EFFECT OF HEAT STRESS DURING THE DRY PERIOD ON DAIRY CATTLE

By

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To my family

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AA	Amino acid
ACTH	Adrenocorticotropin
AEA	Apparent efficiency of absorption
AUC	Area under the curve
BHBA	β hydroxybutyric acid
CONA	Concanavalin A
CPM	Count per minute
CV	Coefficient of variation
DIM	Days in milk
DMI	Dry matter intake
FA	Fatty acid
GH	Growth hormone
GTT	Glucose tolerance test
HPA	Hypothalamic-pituitary-adrenal
IC	Insulin challenge
IG	Immunoglobulin
IGFBP	IGF binding protein
LDPP	Long day photoperiod
NEFA	Non-esterified fatty acids
PBMC	Peripheral blood mononuclear cell
PI-IUGR	Placental insufficiency intrauterine growth retardation
PRL	Prolactin
PRLR	Prolactin receptor
SCC	Somatic cell count

SCS	Somatic cell score
SD	Standard deviation
SDPP	Short day photoperiod
SEM	Standard error of the mean
SI	Stimulation index
THI	Temperature –humidity index
TMR	Total mixed ration
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
WH	Withers height

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In dairy cattle, late gestation is a critical period for fetal growth and physiological transition into the next lactation. Environmental factors, such as temperature and light, exert dramatic effects on the production, health and well-being of animals during this period and after parturition. The aim of this dissertation was to study the effect of heat stress during the dry period on the dairy cow and her offspring. Studies in Chapters 2 and 3 examined the effect of heat stress during the dry period on mammary gland development and gene expression of mammary tissue and lymphocytes. Dry period heat stress compromised mammary growth before parturition relative to heat stress abatement but did not affect the mRNA expression of genes involved in prolactin signaling in the mammary gland. The experiment in Chapter 4 examined the effect of cooling heat-stressed cows during the dry period on the insulin response at peripheral tissues, such as adipose and muscle, of dairy cows across the transition period. Heat stress did not affect the insulin response at peripheral tissues before parturition, but cooling during the dry period had a carryover effect in early lactation cows such that cooled cows had lower insulin response at peripheral tissues in response to the greater milk production compared with heat-stressed cows. In Chapter 5, the effect of late gestation heat stress on the growth and immune function of dairy calves was examined. Relative to those from the cooled dams, calves

from heat-stressed dams had lower birth weight but similar growth rate during the pre-pubertal period and lower passive immunity and impaired cell-mediated immunity before weaning.

In conclusion, heat stress during the dry period impairs mammary gland development before parturition, alters insulin action at peripheral tissues in early lactation and compromises the immune function of offspring.

CHAPTER 1
REVIEW OF LITERATURE

An animal's physiology is influenced by the surrounding environment, which in turn impacts production. One of the well-recognized major environmental factors limiting production of dairy cattle is elevated ambient temperature. Figure 1-1 summarizes the monthly milk production in Florida from 2007 to 2010 and the typical ambient temperature within a year at Okeechobee, Florida, the top-ranked county for dairy cows in Florida. From May to August, milk production in Florida declines as air temperature rises. This phenomenon can be explained by decreased lactational performance due to thermal stress in lactating dairy cows. In addition, it is also observed that the lowest or highest monthly milk production in Florida does not coincide with the highest (July and August) or lowest ambient temperature (January). Instead, cows in Florida produce the lowest amount of milk in September and the most milk in March. Thus, a two-month lag between milk yield and air temperature appears consistently in the annual pattern. This observation suggests that, in addition to a direct effect on milk production during lactation, the thermal status of dry cows has a carryover effect on performance in the subsequent lactation. Indeed, cows exposed to heat stress during the dry period have decreased milk production in the next lactation (Wolfenson et al., 1988; do Amaral et al., 2009). In addition to lactation performance, environmental heat stress also affects the cow immune function, metabolism and reproduction during both lactation and late gestation. Thus, the aim of this review of literature is to describe the effect of heat stress during lactation and late gestation on physiology and production of dairy cattle.

Thermoregulation of Dairy Cows

Homeotherms maintain body temperature by ensuring equilibrium between heat gain and heat loss (Fuquay, 1981). Under heat stress conditions, animals adapt to the increased

environmental heat load by decreasing heat gain and increasing heat loss which is reflected in enhanced heat dissipation to the environment, depressed production, and altered behavior (Fuquay, 1981).

Temperature-humidity index (**THI**) is the most common measure of heat stress in the dairy industry (Armstrong, 1994). It combines the impacts of dry bulb temperature and relative humidity but does not include solar radiation or wind speed. Thus, THI may be a good indicator of heat stress in housing structures but not open lot or pasture based facilities where a black globe humidity index is more appropriate in order to incorporate the effects of solar radiation (Collier et al., 2006). It is commonly considered that THI equal to 72 is the threshold of environmental heat stress in cows (Armstrong, 1994). However, a recent study indicates that, when daily average THI exceeds 68, the milk production of high yield dairy cows decreases relative to those in a thermo-neutral environment (Zimbleman et al., 2009).

In order to cope with the increased heat load, one response to heat stress of cattle is to decrease metabolic heat production by reducing feed intake and production, such as milk yield and growth. In addition, heat exchange between the animal and surrounding environment is also altered in order to dissipate more heat. Animals exchange heat with the environment through sensible heat transfer including radiation, convection and conduction and insensible or latent heat loss by evaporation (Kadzere, et al., 2002). The magnitude of sensible heat exchange is dependent on temperature difference between the body surface of the animal and surrounding environment and evaporative heat loss is temperature independent and associated with humidity. Radiation is of importance in animals raised in open lots, as they receive substantial heat from solar radiation during the day and dissipate heat to the cooler sky at night (Fuquay, 1981). However, for animals housed in barns or buildings, heat exchange through radiation is less

important. For example, in typical free-stall dairy barns in Florida, the black globe temperature is similar to the dry bulb temperature which indicates the physical shade blocks most of the solar radiation (Dikmen and Hansen, 2009). In response to environmental heat stress, the cutaneous blood flow of cattle is increased which brings heat from the body core to the periphery and enhances heat loss through conduction and convection (Choshniak et al., 1982). However, with a high environmental temperature, the capacity for conductive and convective heat loss of heat-stressed animals is minimized due to the negative temperature difference between environmental and body surface temperature and, therefore, evaporative heat loss plays the dominant role in thermoregulation of animals. As ambient temperature increases, evaporative heat loss is initially mediated by sweating and is then followed by increased respiratory heat loss as heat stress becomes more severe (Berman, 2005).

Water intake is also important in the thermoregulation of animals and increases in heat-stressed cattle. In addition to the decline in intake of water through reduced feed, heat-stressed cow lose significant amounts of water through evaporation on the body surface and respiratory tract due to increased evaporative cooling (McDowell et al., 1969). Although a portion of the increase in water consumption of heat-stressed cows is excreted through urine, the water loss through feces is also concurrently decreased in order to support the water necessary for enhanced evaporative cooling (McDowell et al., 1969).

