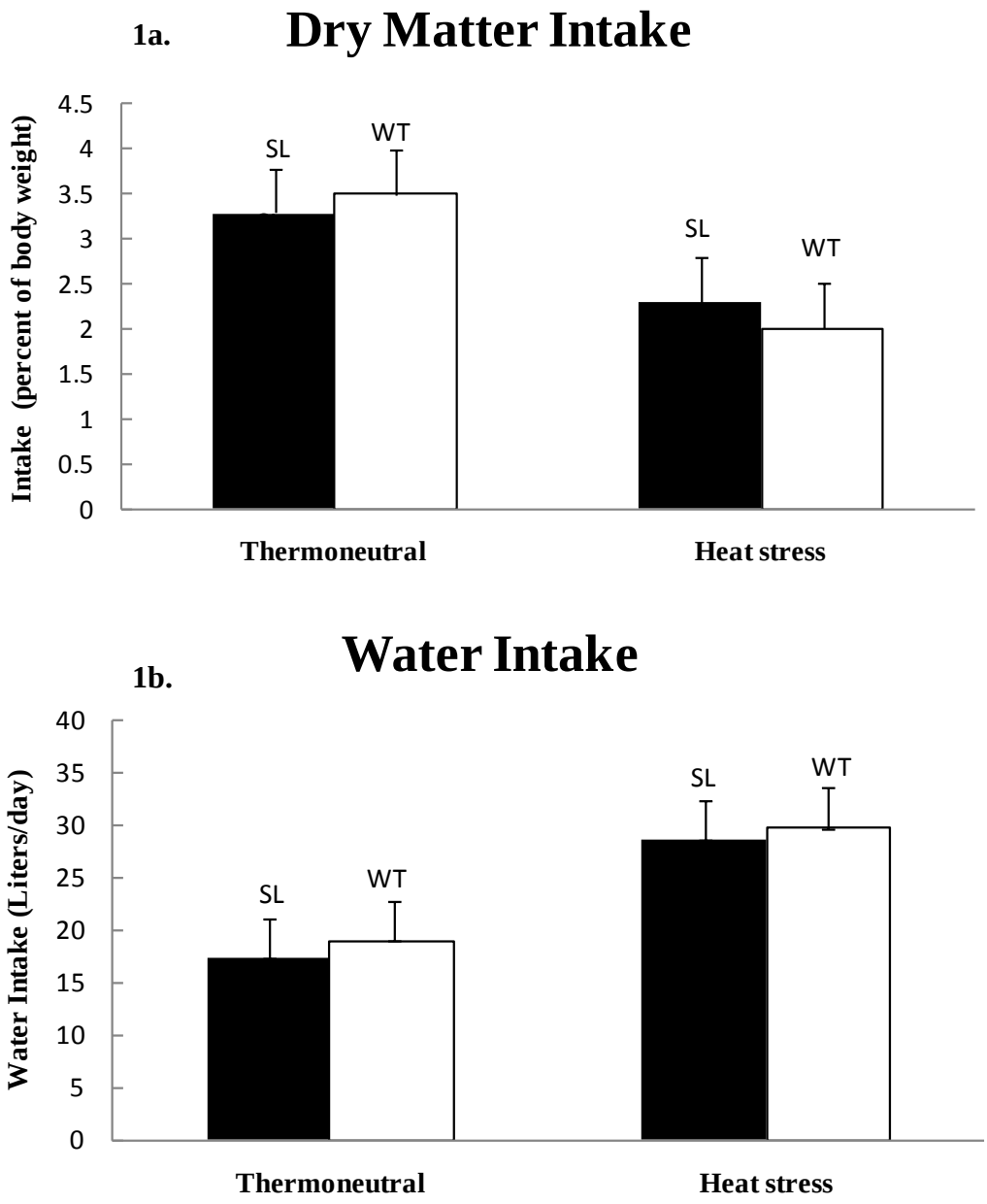


Figure 3-1. Calves expressing the *slick-hair* gene or wild-type under heat stress conditions had decreased DMI (2.29 vs. 3.83%; $P < 0.001$) as percent of body weight (Figure 3-1a). Daily water consumption increased in cattle under heat stress (29.2 vs. 17.8L; $P < 0.04$)(Figure 3-1b).