The adaptation of heat stress in cattle also occurs at the cellular level. One of the most recognized cellular response to hyperthermia is the increase in expression of heat shock proteins (**HSPs**). There are several groups of HSPs according to their molecular weight from 15 to 110 kDa, and HSP70 is the most widely studied among all HSPs and is the most sensitive to temperature (Kregel, 2002). The HSP70 is ubiquitously expressed in bovine tissues (Gutierrez

and Guerriero, 1995) and immune cells (Guerriero and Raynes, 1990; Agnew and Colditz, 2008) and various stressors induce its expression including hyperthermia (Guerriero and Raynes, 1990, Gutierrez and Guerriero, 1995). Although the exact function of the stress induced increase in cellular HSP70 expression is still not entirely understood, it is proposed to act as a molecular chaperone to assist folding of newly-synthesized proteins and repairing and refolding damaged proteins under stressful conditions (Kregel, 2002). Further, as reviewed by Lanneau et al. (2007), part of the cytoprotective function of HSPs is mediated through their anti-apoptotic effects in stressed cells. In primary bovine mammary epithelial cell culture, induction of HSP70 expression by addition of prostaglandin A_1 actually extends the thermo-tolerant capacity and decreases cell apoptosis, which in turn enhance the survival of bovine mammary epithelial cells under high incubation temperature (Collier et al., 2008).

Effects of Heat Stress on Dairy Cattle During Lactation

As reviewed by West (2003) and Bernabucci et al. (2010), environmental heat stress during lactation negatively affects milk production. The magnitude of the negative effect of heat stress on lactation is influenced by stage of lactation and milk production. Early lactation cows are the least adversely affected by heat stress compared with mid and late lactation animals (Maust et al., 1972), which is probably because the extensive tissue mobilization under negative energy balance of early lactation cows offsets the negative effect of heat stress on feed intake (Maust et al., 1972; Bernabucci et al., 2010). Additionally, in the same stage of lactation, cattle with high milk production are more sensitive to heat stress compared with low producing cows, which reflects the increased metabolic heat production with greater milk synthesis (Berman 2005).

The lower energy intake of heat-stressed cows only causes part of the reduction in milk yield observed in lactating cows. For example, mid-lactation heat-stressed cows have 35-50%

lower milk production compared with their pair-fed herdmates (Rhoads et al., 2009; Wheelock et al., 2010). In contrast, force feeding through a rumen cannula of mid-lactation cows only partly reverses the milk production loss under heat stress, which suggests that less available energy is only part of the impact of heat stress on milk production (Wayman et al., 1962). Additionally, heat stress during late lactation reduces milk yield without affecting DMI (Tarazón-Herrera, et al., 1999). In addition to decreased energy intake, heat-stressed lactating cows also have a slower rate of passage of feed (McDowell et al., 1969) and a reduced efficiency of energy utilization for milk production (Wayman et al., 1962; McDowell et al., 1969) which also contribute to the lower milk yield (Fuquay, 1981). In response to elevated temperature in vitro, bovine mammary epithelial cells display abnormal morphology and have reduced cell growth (Collier et al., 2008), which suggests that the hyperthermia induced with environmental heat stress directly compromises mammary cell function. However, in vivo data to support this hypothesis in lactating dairy cows is still not available.

The effect of hyperthermia on metabolic rate is inconsistent. In dairy heifers, exposure to elevated environmental temperature increases metabolic heat production (Purwanto et al., 1994). However, under environmental heat stress, lactating dairy cows experience decreased oxygen consumption which suggests a decrease in metabolic rate (Yousef and Johnson, 1966). These conflicting data may be due to the impact of different life cycle stages of the animals and the magnitude of heat stress (Baumgard and Rhoads, 2011). Relative to cows in a thermo-neutral setting, the energy requirement for maintenance of heat-stressed cows is increased (McDowell et al., 1969) and that primarily results from the activation of physical activities such as panting and sweating to increase heat loss (Baumgard and Rhoads, 2011). Combined with decreased energy intake, energy available for other functions, such as lactation, in heat-stressed animals is limited

in order to prioritize survival (West, 2003). Cellular metabolism of heat-stressed cows is also altered. In mid-lactation, independent of DMI, heat-stressed cows have impaired adipose tissue mobilization (Wheelock et al., 2010; Baumgard et al., 2011) and increased peripheral tissue insulin response (Wheelock et al., 2010) compared with thermo-neutral cows. But if similar metabolic responses to heat stress also occur in early or late lactation cows remains unknown.

The summer season is related to increased disease incidence in dairy cows, which is a function of indirect environmental and direct animal effects (Kadzere et al., 2002). During the summer, elevated ambient temperature and humidity provide a supportive environment for the survival of pathogens compared with the cold and dry conditions of winter (Kadzere et al., 2002). In addition to the increased threat from pathogens, altered immune function in response to environmental heat stress may be a direct reason for the increased disease occurrence in the summer. Surprisingly, compared with the literature related to metabolism and physiology, studies focusing on effects of heat stress on immune function of lactating dairy cows are relatively scant. Peripheral blood mononuclear cells (**PBMC**) isolated from normothermic dairy cows have a lower mitogen-induced proliferative capacity in vitro when incubated under elevated ambient temperatures (Kamwanja et al., 1994; Lacetera et al., 2006). The random migration of neutrophils from normothermic cows in vitro is reduced but cell phagocytosis and oxidative burst are not influenced under high incubation temperatures (Elvinger et al., 1991). When environmental heat stress is applied to cows, neutrophil chemotaxis is depressed in vitro (Elvinger et al., 1991) and heat-stressed lactating cows have a decreased leukocyte migration to the mammary gland in response to a chemotactic challenge of oyster glycogen (Elvinger et al., 1992). Therefore, compromised neutrophil function in vitro and in vivo may partly explain the

increase in somatic cell count (**SCC**) and mastitis incidence in summer (Wegner et al., 1976; Elvinger et al., 1992).

Effects of Heat Stress on Dairy Cattle During Late Gestation

Late gestation is a critical period in the production cycle of food animals. In dairy cattle, the dry period or non-lactating interval before parturition is also of importance in regard to mammary gland remolding, immune function, the metabolic transition from pregnancy to lactation and fetal growth.

Mammary Biology During the Dry Period

In dairy cattle, the dry period or non-lactating period late in gestation is important to achieve maximal milk production in the next lactation. Numerous studies support the concept that a 45 to 60 day interval before parturition when milking is absent is ideal for dairy cows, as dry periods greater than 70 d or less than 30 d are detrimental to yield in the next lactation. (Annen et al., 2004, Pezeshki et al., 2007). In the absence of a dry period, milk production in the next lactation drops 20-25% compared with cows that experience a dry period (Bachman and Schairer, 2003; Grummer and Rastani, 2004). Additionally, the importance of dry period with regard to milk yield is centered on the mammary gland rather than supportive tissues (Capuco et al., 1997).