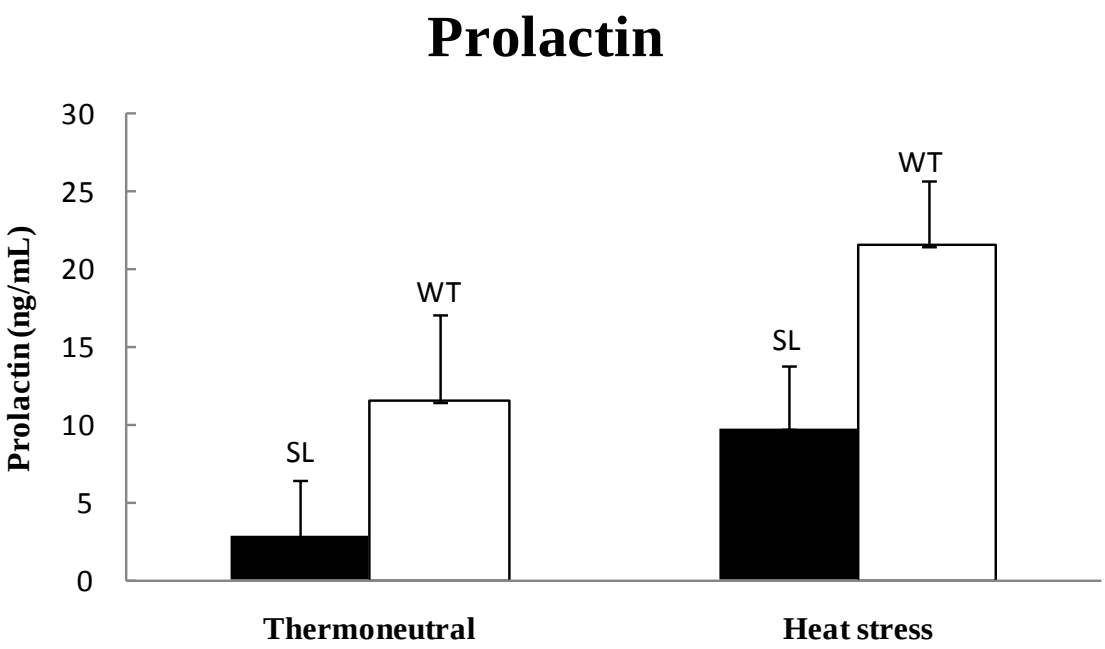
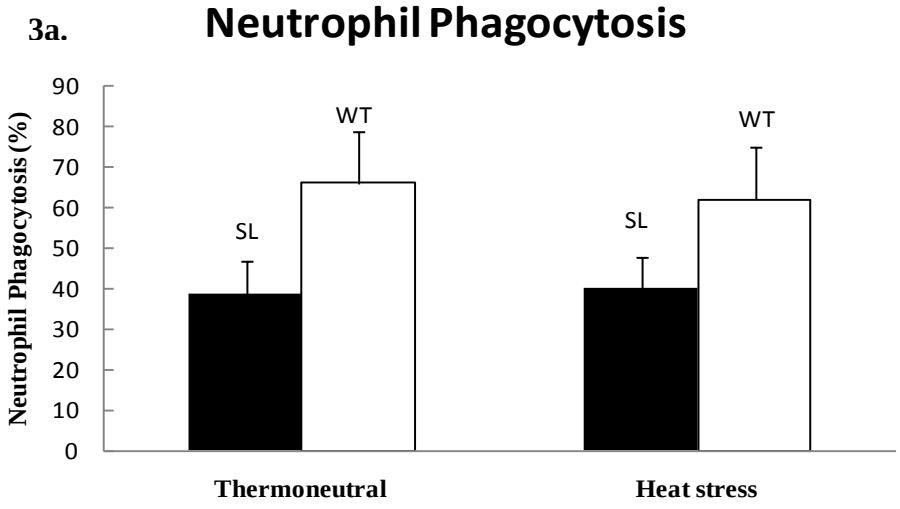


Figure 3-2. Calves exposed to heat stress, comparison of slick to wild-type calves. Heat stress conditions increased prolactin concentrations (15.76 vs. 7.38 ng/ml; SEM = 2.80 ng/ml d; $P = 0.03$) compared to a thermoneutral climate. Calves expressing the *slick-hair* gene had lower prolactin concentrations (6.01 vs. 17.13 ng/ml; SEM = 3.12 ng/ml; $P = 0.02$) when compared with wild-type calves.



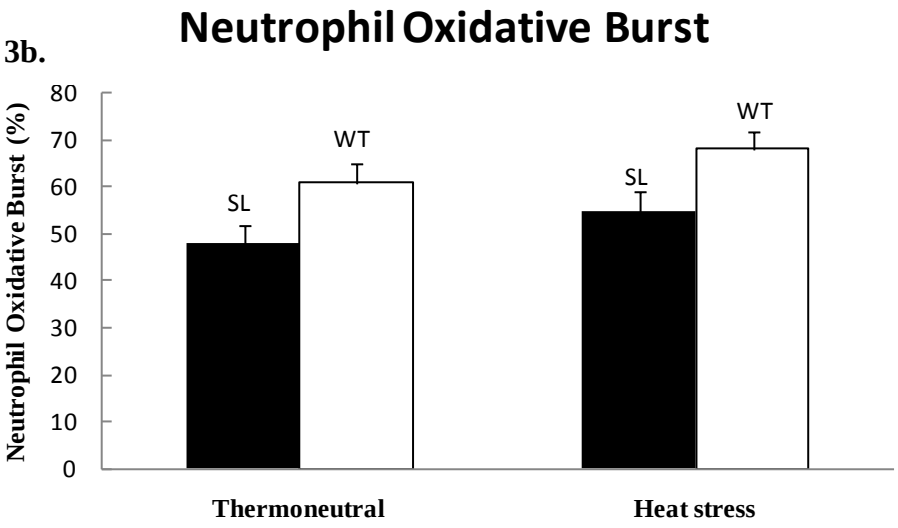


Figure 3-3. Neutrophil function, measured by phagocytosis (52.7 vs. 51.1%; SEM = 3.08; *P*< 0.73) and oxidative burst (59.2 vs. 61.4%; SEM = 3.41; *P*< 0.65), did not differ between thermal treatments. Calves expressing the *slick-hair* gene did have a suppression of the percent of neutrophils undergoing phagocytosis (39.9 vs. 64.0%; SEM = 3.56; *P*< 0.001, Figure 3a) and oxidative burst (50.93 vs. 69.6%; SEM = 3.56; *P*< 0.01) relative to wild type calves (Figure 3b).

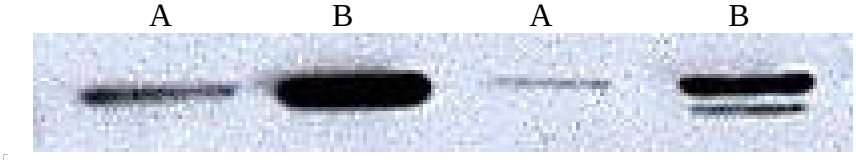


Figure 3-4. Calves exposed to heat stress did not drastically differ in concentrations of PRL-R protein. However, slick hair calves (B) had greater protein concentrations compared with that of wild-type (A) as represented in the top panel. The bottom panel represents the β-actin control.

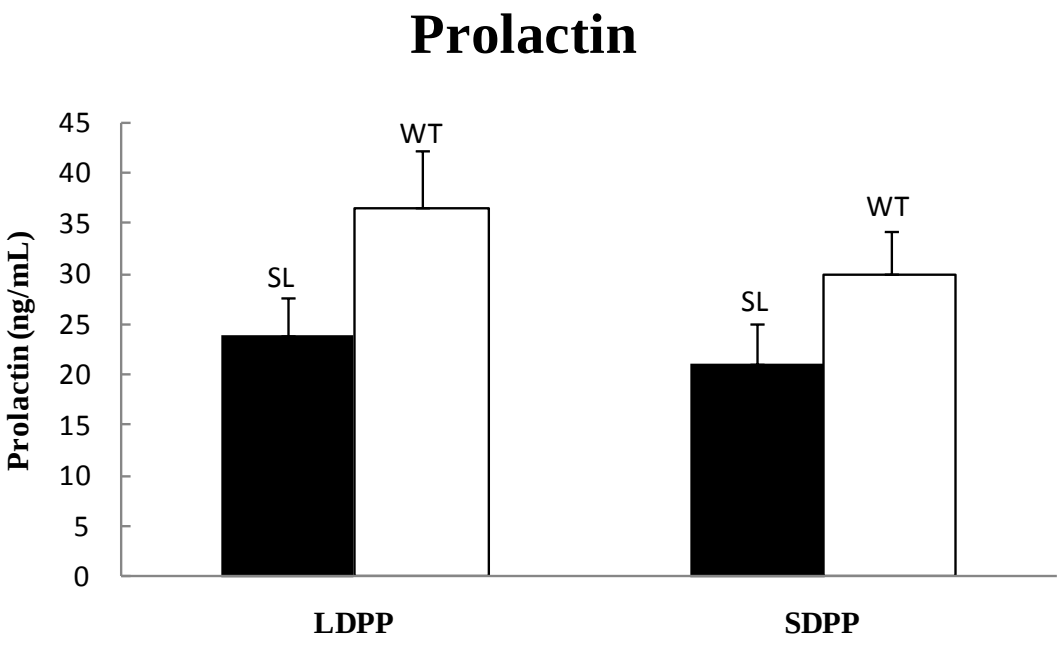


Figure 3-5. LDPP increased prolactin concentrations (35.8 vs. 22.3 ng/ml; SEM = 3.54 ng/ml ; *P* = 0.03) compared to SDPP conditions. Calves expressing the *slick-hair* gene had lower prolactin concentrations (23.5 vs. 34.7 ng/ml; SEM = 3.92 ng/ml; *P* = 0.09) when compared with wild-type calves (Figure 4-1b).

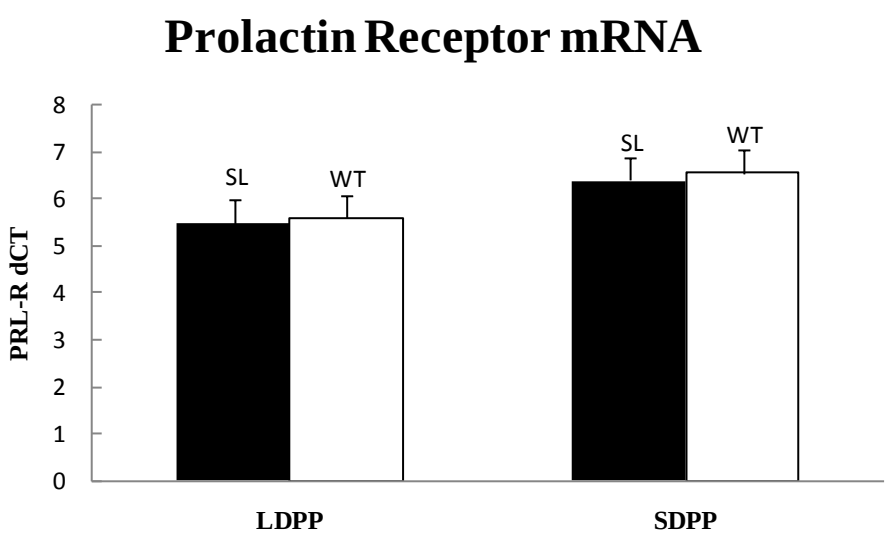


Figure 3-6. Real time reverse transcriptase polymerase chain reaction was used to quantify PRL-R mRNA transcription. There were no significant differences between slick-hair and wild type calves (6.19 vs. 5.97 dCT; SEM = 1.41 dCT; *P* = 0.80). However, lower expression of PRL-R was observed in calves exposed to LDPP when compared with SDPP (5.60 vs. 6.49 dCT; SEM = 1.4 dCT; *P* = 0.04).

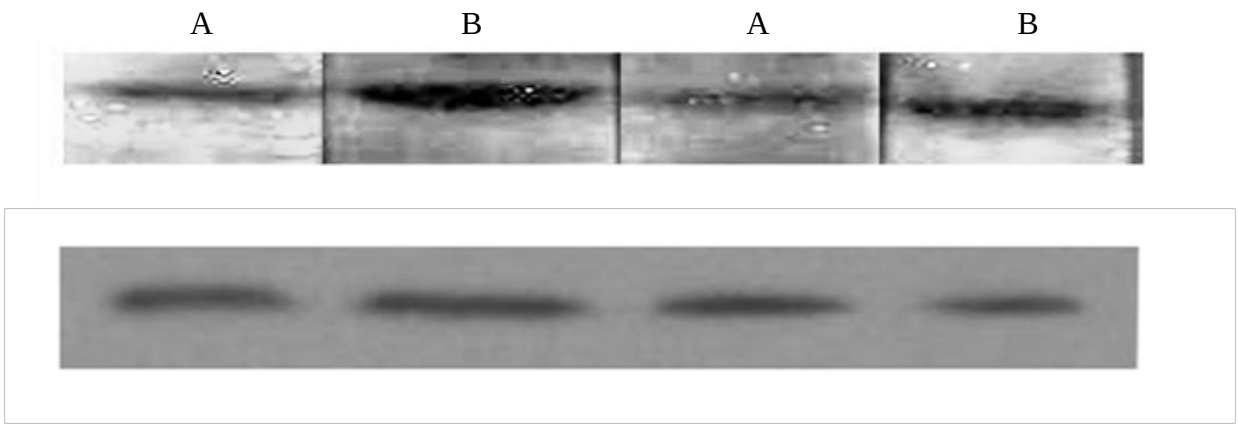


Figure 3-7. Relative protein abundance of PRL-R in wild-type and slick calves exposed to long and short days (LDPP vs. SDPP). The top panel represents PRL-R protein whereas the bottom panel represents the β-actin control. Calves exposed to LDPP did not differ in expression of PRL-R protein relative to abundance under SDPP. However, slick hair calves had greater protein concentrations (B) compared to that of wild-type (A).