During the dry period, the alveolar structure of the mammary gland undergoes series of morphological changes. Whereas the tissue area occupied by mammary epithelial cells is not influenced by advancing gestation, the luminal area of mammary tissue decreases to a minimum in the middle of dry period and reaches the peak before parturition. The stromal area of mammary tissue is inversely related to luminal area (Capuco and Akers, 1999). These morphological changes of mammary tissue during the dry period are consistent with the initial absorption of luminal secretions in the initial phase of dry period followed by accumulation of

mammary secretion during colostrogenesis and last phase of lactogenesis as parturition approaches (Capuco et al., 1997).

In addition to structural changes at the tissue level, individual mammary cells undergo extensive growth and cell turnover. During the prepartum period, the mass and DNA content of mammary parenchymal tissue increase as gestation advances which indicates an increase in mammary cell number during the dry period (Capuco et al., 1997). This extensive mammary growth in the dry period results from increased mammary epithelial cell proliferation, especially during the second half of the dry period (Capuco et al., 1997). Further, following milk stasis, mammary involution is initiated and is accompanied by an induction of mammary cell apoptosis (Wilde et al., 1997, Sorensen et al., 2006). The increase in programmed cell death of mammary cells at the beginning of the dry period is important to eliminate senescent mammary cells from the previous lactation while the extensive mammary growth in the second half of the dry period replaces the epithelial component (Capuco et al., 2001).

In addition to extensive mammary gland remodeling during the dry period, suppressed acquired and innate function characterizes the transition period from late gestation to early lactation (Van Kampen and Mallard, 1997; Mallard et al., 1998; Herr et al., 2011). Negative energy balance and increased health and metabolic disorders are also associated with the transition period (Goff and Horst, 1997; Mallard et al., 1998; Drackley, 1999). Further, the fetus grows at the fastest rate and accumulates ~60% of its birth weight during the last two months of gestation (Bauman and Currie, 1980). Given the physiological challenges cows experience under the best of conditions, insults from environment such as heat stress are expected to exert additional negative influences on the cow during this critical period.

Heat Stress Effects on the Dam

Relative to lactating cows, dry cows generate less metabolic heat (West; 2003) and have a higher upper critical temperature (Hahn; 1997). But, environmental thermal stress still dramatically influences the performance of cows during late gestation and that carries over to the next lactation. In addition, maternal thermal stress alters fetal growth and affects postnatal calf development.

Late gestation heat stress effects on lactation performance

Late gestation heat stress has profound effects on milk production in the subsequent lactation in ruminants (Wolfenson et al., 1988; Ocfemia et al., 1993; do Amaral et al., 2009). Depending on the method and duration of prepartum cooling, dairy cattle have different milk yield responses in the next lactation. With limited cooling, such as shade (Collier et al., 1982b) or short interval soaking in the middle of the day (Avendaño-Reyes, et al., 2006), only modest increases in subsequent milk production were observed and the difference was not statistically significant. However, when more extensive cooling (shade, fans and sprinklers) was provided to dry cows, milk production in the subsequent lactation was significantly improved (Wolfenson et al., 1988; do Amaral et al., 2011). The duration of prepartum cooling during environmental heat stress may also affect future lactation performance. Moore et al. (1992) studied the relationship between climatological data and milk production of cows that calved from July to September in Mississippi using regression analysis and found that exposure to heat stress during last 30 d of gestation was too short to elicit any effect on the milk production in next lactation. However, in a controlled study (Urdaz et al., 2006), actively cooled cows in the last 28 d of gestation had improved milk yield in the first 60 d of lactation compared with non-cooled cows.

Mechanisms related to compromised subsequent lactation performance by dry period heat stress are still not clear. Collier et al. (1982b) reported that the retarded placental development

resulting from late gestation heat stress was directly related to impaired mammary function because of the linear relationship between calf birth weight and milk yield of the dams, however, no significant difference of milk production was observed in that study. Thus, the reduced placental function may not be the only explanation of the significant decrease in milk production following prepartum heat stress observed by others (Wolfenson et al., 1988; do Amaral et al., 2011). Shorter gestation length is a common outcome of late gestation heat stress in cows and may also account for the decrease in milk production in the next lactation because it is possible that heat-stressed cows have a more limited period of mammary gland growth while dry. However, results from induced parturition studies (Schmitt et al., 1975; Beardsley et al., 1976; Bremmer et al., 1999) suggest that a reduction of several days in gestation length only decreases the milk yield in early lactation rather than the entire lactation. Mammary gland development in the dry period is important for productive performance in the next lactation (Capuco et al., 1997) and modified mammary remodeling in the dry period could dramatically affect the subsequent lactation curve, such as is observed with photoperiod manipulation (Auchtung et al., 2005; Wall et al., 2005). Thus, it is possible that environmental heat stress exerts negative effects on mammary growth in the dry period; however, data to support this possibility is currently not available.

The hormone PRL plays important roles in lactogenesis and mammogenesis in cattle (Tucker, 2000) and altered PRL signaling mediates photoperiodic effects on the mammary gland development during the dry period (Wall et al., 2005) and affects future milk production (Auchtung et al., 2005). Dairy cows exposed to short day photoperiod (**SDPP**, 8 h light: 16 h dark) during the entire dry period produce more milk in the subsequent lactation compared with those under long day photoperiod (**LDPP**, 16 h light: 8 h dark) (Miller et al., 2000; Auchtung et

al., 2005). This improved lactation performance with SDPP is mediated by enhanced mammary gland remodeling through increased mammary cell proliferation and decreased cell apoptosis during the dry period (Wall et al., 2005). Additionally, in response to SDPP, circulating PRL concentration is decreased (Miller et al., 2000; Auchtung et al., 2005), but there is a concomitant increase in PRL receptor (**PRLR**) gene expression in mammary tissue and lymphocytes relative to LDPP (Auchtung et al., 2005).

Similar to LDPP, heat stress in cattle also increases circulating concentrations of PRL (Collier et al., 1982a; do Amaral et al., 2009). The inverse relationship between circulating PRL and *PRLR* gene expression observed with photoperiod manipulation also occurs in the liver and lymphocytes of late gestation heat-stressed cows (do Amaral et al., 2010; 2011). Additionally, the increased blood estrone-sulfate and decreased progesterone concentrations in heat-stressed dry cows (Collier et al., 1982b) are also related to decreased PRL signaling in the mammary gland (Tucker, 2000). Therefore, it is reasonable to hypothesize that decreased PRL signaling in the mammary gland occurs in heat-stressed dry cows and exerts some effect to impair subsequent lactational performance (Dahl, 2008).