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

Mechanistically it was hypothesized that PRL signaling was a mediator of immune status responses to heat stress in cattle. Indeed, circulating PRL concentrations differed between wild-type and slick Holstein heifer calves, in that wild-type calves had greater PRL concentrations relative to slick calves under thermoneutral and under heat stress conditions. Similar increases in PRL have been observed during heat stress in bull calves and mature dairy cows (Tucker et al., 1990; Amaral et al. 2009), but this is the first observation of the effect of ambient temperature on PRL in slick cattle.

Of interest, the *slick hair* gene has been identified as being mapped to chromosome 20 near the PRL-R complex. Further, a previously identified inverse relationship between PRL and PRL-R mRNA exists under temperature and light treatments that affected circulating PRL. Indeed, LDPP and heat stress increase PRL which drives a decrease in expression of PRL-R in various tissues (Auchtung et al., 2003; Amaral et al. 2009). In the present study, no difference was observed in PRL-R mRNA analyzed in lymphocytes isolated from slick and wild-type calves, regardless of the temperature treatment. One possibility is that young calves are less responsive than mature cows, yet long day induced PRL increments suppress PRL-R in calves (Auchtung et al., 2003). Because both a long- and short-form of PRL-R is expressed, another explanation could be the PRL is influencing the ratio of long to short PRL-R mRNA expression. Because total PRL-R mRNA was measured rather than separating the forms in the present experiments, testing of that hypothesis requires further study.

Despite a lack of effect on PRL-R mRNA, a genotype effect was observed on PRL-R protein expression. Specifically, slick haired calves expressed more PRL-R protein than wild-type. This is a novel observation and suggests that the slick genotype confers a different level of control on PRL signaling. Because slick calves had lower circulating PRL concentrations, the

response of PRL-R protein abundance supports the concept of a negative relationship between ligand and signal transduction via the PRL-R. It is of interest to consider how genotype might affect protein expression independent of an influence on PRL-R mRNA expression.

With regard to immune status, the change in PRL concentrations and PRL-R abundance did not elicit a lymphocyte proliferation response, but genotype did influence the percentage of neutrophils undergoing oxidative burst and phagocytosis. Relative to the wild-type calves, slick calves had lesser immune responses in vitro, which suggests a less sensitive immune surveillance system or potentially a less robust immune system overall. The lower immune response despite greater PRL-R protein abundance does not support the hypothesis of a direct effect of PRL signaling on immune function, but must be considered in the context of genotype. That is, there was no effect of heat stress on immune measures in either line of calves, thus, PRL signaling mechanisms may not contribute to the differences observed in slick calves relative to wild-type animals.

The impact of photoperiod is well documented across species and is perhaps the most consistent response to an environmental variable (Dahl et al., 2000). Thus, the observed increase in circulating PRL was expected under LDPP conditions relative to SDPP exposure. However, PRL was also higher in wild-type when compared with *slick* animals. This supports the observation of Hammond et al. (1996), wherein Romosinuano cattle, a heat-tolerant *Bos taurus* breed comparable to Senepol cattle, had lower PRL concentrations relative to Angus cattle. Because the PRL genotype effect was independent of photoperiod and as described earlier, temperature, with wild-type calves having higher PRL relative to slick calves, it appears that the slick genotype confers a homeostatic decrease in PRL release compared with wild-type animals.

In the present studies, the difference observed in PRL-R mRNA expression in lymphocytes from slick and wild-type calves confirm previous findings that circulating PRL is inversely related to PRL-R expression in lymphocytes and other tissues (Amaral et al., 2009, Auchtung et al., 2003). The *slick hair* gene has been identified as being mapped to chromosome 20 near the PRL-R complex (Mariasegaram et al., 2007). In contrast to the genotype effect on circulating PRL, however, no effect of genotype was observed in PRL-R mRNA expression. Considering the aforementioned inverse relationship between PRL and PRL-R mRNA, it was expected that the mRNA expression would be greater in slick animals due to lower circulating PRL, yet there was no difference observed due to genotype. A possible explanation for the lack of response in slick animals could be related to a reduced sensitivity to PRL as a negative feedback regulator, although that possibility requires further study.

In Experiment 1, slick calves had lower circulating PRL concentrations, the response of slick calves with elevated PRL-R protein abundance supports the concept of a negative relationship between PRL and PRL-R. Relative to the wild-type calves, slick calves had lesser immune responses in vitro, which suggests a less sensitive immune surveillance system or potentially a less robust immune system overall. However, the lower immune response despite greater PRL-R protein abundance does not support the hypothesis of a direct effect of PRL signaling on immune function. Further research is recommended to identify possible mechanisms affecting PRLR expression between the two genotypes.

In Experiment 2, the results of the study confirm the inverse relationship between PRL and daylength, and extend the previous findings of inverted relationship of PRL to PRL-R mRNA to actual protein abundance. In addition, evidence is provided of a substantial difference between wild-type and slick genotype calves with regard to circulating PRL and PRL-R protein

abundance in lymphocytes. The lack of effect of photoperiod on immune measures is in contrast to earlier work and requires additional study to evaluate differences in responsiveness due to age or physiological state.

LIST OF REFERENCES

Ahern, B., S. Mansson, R.L. Gingerich, and P.J. Havel. 1997. Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting. Am. J. Phys. 273:R113-R120.

Amaral do B.C., E.E. Connor, S. Tao, J. Hayen, J. Bubolz, and G.E. Dahl. 2009. Heat stress abatement during the dry period: does cooling improve transition into lactation? J. Dairy Sci. 92:5988-5999.

Amaral do B.C., E.E. Connor, S. Tao, J. Hayen, J. Bubolz, and G.E. Dahl. 2010. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes.Domest. Anim. Endo. 38:38-45.

Akers, R.M. 1985. Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. J. Dairy Sci. 68:501-519.

Auchtung, T.L., P.E. Kendall, J. Salak-Johnson, T.B. McFadden, and G.E. Dahl. 2003. Photoperiod and bromocriptine treatment effects on expression of prolactin receptor mRNA in bovine liver, mammary gland and peripheral blood lymphocytes. J. Endocrinol. 179:347-356.

Auchtung, T.L., J.L. Salak-Johnson, D.E. Morin, C.C. Mallard, and G.E. Dahl. 2004. Effects of photoperiod during the dry period on cellular immune function of dairy cows. J. Dairy Sci. 87:3683-3689.

Auchtung, T.L. and G.E. Dahl. 2004. Prolactin mediates photoperiodic immune enhancement: Effects of administration of exogenous prolactin on circulating concentrations, receptor expression, and immune function in steers. Biol. Reprod. 71:1913-1918.

Auchtung, T.L., A.G. Rius, P.E. Kendall, T.B. McFadden, and G.E. Dahl. 2005. Effects of photoperiod during the dry period on prolactin, prolactin receptor, and milk production of dairy cows. J. Dairy Sci. 88:121-127.

Avendaño-Reyes, L., F.D. Alvarez-Valenzuela, A. Correa-Calderón, J.S. Saucedo-Quintero, P.H. Robinson, and J.G. Fadel. 2006. Effect of cooling Holstein cows during the dry period on postpartum performance under heat stress conditions. Livestock Science. 281:2535-2547.

Ben-Jonathan, N. and R. Hnasko, 2001. Dopamine as a prolactin inhibitor. Endocr. Rev. 22:724-763.

Bhat, G.K., M.L.Hamm, J.U. Igietseme and D.R. Mann. 2003. Does leptin mediate the effect of photoperiod on immune function in mice? Biol. Reprod. 69: 30-36.

Bilbo, S.D., F.S. Dhabhar, K. Viswanathan, A. Saul, S.M. Yellon, and R.J. Nelson 2002a. Short day lengths augment stress-induced leukocyte trafficking and stress-induced enhancement of skin immune function. Proc. Nat. Acad. Sci. 99: 4067-4072.

Bilbo, S.D., D.L. Drazen, N. Quan, L. He, and R.J. Nelson. 2002b. Short day lengths attenuate the symptomsof infection in Siberian hamsters. Proc. R. Soc. Lond. B 269: 447-454.

Bouchard. B., C.J. Ormandy, J.P. Di Santo, and P.A.Kelly. 1999. Immune system development and function in prolactin receptor-deficient mice. J. Immunol. 163:576-582.

Bauman, D.E., and R.G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. Ann. Rev. Nutr. 13:437-461.

Buchanan, B.A.,L.T. Chapin, and H.A. Tucker. 1992. Prolonged suppression of serum concentration of melatonin in heifers. J. Pineal Res. 12: 181-189.

Chen, W.Y., P. Ramamoorthy, N. Chen, R. Sticca, and T.E. Wagner. 1999. A human prolactin antagonist, hPRL-G129R, inhibits breast cancer cell proliferation through induction of apoptosis. Clin. Cancer Res. 5:3583-3593.

Cho, S.J., D.L. Hard, L.S. Carpenter, M.A. Diekman,and L.L. Anderson. 1998. Seasonal regulation of prolactin secretion in hypophyseal stalk transected beef calves. Anim. Repro. Sci.52:253-265.

Clevenger, C.V., D.O. Freier, and J.B. Kline. 1998. Prolactin receptor signal transduction in cells of the immune system. J. Endocrinol. 157:187-197.

Collier, R.J., S.G. Doelger, H.H. Head, W.W. Thatcher, and C.J. Wilcox. 1982. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. J. Anim. Sci. 54:309-319.

Cook, N.B., T.B. Bennett, K.M. Emery, and K.V. Nordlund. 2002. Monitoring nonlactating cow intramammary infection dynamics using DHI somatic cell count data. J. Dairy Sci. 85:1119-1126.

Correa-calderon, A., D. Armstrong, D. Ray, S. DeNise, M. Enns, and C. Howison. 2004. Thermoregulatory responses of Holstein and Brown Swiss heat-stressed dairy cows to two different cooling systems. Int. J. Biometeorology 48:142-148.

Dahl, G.E., T.H. Elsasser, A.V. Capuco, R.A. Erdman, and R.R. Peters. 1997. Effects of a long daily photoperiod on milk yield and circulating concentrations of Insulin-like growth factor-I. J. Dairy Sci. 80:2784-2789.

Dahl, G.E., B.A. Buchanan, and H.A. Tucker. 2000. Photoperiodic effects on dairy cattle: A review. J. Dairy Sci. 83: 885-893.

Davis, S.L. 1998. Environmental modulation of the immune system via the endocrine system. Domest. Anim. Endocrinol. 15:283-289.

Demas, G.E., and R.J. Nelson. 1998. Photoperiod, ambient temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). J.Biol. Rhythms 13:253-262.

Demas, G.E., D.L. Drazan, A.M. Jasnow, T.J. Bartness, and R.J. Nelson. 2002. Sympathoadrenal system differentially affects photoperiodic changes in humoral immunity of Siberian hamsters. J.Neuroendocrinol. 14:29-35.

Detilleux J.C., K.J. Koehler, A.E. Freeman, M.E. Kehrli, and D.H. Kelley. 1994. Immunological parameters of periparturient Holstein cattle: genetic variation. J. Dairy Sci. 77:2640-2650.