Late gestation heat stress effects on health and immune function

In addition to the compromised lactational performance, late gestation heat stress influences animal health and immune function during the transition period. Few studies have explored the relationship between heat stress during late gestation and the incidence of disease in the postpartum period. Urdaz et al. (2006) reported that, compared with heat-stressed cows, those under evaporative cooling during the last 28 d of gestation had similar incidence of common postpartum health disorders (i.e. displaced abomasum, retained placenta, metritis and milk fever) in the first 60 days in milk (**DIM**). However, in a larger scale study that included more than 2600 calving records over three consecutive years on a commercial dairy located in Florida,

Thompson et al. (2011) studied seasonal effects during the dry period on the occurrence of health disorders in the first 60 DIM and found that cows dried off in hot months (June, July and August) had higher incidences of mastitis, respiratory problems and retained fetal membranes in early lactation compared with those dried in cool months (December, January and February). Because day length during the hot months (~14 h) is longer than cool months (~11 h) in Florida, photoperiod may contribute to the observed increase in disease incidence and cannot be excluded because exposure to SDPP during the dry period is related to the enhanced immune function (Auchtung et al., 2004b). Although the results presented by Thompson et al. (2011) are confounded with the seasonal effects during early lactation, the compromised immune function during the transition period due to late gestation heat stress may partly result in the observation of an increased occurrence of health disorders in early lactation of cows dried off in hot weather. The immune system includes the non-specific innate immune function that is the first line of defense to pathogens in the body and the specific adaptive immune function that generates memory of pathogen exposure. do Amaral et al. (2010, 2011) examined effects of late gestation heat stress on both arms of the immune system. To evaluate innate immunity, blood was drawn from prepartum heat-stressed or cooled cows at dry-off, -20, 2 and 20 d relative to calving and ability of neutrophils to phagocytize and destroy pathogens via oxidative burst were evaluated. There were no differences between treatments during the dry period, however, residual effects of late gestation heat stress on neutrophil function were observed in early lactation such that heat-stressed cows had impaired neutrophil phagocytosis and oxidative burst relative to cooled cows (do Amaral et al., 2011). In contrast, acquired immunity during the transition period is improved by heat stress abatement. Relative to those from non-cooled cows during the dry period, PBMC isolated from cooled cows have improved proliferation and tumor necrosis factor (**TNF-α**)

production in response to a mitogen in vitro during the transition period (do Amaral et al., 2010). Additionally, cooled cows have greater immunoglobulin G (**IgG**) production against ovalbumin challenge relative to non-cooled cows during the dry period but not in early lactation. These data reflect improved humoral immunity when heat stress abatement is applied in late gestation (do Amaral et al., 2011). However, cytokine gene expression in PBMC collected from heat stressed versus cooled dry cows has never been evaluated.

The compromised immunological responses observed during the transition period following late gestation heat stress may be due to altered PRL signaling in immune cells. Both innate and adaptive immune function are modulated by PRL (Bole-Feysot et al., 1998; López-Meza et al., 2010) and PRL is associated with multiple autoimmune diseases (Orbach and Shoenfeld, 2007). In dairy calves, manipulation of PRL signaling through the inverse relationship between circulating PRL and *PRLR* gene expression in immune cells alters cellular immune function in vitro (Auchtung et al., 2003; Auchtung and Dahl, 2004a). Moreover, the enhanced PRL signaling in immune cells is associated with an increase in cellular immune function during the transition period of cows exposed to prepartum SDPP relative to those on LDPP (Auchtung et al., 2004b, Dahl, 2008). Similar to the photoperiodic effect, heat-stressed cows had increased circulating concentration of PRL and decreased *PRLR* gene expression in lymphocytes during the transition period relative to those under heat stress abatement when dry (do Amaral et al., 2010). Therefore, altered PRL signaling may be partly responsible for the different immunological responses between prepartum heat-stressed and cooled cows during the transition period (do Amaral et al., 2010).

Late gestation heat stress effects on metabolism

Similar to lactating cows, heat stress during the dry period decreases DMI (Adin et al., 2009; do Amaral et al., 2009). As a result of the reduced energy intake, heat-stressed dry cows

have lower body weight gain in late gestation compared with those under heat stress abatement (Collier et al., 1982a; do Amaral et al., 2009, 2011). Despite the lower feed intake, late gestation heat stress had no effect on circulating concentrations of glucose (Collier et al., 1982b; do Amaral et al., 2009), non-esterified fatty acids (**NEFA**, Urdaz et al., 2006; do Amaral et al., 2009) and insulin (Collier et al., 1982b) before calving relative to cows that are cooled.

In mid-lactation, heat-stressed cows experience negative energy balance and have compromised adipose tissue mobilization, lower circulating NEFA and enhanced insulin response at peripheral tissues compared with their pair-fed herdmates under thermo-neutrality (Wheelock et al., 2010; Baumgard et al., 2011). These metabolic responses to heat stress at mid-lactation indicate heat-stressed cows have a preference to use glucose as the major energetic substrate in peripheral tissues at the expense of milk production. Given the fact that non-lactating dry cows and mid-lactation cows are in different energetic states (Bell, 1995; Drackley et al., 2001; NRC, 2001), it is questionable if similar metabolic responses occur in late gestation dry cows under heat stress relative to mid-lactation cows.

The thermal status of cattle during the dry period has carryover effects on metabolic responses in early lactation. Circulating NEFA and β hydroxybutyric acid (**BHBA**) increase at parturition and early lactation in heat stress abated dry cows relative to non-cooled cows (do Amaral et al., 2009). The altered blood metabolite profile suggests more active adipose tissue mobilization in cooled cows relative to heat-stressed cows (Bauman and Currie, 1980; Vernon and Pond, 1997). Relative to samples from heat-stressed cows, do Amaral et al. (2009) reported that milk fat and hepatic tissue of cooled cows contained higher proportions of preformed fatty acids in early lactation which also reflects more extensive body fat mobilization and higher circulating NEFA in cooled cows. Early lactation is characterized by decreased circulating

insulin and suppressed insulin sensitivity at peripheral tissues (Drackley, et al., 2001) and the accelerated adipose tissue mobilization of cooled cows in early lactation probably indicates the insulin response in peripheral tissues is further modified by prepartum heat stress. However, these areas have never been studied to date.

Late gestation heat stress effects on reproduction

It is well known that heat stress during lactation has detrimental effects on reproductive performance but studies related to the impact of late gestation heat stress on reproduction in the next lactation are limited and the existing data are inconsistent. Comparison of cows dried in hot months (June, July and August) versus cool months (December, January and February) under commercial management in Florida revealed effects of season of the dry period on reproductive performance in the subsequent lactation (Thompson et al., unpublished data). Indeed, cows dried in hot months had an increased number of breedings, days to first breeding and days to pregnancy diagnosis during the first 150 DIM in the subsequent lactation relative to those dried in cool months. Although confounded with seasonal effects during the lactation, these results may be partly explained by the carryover effects ambient heat during the dry period on reproductive performance in the next lactation (Thompson et al., unpublished data). However, studies conducted in Mississippi suggest that there is no correlation between late gestation heat stress and reproductive performance in the next lactation (Moore et al., 1992; Avendaño-Reyes et al., 2010). These conflicting results were also observed in controlled studies. For example, Avendaño-Reyes et al. (2006) reported that compared with those provided with cooling during the dry period, heat-stressed cows had more days open and increased services per conception in the subsequent lactation. In contrast, Adin et al. (2009) reported no differences with regard to reproductive traits between cows heat-stressed or cooled prepartum. Lewis et al. (1984) studied the residual effects of prepartum heat stress on uterine and ovarian development and function in

early lactation and found that late gestation heat stress was related to increased systemic 13,14-dihydro-15-keto-prostaglandin F2α concentrations, accelerated uterine involution and smaller corpora lutea, but there were no differences for days open and services per conception between treatments. Therefore, the impact of dry period heat stress on subsequent reproductive outcomes remains unclear.