Di Carlo, R., C. Bole-Feysot, O. Gualillo, R. Meli, M. Nagano, and P.A. Kelly. 1995. Regulation of prolactin receptor mRNA expression in peripheral lymphocytes in rats in response to changes in serum concentrations of prolactin. Endocrinology 136: 4713-4716.

Dikemen, S., E. Alava, E. Pontes, J.M. Fear, B.Y. Dikmen, T.A. Olson, and P.J. Hansen. 2008. Differences in thermoregulatory ability between slick haired and wild type lactating Holstein cows in response to acute heat stress. J. Dairy Sci. 91: 3395-3402.

Djiane, J., L.M. Houdebine, P.A. Kelly, B, Teyssot, and I. Dusanter, 1983. Down-regulation of prolactin receptors and biological effects of the prolactin in the mammary gland. Evidencefor the existence of a prolactin second messenger. Prolactin and Prolactinomas;ed.G.Tolis et al.,Raven Press NY.

Dorshkind, K., and N.D. Horseman. 2001. Anterior pituitary hormones, stress, and immune system homeostasis. Bioessays 3:288-294.

Dowell, S.F. 2001. Seasonal variation in host susceptibility and cycles of certain infectious diseases. Emer. Infect. Dis. 7: 369-374.

Drazen, D.L., G.E. Demas, and R.J. Nelson. 2001. Leptin effects on immune function and energy balance are photoperiod dependent in Siberian hamsters. Endocrinology 142: 2768-2775.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Edmonson, A. J., I.J. Lean, L.D. Weaver, T. Farver, and G. Webster. 1989. A body condition score chart for Holstein dairy cows. J. Dairy Sci 72:68-78.

Elenkov, I. J., E. L. Webster, D. J. Torpy, and G. P. Chrousos. 1999. Stress, corticotrophinreleasing hormone, glucocorticoids, and the immune/inflammatory response: acute and chronic effects. Ann. NY. Acad. Sci. 876:1-11.

Elvinger, F., P.J. Hansen, and R.P. Natzke. 1991. Modulation of function of bovine polymorphonuclear leukocytes and lymphocytes by high temperatures in vitro and in vivo. Am. J. Vet. Res. 52:1692-1698.

Feng, J.C., T.T. Loh, and H.P. Sheng. 1998. Lactation increases prolactin receptor expression in spleen and thymus of rats. Life Sci. 63:111-119.

Fitzgerald, B.P., L.A. Davison, and C.J. Mcmanus. 2000. Evidence for a seasonal variation in the ability of exogenous melatonin to suppress prolactin secretion in the mare. Dom. Anim. Endocrinol. 18:395-408.

Forman, B. M., J. Chen, and R.M. Evans. 1996. The peroxisome proliferator-activated receptor: ligands and activators. Ann. NY Acad. Sci. 804: 266-275.

Gouilleux, F.,D. Moritz, M. Humar, R. Moriggl,S. Berchtold, and B. Groner. 1995. Prolactin and interleukin-2 receptors in T-lymphocytessignal througha MGF-STAT5-like transcription factor. Endocrinology 136: 5700-5708.

Goupille, O., J.V. Barnier, B. Guibert, J. Paly, and J. Djiane. 2000. Effect of PRL on MAPK activation: negative regulatory role of the C-terminal part of the PRL receptor. Mol. Cell. Endocrinol. 159:133-146.

Hahn, G.L. 1995. Environmental management for improved livestock performance, health and well-being. Jpn. J. Livest.Manage. 30: 113-127.

Hahn, G.L. 1999. Dynamic responses of cattle to thermal heat loads. J. Anim. Sci. 77:10-12.

Hammond, A.C., T.A. Olson, C.C. Chase, Jr. E.J. Bowers, R.D. Randel, C.N. Murphy, D.W. Vogt, and A. Tewolde. 1996. Heat tolerance in two tropically adapted Bos taurus breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J. Anim. Sci. 74:295-303.

Hanew, K.,M. Shiino, and E.G. Rennels. 1980. Effect of indoles, AVT, oxytocin, and AVP on prolactin secretion in rat pituitary clonal (2B8) cells. Proc. Soc. Exp. Biol. Med. 164:257-261.

Hazelrigg, D.G., M.H. Hastings, and P.J. Morgan. 1996. Production of a prolactin releasing factor by the ovine pars tuberalis. J. Neuroendocrinol. 8:489-492.

Jahn, G.A., M. Edery, L. Belair, P.A. Kelly, and J. Djiane. 1991. Prolactin receptor gene expression in rat mammary gland and liver during pregnancy and lactation. Endocrinology 128:2976-2984.

Johnson, H.D. 1967. Climate effects on physiology and productivity of cattle. Ground Level Climatology. R.H. Shaw (ed). Amer. Assoc. Adv. Sci. Pub. 86. Washington, DC.

Johnson, H.D. 1980. Environmental management of cattle to minimize the stress of climatic change. Int. J. Biometeorol. 24:65-78.

Kampen, A. H., T. Tollersrud, S. Larsen, J.A. Roth, D.E. Frank, and A. Lund. 2004. Repeatability of flow cytometric and classical measurement of phagocytosis and respiratory burst in bovine polymorphonuclear leukocytes. Vet. Immunol. Immunopathol. 97:105–114.

Kamwanja, L. A., C. C. Chase Jr., J. A. Gutierrez, V. Guerriero Jr., T. A. Olson, A. C. Hammond, and P. J. Hansen. 1994. Response of bovine lymphocytes to heat shock as modified by breed and antioxidant status. J. Anim. Sci. 72:438-444.

Kelley, K.W., R.E. Greenfield, J.F. Everman, and S.M. Parish. 1980. Immunosuppression in calves caused by heat and cold stress. 1980. American Society Animal Science, Meeting Abstracts vol. 102, p. 147.

Kelley, P.A., J. Dijiane, M.C. Postel-Vinay, and M. Edery. 1991. The prolactin/growth hormone receptor family. Endocr. Rev. 12:235-251.