Maternal Heat Stress Effects on Offspring

In addition to the substantial influence on the dam, maternal heat stress during late gestation also impacts the fetus. Further, prenatal stress exerts carryover effects on the offspring in postnatal life. Related studies in dairy cattle are rare, but more data in other farm animals provide information and may be informative for dairy cattle.

Fetal thermogenesis and thermoregulation

Across many species under thermo-neutral conditions, the fetus has a consistently higher body temperature relative to its dam (Laburn et al., 2000; Asakura, 2004). In pregnant sheep and goats during late gestation, fetal body temperature is approximately 0.6 ^{o}C higher than maternal core body temperature (Laburn et al., 1992; 2002; Faurie et al., 2001). Data related to bovine fetal thermoregulation are limited; however, the higher rectal temperature (~ 39.5 ^{o}C) of newborn calves at birth relative to mature cows (Vermorel et al., 1983) also indicates higher body temperature of bovine fetus in utero compared with maternal temperature. Fetal thermoregulation is inhibited in utero and the body temperature of the fetus is dependent on fetal metabolic heat production and heat transfer with the dam. The fetus exchanges heat with dam largely through the fetal-placental circulation which accounts for ~ 85% of total fetal heat loss; the remaining heat is exchanged conductively through fetal membranes, amniotic fluid and the uterine wall (Laburn et al., 2000; Asakura, 2004). In addition to heat exchange with the dam, the fetus has a twofold higher metabolic rate than the dam, which generates a large amount of heat and accounts

for the higher body temperature (Schröder and Power, 1997; Laburn et al., 2000; Asakura, 2004). Moreover, the metabolic rate of placenta is high and also contributes to the difference in body temperature between the dam and fetus (Schröder and Power, 1997; Asakura, 2004).

Under maternal heat stress, fetal body temperature increases as does maternal temperature, but the rate of the increase is slower than that of the dam (Laburn et al., 1992; 2002; Faurie et al., 2001). The smaller elevation in fetal body temperature may reflect the reduced fetal metabolic heat production that results from decreased uterine blood flow and oxygen delivery (Schröder and Power, 1997). Therefore, although not at the same rate as the maternal temperature, fetal body temperature still increases dramatically under maternal heat stress.

Maternal heat stress effects on placental function

Heat stress during gestation is associated with reduced placental weight (Collier et al., 1982b; Bell et al., 1989), which is related to a decrease in tissue size rather than the number of placentomes (Early et al., 1991). Compared with those from animals under thermo-neutrality, the placenta from the hyperthermic animals had decreased total DNA, RNA and protein content but concentrations were similar, which indicates that the reduced placenta mass is due to smaller cell number rather than cell size (Early et al., 1991). Additionally, decreased circulating placental hormones in heat-stressed animals (i.e. estrone sulfate, Collier et al., 1982a; placental lactogen, Bell et al., 1989; pregnancy associated glycoprotein, Thompson et al., unpublished data) relative to those under thermo-neutrality or heat stress abatement also reflect compromised placental development. Moreover, a decrease in total uterine and umbilical blood flow (Dreiling et al., 1991; Reynolds et al., 2006) and compromised placental vascularization (Regnault et al., 2003) are also observed in heat-stressed animals.

In pregnant animals, the oxygen and nutrient supply of the fetus rely on the placenta (Bell and Ehrhardt, 2002). With compromised placental growth and vascularization, it is expected that

placental oxygen and nutrient transfer to the fetus are also impaired under heat stress. Literature related to late gestation heat stress on placental function is scarce, however, given the same endpoint (i.e. retarded fetal growth), the hyperthermia-induced placental insufficiency intrauterine growth retardation (**PI-IUGR**) sheep model provides some clues with regard to the maternal-fetal oxygen and nutrient exchange during heat stress (Morrison, 2008).

Maternal-fetal oxygen transport occurs by simple diffusion. Uterine blood flow, placental size, placental vascular resistance and the maternal-fetal oxygen concentration gradient play deterministic roles in oxygen exchange (Reynold et al., 2006; Yates et al., 2011). In the heat-stressed PI-IUGR sheep, the increased placental resistance due to impaired placental angiogenesis impedes transplacental oxygen diffusion (Regnault et al., 2003). However, with the increased maternal-fetal blood oxygen concentration gradient, the oxygen diffusion capacity per unit of placental mass in the PI-IUGR sheep is actually similar to control ewes under thermo-neutrality (Regnault et al., 2003). Collectively, the decreased placenta size in the hyperthermic animals determines that the total umbilical oxygen uptake is reduced and the fetus is exposed to a hypoxic state. Glucose is the primary energetic substrate in the fetus and placenta and fetal glucose is almost exclusively supplied by maternal circulation through the placenta under normal conditions (Bell and Ehrhardt, 2002; Hay, 2006). In contrast to simple oxygen diffusion, however, transplacental glucose transport in ruminants is mediated by facilitated diffusion through glucose transporters 1, 3 and 8 (Bell and Ehrhardt, 2002; Limesand et al., 2004; Hay, 2006). Therefore, maternal-fetal glucose exchange is dependent on the maternal-fetal glucose concentration gradient and the placental transport capacity (Bell and Ehrhardt, 2002; Hay, 2006). In addition to the decreased placenta size and surface area in PI-IUGR ewes, the glucose transport capacity per unit of placenta mass is also reduced (Bell and Ehrhardt, 2002; Limesand

et al., 2004) because of the lower glucose transporter expression in placental tissue (Limesand et al., 2004; Yates et al., 2011). Even though the maternal-fetal glucose concentration gradient is increased in hyperthermic animals (Limesand et al., 2004), the impaired placental glucose transport capacity reduces the absolute total maternal-fetal glucose exchange (Bell and Ehrhardt, 2002; Hay, 2006; Yates et al., 2011). Because the concentration of most amino acids (**AA**) in the fetal circulation is higher than the dam, energy-dependent AA transporters are required in the placenta in order for active maternal-fetal AA transport to occur (Bell and Ehrhardt, 2002; Regnault et al., 2005). In PI-IUGR sheep, the transplacental AA transport is impaired due to the reduced transport capacity that results from the smaller placental size and surface area and the compromised transport capacity per unit of placenta mass (de Vrijer et al., 2004; Regnault et al., 2005). Moreover, the reduced maternal concentrations of AA due to decreased feed intake in the hyperthermic ewes may also affect maternal-fetal AA transport (de Vrijer et al., 2004) because reduced maternal circulating AA concentration also negatively impacts transplacental AA transport (Regnault et al., 2005).

Maternal heat stress effects on growth and metabolism of offspring during pre- and postpartum periods

Heat stress in late gestation decreases birth weight of newborn farm animals, which reflects compromised fetal development in utero (Table 1-1). The hyperthermia related IUGR is a result of several factors. First, heat stress during gestation decreases gestation length (Table 1-1). In the last two months of gestation in dairy cattle, the bovine fetus grows at a rapid rate that accounts for 60% of the total fetal body size at birth (Bauman and Currie, 1980). A reduction of several days in gestation length could account for a portion of the birth weight differences between calves from cows cooled and heat-stressed during late gestation. As discussed previously, in response to maternal heat stress, an increase in fetal body temperature may also have a direct

effect to retard fetal growth (Bell et al., 1989). However, the data supporting this possibility is still lacking in cattle. A decrease in energy intake is a hallmark of the heat stress response in animals and malnutrition is a factor in reduced fetal growth (Wu et al., 2006). Severely restricted energy intake in the last trimester of beef cows dramatically decreases calf birth weight (Tudor, 1972), yet, a moderate decrease in energy intake in late gestation has no effect on calf birth weight (Hough et al., 1990; Janovick and Drackley, 2010). Additionally, fetal growth retardation related to maternal hyperthermia in late gestation sheep is independent of maternal nutrition (Brown et al., 1977). Therefore, if the small decrease in DMI of heat-stressed animals during late gestation plays an important role in compromised fetal growth is open to question.

Placental development and function are determinants of fetal growth. Maternal hyperthermia related placental insufficiency is deleterious to fetal development and may also have profound effect on postnatal growth. In the PI-IUGR sheep model, in response to the hypoxia and nutrient restriction, the IUGR fetus develops series of endocrine and metabolic adaptations to survive at the expense of growth. Compared with the normothermic control, the IUGR sheep fetus has lower circulating glucose, insulin and IGF-I (Limesand et al., 2005; 2006; Thorn et al., 2009) but increased catecholamine, epinephrine and norepinephrine concentrations (Leos et al., 2010). In addition, heat stress impairs pancreatic β-cell growth (Limesand et al., 2005), compromises insulin synthesis and secretion (Limesand et al., 2006) and enhances insulin sensitivity at peripheral tissues (Limesand et al., 2006, Thorn et al., 2009). In order to cope with the decreased nutrient supply, the IUGR fetus stimulates hepatic gluconeogenesis and decreases the rate of whole body glucose oxidation (Limesand et al., 2007). Under hypoxia, the anaerobic glucose metabolism in an IUGR fetus is enhanced and thus a large amount of lactate is produced (Limesand et al., 2007) which, together with increased circulating gluconeogenic AAs

(Limesand et al., 2006), serve as the substrates for hepatic gluconeogenesis. As a result, AA oxidation at peripheral tissues increases and, coupled with the decrease in placental AA transport and lower circulating IGF-I, results in impaired protein accretion in IUGR fetus (Thorn et al., 2009; Yates et al., 2011). Even though the hyperthermia induced PI-IUGR sheep model encompasses early fetal development whereas the cow model is applied later in gestation, using the same endpoints, such as shorter gestation length and lower birth weight, (do Amaral et al., 2009; Chen et al., 2010), similar fetal endocrine and metabolic responses may be observed. Further studies are required to confirm the responses in the bovine model.

Fetal adaptations to heat stress in the IUGR model may also affect the postnatal growth of lambs. Relative to controls, the IUGR lamb has higher insulin response to glucose (Yates, et al., 2011) and lower lipolytic response to adrenergic stimulation (Chen et al., 2010). Despite these metabolic differences, the overall postnatal growth rate is similar to normothermic control lambs (Chen et al., 2010). However, whether these metabolic responses in IUGR lambs induce changes in body composition, such as accelerated fat deposition, is unknown.

The effect of late gestation heat stress on dairy calves has never been evaluated. It is of interest, however, because it is the growth of the dairy heifer that determines the future performance and profitability of the farm. For example, increasing fat accumulation during the pre-pubertal period has significant effect on first lactation milk yield (Capuco et al., 1995; Silva et al., 2002) because mammary gland development is reduced when high-energy diets are fed in excess. If similar shifts in body composition occur with in utero heat stress is currently unknown.

Maternal heat stress effects on immune function of offspring

In addition to the dramatic effects on fetal and neonatal growth, maternal heat stress also influences immune function of the offspring. Passive immunity is of particular importance to

neonatal survival of farm animals and is altered by maternal heat stress. When sows were exposed to heat stress during last two weeks of gestation, their suckling piglets had lower circulating IgG compared to piglets from sows under thermo-neutrality (Machado-Neto et al., 1987). When feeding pooled colostrum to newborn calves, those from heat-stressed dams only have slightly lower blood IgG concentration compared with calves from cooled dams (Stott, 1980). However, the author attributed the compromised IgG transfer of calves from heat-stressed cows to the direct effect of thermal stress on neonates at birth and short period after birth rather than maternal heat stress. Thus, if the maternal heat stress has similar detrimental effect on the passive immune transfer in dairy cows to that of pigs is still in question.

Prenatal stressors modify T and B-cell function of the offspring (Merlot et al., 2008). For example, prenatal social stress in pregnant sows increases the in vitro lymphocyte proliferation in response to a mitogen in piglets in early life (Couret et al., 2009). Therefore, it is expected that maternal heat stress may also have carryover effects on cell-mediated immune function in the calf, yet that is not known at present.

Summary

It is well established that heat stress during the dry period in dairy cattle has a profound negative effect on milk production in the subsequent lactation. In addition, dry period heat stress compromises the immune status of cows during the transition period and exerts carryover effects on maternal metabolism in early lactation. Emerging evidence in other farm species indicates that heat stress effects during gestation carry over in offspring. However, there are still many questions remaining, for example:

Does heat stress during the dry period directly affect mammary gland development?

Does PRL signaling play a role in the mammary gland due to heat stress in the dry period?

Is insulin sensitivity or metabolic responses at peripheral tissues during the transition period influenced by dry period heat stress?

Does late gestation heat stress in dairy cattle have any carryover effect on the growth and immune function of the calf?

All the experiments in Chapters 2-5 of this dissertation were designed and conducted in order to answer the questions described above. Contents of this Literature Review (Chapter 1) are the basis of an "Invited Review" to be published in the Journal of Dairy Science. Chapter 2 was published and Chapter 4 is "In Press" in the Journal of Dairy Science. Chapters 3 and 5 have been submitted to the Journal of Dairy Science.

Table 1-1. Summary of studies on effects of late gestation heat stress on gestation length, fetal/newborn weight in ruminants.