Kume, K., M.J. Zylka, S. Sriram, L.P. Shearman, D.R. Weaver, X. Jin, E.S. Maywood, M.H. Hastings, and S.M. Reppert. 1999. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98:193-205.

Lacetera, N., U. Bernabucci, B. Ronchi, D. Scalia, and A. Nardone. 2002. Moderate summer heat stress does not modify immunological parameters of Holstein dairy cows. J. Biometeorology 46:33-37.

Lacetera, N., U. Bernabucci, D. Scalia, B. Ronchi, G. Kuzminsky, and A. Nardone. 2005. Lymphocyte function in dairy cows in hot environment. Int. J. Biometeorol. 50:105-110.

Lacetera, N., U. Bernabucci, D. Scalia, L. Barisico, P. Morera, and A. Nardone. 2006. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. J. Dairy Sci. 89:4606-4612.

Lebrun, J.J., S. Ali, L. Sofer, A. Ullrich, and P.A. Kelly. 1994. Prolactin-induced proliferation involves tyrosine phosphorylation of the prolactin receptor and its associated tyrosine kinase Jak-2.J. Biol. Chem. 269:14021-14024.

Lee, W.L., Harrison, R.E., and S. Grinstein. 2003. Phagocytosis by neutrophils. Microbes and Infection. 5:1299-1306.

Leining, K. B., R.A. Bourne, and H.A. Tucker. 1979. Prolactin response to duration and wavelength of light in prebubertal bulls. Endocrinology 104:289-294.

Lichtman, A.H., Segel,G. B. and Lichtman, M.A. 1983. The role of calcium in lymphocyte proliferation. J.Amer. Soc.Hematology. 61: 413-416

Lincoln, G.A., and M. Richardson. 1994. Photoperiodically-induced cycles in the secretion of prolactin in hypothalamopituitary disconnected rams-evidence for translation of themelatonin signal in the pituitary gland. J. Neuroendocrinol. 6:251-261.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS for mixed models. SAS Institute Inc., Cary, NC.

Loor, J.J., H.M. Dann, N.A. Guretzky, R.E. Everts, R. Oliveira, C.A. Green, N.B. Litherland, S.L. Rodriguez-Zas, H.A. Lewin, and J.K. Drackley. 2006. Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. Physiol. Genomics. 27:29-41.

Mallard, B.A., L.C. Wagter, M.J. Ireland, and J.C. Dekkers. 1997. Effects of growth hormone, insulin-like growth factor-I, and cortisol on periparturient antibody response profiles of dairy cows. Vet. Immunol. Immunopathol. 60:61-76.

Mariasegaram, M., C.C. Chase Jr., J.X. Chaparro, T.A.Olson, R.A. Brenneman and R.P.Niedz. 2007. The slick hair coat locus maps to chromosome 20 in Senepol-derived cattle. Animal Genetics. 38: 54-59.

Marsh, W. H, B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. J. Clin. Chem. 6:624-627.

Mizoguchi, Y., J.Y. Kim, J.Enami, and S.Sakai.1997. The regulation of the prolactin receptor gene expression in the mammary gland of early pregnant mouse. Endocr. J. 44:53-58.

Morgan, P.J. 2000. The pars tuberalis: The missing link in the photoperiodic regulation of prolactin secretion? J. Neuroendorcrinol. 12:287-295.

Munck, A., P.M. Guyre, and N.J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev. 5:25-44.

Nelson, R.J., and G.E. Demas. 1996 Seasonal changes in immune function. Quart.Rev.Biol. 71:511-548.

Nixon, A.J., C.A. Ford, J.E. Wildermoth, A.J. Craven, M.G. Ashby, and A.J. Pearson. 2002. Regulation of prolactin receptor expression in ovine skin in relation to circulating prolactin and wool follicle growth status. J. Endocrinol. 172:605-614.

Olson, T.A., C. Lucena, C.C. Chase, and A.C. Hammond. 2003. Evidence of a major gene influencing hair length and heat tolerance in *Bos taurus* cattle. J. Anim. Sci. 81:80-90.

Ostrom, K.M. 1990. A review of the hormone prolactin during lactation. Prog. Food Nutr. 12:1-17.

Ouhtit, A., G. Morel, and P.A. Kelly. 1993. Visualization of gene expression of short and long forms of prolactin receptor in the rat. Endocrinology. 133:135-144.

Padmanabhan, V., E.M. Convey, and H.A. Tucker. 1979. Pineal compounds alter prolactin release from bovine pituitary cells. Proc. Soc. Exp. Biol. Med. 160:340-343.

Parkins, J., and B. Cohen. 2001. An overview of the immune system. The Lancet. 357:1777-1789.

Paula-Lopes, F.F., Y.M. Al-Katanani, A.C. Majewski, L.R. McDowell and P.J. Hansen. 2002. Manipulation of antioxidant status fails to improve fertility of lactating dairy cows or survival of heat-shocked Embryos. J. Dairy Sci. 86:2343-2351.

Peters, R.R. and H.A. Tucker, 1978. Prolactin and growth hormone responses to photoperiod in heifers. Endocrinology 103: 229-234.

Peters, R.R., L.T. Chapin, R.S. Emery, and H.A. Tucker. 1981. Milk yield, feed intake, prolactin, growth hormone, and glucocorticoid response of cows to supplemented light. J. Dairy Sci. 64:1671-1678.

Pevet, P. 2003. Melatonin: from seasonal to circadian signal. J. Neuroendocrinol. 15:422-426.

Pezet,A., H. Favre, P.A. Kelly, and M. Edery. 1999. Inhibition and restoration and restoration of prolactin signal transduction by suppressors of cytokine signaling. J. Biol. Chem. 274:24497-24502.

Plaut, K., D.E. Bauman, N.Agergaard, and R.M. Akers. 1987. Effects of exogenous prolactin administration of lactation performance of dairy cows. Dom. Anim. Endocrinol. 4:279-290.