Species	Treatment Length, d	Parturition Procedure	Gestation Length, d		Fetal/Newborn Weight, kg			Reference
			HT	Con.	HT	Con.	Diff.	
Sheep	25-53	Normal	---	---	3.18*	4.57	30%	Brown et al., 1977
Sheep	75	Slaughtered	139	139	3.39	4.07	17%	Bell et al., 1989
Sheep	30	C- section	140	140	2.46*	3.00	18%	Dreiling et al., 1991
Cattle	3rd Trimester	Normal	281	281	36.6*	39.7	8%	Collier et al., 1982a
Cattle	Dry	Normal	---	---	40.6*	43.2	8%	Wolfenson et al., 1988
Cattle	Dry	Normal	---	---	33.7†	37.9	11%	Avendaño-Reyes et al., 2006
Cattle	Dry	Normal	274	278	40.8*	43.6	6%	Adin et al., 2009
Cattle	Dry	Normal	---	---	31.0*	44.0	30%	do Amaral et al., 2009
Cattle	Dry	Normal	---	---	39.5*	44.5	11%	do Amaral et al., 2011

*$P \leq 0.05$; †$P \leq 0.10$

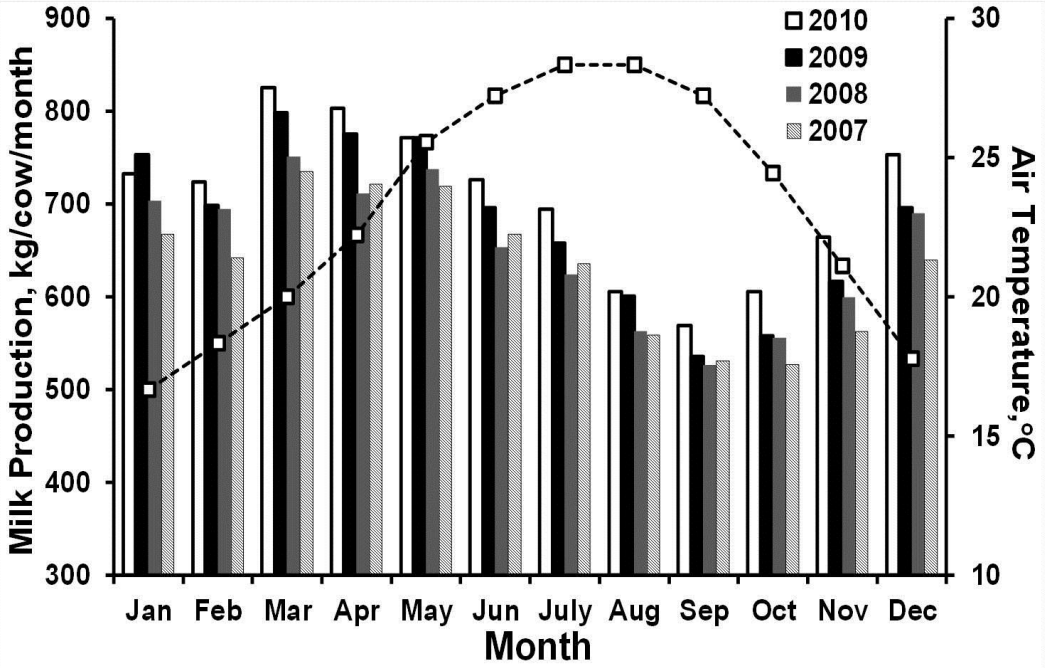

Figure 1-1. Monthly milk production in Florida (Bar graph, data from Florida livestock, dairy, and poultry summary, 2010, National Agricultural Statistics Service-USDA) and typical air temperature within a year in Okeechobee, Florida (Line graph, data from http://www.weather.com/weather/wxclimatology/monthly/34972).

CHAPTER 2
EFFECT OF HEAT STRESS DURING THE DRY PERIOD ON MAMMARY GLAND DEVELOPMENT

Abstract

Heat stress during the dry period negatively impacts hepatic metabolism and cellular immune function during the transition period, and milk production in the subsequent lactation. However, the cellular mechanisms involved in the depressed mammary gland function remain unknown. The objective of the present study was to determine the effect of heat stress during the dry period on various indices of mammary gland development of multiparous cows. Cows were dried off approximately 46 d prior to expected calving and randomly assigned to two treatments, heat stress (HT, n = 15) or cooling (CL, n = 14) based on mature equivalent milk production. Cows in the CL treatment were provided with sprinklers and fans that came on when ambient temperatures reached 21.1 ^{o}C, whereas HT cows were housed in the same barn without fans and sprinklers. After parturition, all cows were housed in a free-stall barn with cooling. Rectal temperatures were measured twice daily (0730 and 1430 h) and respiration rates recorded at 1500 h on a Mon-Wed-Fri schedule from dry off to calving. Milk yield and composition were recorded daily up to 280 days in milk. Daily dry matter intake was measured from dry off to 42 d postpartum. Mammary biopsies were collected at dry off, -20, 2 and 20 d relative to calving from a subset of cows (HT, n = 7, CL, n = 7). Labeling with Ki67 antigen and terminal deoxynucleotidyl transferase dUTP nick end labeling were used to evaluate mammary cell proliferation and apoptosis, respectively. Average temperature-humidity index during the dry period was 76.6 and not different between treatments. Compared with CL cows, HT cows had higher rectal temperatures in the morning (38.8 vs. 38.6 ^{o}C) and afternoon (39.4 vs. 39.0 ^{o}C), greater respiration rate (78.4 vs. 45.6 breath/min), and decreased dry matter intake (8.9 vs. 10.6 kg/d) when dry. Relative to HT cows, CL cows had greater milk production (28.9 vs. 33.9 kg/d),

lower milk protein concentration (3.01 vs. 2.87%) and tended to have lower somatic cell score (3.35 vs. 2.94) through 280 days in milk. Heat stress during the dry period decreased mammary cell proliferation rate (1.0 vs. 3.3%) at -20 d relative to calving compared with CL cows. Mammary cell apoptosis was not affected by prepartum heat stress. We conclude that heat stress during the dry period compromises mammary gland development before parturition which decreases milk yield in the next lactation.

Introduction

The dry period in dairy cattle is critical for maximal milk production in the subsequent lactation, as its absence is associated with a significant reduction in milk yield (Bachman and Schairer, 2003; Grummer and Rastani, 2004). During the dry period, mammary tissue undergoes extensive growth and cell turnover (Capuco et al., 1997; Sorensen et al., 2006). This process is necessary to compensate for mammary cell loss during the previous lactation (Capuco et al., 2001) and to replace senescent secretory epithelial cells (Capuco et al., 1997). Because the lactation curve is a function of mammary cell number and secretory capacity per cell (Capuco et al., 2003), manipulation during the dry period that enhances mammary growth may be a promising approach to improve lactation performance in dairy cows.