Prendergest, B.J., K.E. Wynne-Edwards, S.M. Yellon, and R.J. Demas. 2002. Photofractoriness of immune function in the male siberian hamsters (Phodopus sungorus). J. Neuroendocrinol. 14:318-329.

Reber, P.M. 1993. Prolactin and immunomodulation. Am. J. Med. 95:637-644.

Royster, M., P. A. Kelly, and M. Freemark. 1995. The prolactin receptor in the fetal rat: Cellular localization of messenger ribonucleic acid, immunoreactive protein, and ligand-binding activity and induction of expression in late gestation. Endocrinology 136: 3892-3900.

Saad, A. M., C. Concha, and G. ÅStröm. 1981. Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. J.Vet. Med., Series B, 36: 337–345.

Sanchez-Barcelo, E.J., M.D. Mediavilla, S.A. Zinn, B.A. Buchanan, L.T. Chapin, and H.A. Tucker. 1991. Melatonin suppression of mammary growth in heifers. Biol. Reprod. 44: 875-879.

Sapolsky, R.M. 2001. Depression, antidepressants, and the shrinking hippocampus. Proc. Nat.Acad. Sci. 98:12320-12322.

Schuler, L.A., R.J. Nagel, J. Gao, N.D. Horseman, and M.A.Kessler. 1997. Prolactin receptor heterogeneity in bovine fetal and maternal tissues. Endocrinology 138: 3187-3194.

Sloane, E.D., C.C. Muscoplat, J.M. Kanene, C.O. Thoen and D.W. Johnson. 1978. In vitro stimulation of bovine peripheral blood lymphocytes: Comparison of round-and flat-bottom microtiter plates for detection of tuberculin hypersensitivity. J. Clin. Micro. 7:172-175.

Soper, F. F., C.C. Muscoplat, and D.W. Johnson. 1978. In vitro stimulation of bovine peripheral blood lymphocytes: analysis of variation of lymphocyte blastogenic response in normal dairy cattle. Am. J. Vet. Res. 39:1039-1042.

Stanisiewski, E.P., L.T. Chapin, N.K. Ames, C.A. Blaze, and H.A. Tucker. 1988a. Effect of pinealectomy on prolactin, testosterone and luteinizing hormone concentration in plasma of bull calves exposed to 8 or 16 hours of light per day. J. Anim Sci. 66:464:469.

Stanisiewski, E.P., L.T. Chapin, N.K. Ames, S.A. Zinn, and H.A. Tucker. 1988b. Melatonin and prolactin concentrations in blood of cattle exposed to 8,16, or 24 hours of daily light. J. Anim. Sci. 66: 727-734.

Stehle, J.H., C. Von Gall, and H.W. Korf. 2003. Melatonin. A clock-output, a clock-input. J. Neuroendocrinol. 15: 383-389.

Sweeney, T., A. Donovan, F.J. Karsch, J. Roche, and D. O'Callaghan. 1997. Influence of previous photoperiodic exposure on the reproductive response to a specific photoperiod signal in ewes. Biol Reprod. 56:916-920.

Sweeney, T., G. Kelly, and D.O'Callaghan. 1999. Seasonal variations in long-day stimulation of prolactin secretion in ewes. Biol. Reprod. 56:128-133.

Telleria, C.M., T.G. Parmer, L. Zhong, D.L. Clarke, C.T. Albarracin, W.R. Duan, D.I.H. Linzer, and G. Gibori. 1997. The different forms of the prolactin receptor in the rat corpus luteum: Developmental expression and hormonal regulation in pregnancy. Endocrinology 138: 4812-4820.

Tournier, B.B., J.S. Menet, H. Dadente, V.J. Poirel, A. Malan, M. Mason-Pevet, P. Pevet, and P Vuillez. 2003. Photoperiod differentially regulate clock genes' expression in the suprachiasmatic nucleus of Syrian hamsters. Neuroscience 118:317-322.

Tucker, H.A. 1994. Lactation and it's hormone control. In The *Physiology of Reproduction*, edn 2. Pp 1065-1089. eds E. Knobil & J.D. Neill. New York: Raven Press Ltd.

Yellon, S.M., O.R. Fagoaga, and S.L. Nehlsen-Cannarella. 1999a. Influence of photoperiod on immune cell functions in the male Siberian hamster. Am. J. Physiol. 276:R97-R102.

Yellon, S.M., L.A. Teasley, O.R. Fagoaga, H.C. Nguyen, H.N. Truong, and S.L. Nehlsen-Cannarella. 1999b. Role of photoperiod and the pineal gland in T cell-dependent humoral immune reactivity in the Siberian hamster. J. Pineal Res. 27: 243-248.

Yu-lee, L.Y. 2002. Prolactin modulation of immune and inflammatory responses. Rec. Prog. Hormone Res. 57:435-455.

Zhou, S., F.R.A. Cagampang, J.A. Stirland, A.S.I. Loudon, and S.J. Hopkins. 2002. Different photoperiods affect proliferation of lymphocytes but not expression of cellular, humoral, or innate immunity in hamsters. J. Biol. Rhythms 17:392-405.

Zinn, S.A., L.T. Chapin, K.J. Lookingland, K.E. Moore, and H.A.Tucker. 1991. Effects of photoperiod on lactotrophs and on dopaminergic and 5-hydroxytryptaminergic neurons in bull calves. J. Endocrinol. 129:141-148.

Zwahlant, R.D., and D.R. Roth. 1990. Chemotactic competence of neutrophils from neonatal calves. Inflammation 14: 109-123.

BIOGRAPHICAL SKETCH

Jacob William Bubolz was born in Manitowoc, Wisconsin. In 2007, he graduated from the University of Wisconsin–Platteville where he earned a Bachelor of Science in Animal science. In 2008 he began his graduate work at the University of Florida under the supervision of Dr. Geoffrey Dahl. Jacob's master's program focused on Animal Science with an emphasis in dairy cattle environmental physiology. Jacob is currently employed at Pfizer Animal Health as a veterinary clinical research associate in the area of clinical development in swine biologics.