Environmental factors, such as photoperiod and temperature, can be manipulated during the dry period and influence a cow's performance in the subsequent lactation. Cows exposed to SDPP when dry produce more milk in the next lactation (Miller et al., 2000; Auchtung et al., 2005) and have improved immune function (Auchtung et al., 2004b) compared with cows under a LDPP. Short days also enhance mammary gland remodeling through increased mammary cell proliferation and decreased epithelial cell apoptosis relative to LDPP (Wall et al., 2005). This increased mammary growth occurs during a specific window of the dry period (i.e. 3-6 weeks before calving; Wall et al., 2005). There is strong evidence that the observed photoperiodic

effects are related to altered PRL signaling (Dahl, 2008), particularly the inverse relationship between circulating PRL and *PRLR* expression in multiple tissues. Indeed, SDPP decreases circulating PRL concentrations and increases *PRLR* gene expression in immune cells (Auchtung et al., 2005), hepatic tissue (Auchtung et al., 2003), and the mammary gland (Auchtung et al., 2005) compared with LDPP.

Heat stress is another environmental factor that can affect cow productivity. For example, heat stress in lactating cows is detrimental to milk production and reproductive performance (West, 2003; Collier et al., 2006). Moreover, heat stress during the dry period has a tremendous carryover effect into the postpartum period. In addition to decreased milk production (Wolfenson et al., 1988; Avendaño-Reyeset al., 2006; do Amaral et al., 2009), heat stress during the dry period negatively affects hepatic metabolism in early lactation, which compromises the transition into lactation from the non-lactating state (do Amaral et al., 2009). Immune function also is decreased by heat stress during the transition period. Relative to heat stress, cooled dry cows have greater lymphocyte proliferation (do Amaral et al., 2010), neutrophil phagocytosis and oxidative burst, as well as IgG production in response to ovalbumin (do Amaral et al., 2011). Similar to photoperiod, heat stress during the dry period increases circulating PRL concentrations (do Amaral et al., 2009), and decreases *PRLR* gene expression in lymphocytes (do Amaral et al., 2010) and hepatic tissue (do Amaral et al., 2009) compared with heat stress abatement. However, studies related to effects of heat stress on mammary gland growth and development are limited. Adin et al. (2009) concluded that cows cooled during the dry period had greater mammary epithelial cell proliferation during the close-up period based on increased expression of the hormone-sensitive lipase gene in the mammary gland compared with non-cooled cows. However gene expression profile of a lipid synthetic pathway is more associated

with cell secretory state than mammary gland growth. Our hypothesis was that heat stress during the dry period compromised mammary gland growth compared with heat stress abatement, and thus decreased subsequent milk yield. Therefore, the objective of the current study was to directly evaluate the effect of heat stress during the dry period on mammary gland development of dairy cows.

Materials and Methods

Animal, Housing, Experimental Design

This study was conducted at the Dairy Unit of University of Florida (Hague, Florida) from May to November, 2009. All the treatments and procedures were approved by the University of Florida Institute of Food and Agricultural Sciences Animal Research Committee. All cows were dried off at ~46 d relative to expected calving by cessation of milking and intramammary infusion with antibiotic (Quartermaster, Pfizer Animal Health, Kalamazoo, MI) to each quarter. At dry off, cows were assigned randomly to one of two treatments, heat stress (HT, n = 15) or cooling (CL, n = 14), based on mature equivalent milk production from the just completed lactation. Parity between HT and CL cows (1.6 ± 0.3 vs. 1.9 ± 0.3, respectively; $P = 0.5$) was similar.

Dry cows were housed in a sand-bedded free stall barn with the stall areas for CL cows equipped with sprinklers (Rainbird Manufacturing, Glendale, CA) and fans (J&D Manufacturing, Eau Claire, WI) whereas those of the HT cows were not. When ambient temperature exceeded 21.1^{o}C, fans automatically turned on and sprinklers were activated for 1.5 min at 6 min intervals. Photoperiod (14 h light: 10 h dark) of the barn for dry cows on both treatments was controlled using metal halide lights. The lights provided approximately 250 lx intensity at eye level of cows and were kept on from 0600 to 2000 h. After calving, all cows were housed in the same sand-bedded free stall barn with sprinklers and fans for cooling.

Air temperature and relative humidity of each pen in the barn for dry cows were recorded every 15 min by Hobo Pro series Temp probes (Onset Computer Corporation, Pocasset, MA). THI was calculated based on the equation reported by Dikmen et al. (2008): THI = (1.8×T+32) - ((0.55-0.0055×RH) × (1.8×T - 26)), where T = air temperature (^{o}C) and RH = relative humidity (%). During the dry period, rectal temperatures were measured twice daily (0730 and 1430 h) using a GLA M700 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA) and respiration rate was measured by counting the flank movements for one minute in the afternoon (1500 h) thrice weekly (Mon-Wed-Fri). Dry cows were fed once a day (0900 h) and milking cows were fed twice daily (0800 and 1300 h). Diet composition for both the prepartum and postpartum periods is in Table 2-1. Estimates of DMI were recorded from dry off until 42 days postpartum using the Calan gate system (American Calan Inc., Northwood, NH). Daily water consumption for each pen was recorded during the dry period and water intake for individual cow per day was estimated by dividing water consumption by number of cows in each pen. Cows were milked twice daily (0800 and 2000 h) and milk production was recorded daily up to 280 DIM. Cows were weighed and body condition scored at dry off, -32, -18, 0, 14, 28 and 42 d relative to calving before feeding when dry and after morning milking during lactation. Prepartum cumulative body weight (**BW**) and body condition score (**BCS**) change were calculated by subtracting values at -32, -18 d relative to calving and calving from values at dry off. Postpartum cumulative BW and BCS change were calculated by subtracting values at 14, 28 and 42 d relative to calving from values at calving.

Milk Composition

Percentages of milk fat, protein, lactose and SCC were measured at each milking by AfiLab^{TM} real time milk analyzer (Kibbutz Afikim, Israel) until 280 DIM. The AfiLab^{TM} milk analyzer is based on the optical characteristics of light scattering off matter such as milk fat,

protein etc. The values obtained using AfiLab^{TM} are well correlated with DHIA measures (De Vries et al., 2009). Somatic cell score was calculated based on the equation: Somatic cell score (**SCS**) = (Log10(SCC/12.5))/Log10(2).

Blood Sampling and Hormone Analysis

Blood samples for PRL analysis were collected once daily (0745 h) at dry off, -32, -18, -7 d relative to calving. Blood was collected from coccygeal vessels into sodium-heparinized Vacutainer^{TM} (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed in ice and centrifuged at 2,619 × g at 4 ^{o}C for 30 min within 1 h after collection. After centrifugation, plasma samples were frozen at -20 ^{o}C until analysis. Concentrations of PRL in plasma were determined by radioimmunoassay (Miller et al., 2000).

Mammary Tissue Samples

Mammary biopsies were collected at dry off, -20 d relative to expected calving, 2 and 20 d relative to actual calving from a subset of animals (n = 14, 7 cows per treatment). The actual biopsy dates were -39 ± 1.8, -16 ± 1.5, 3 ± 0.3, and 20 ± 0.4 d relative to calving. At the time of biopsy, all the mammary gland quarters biopsied were healthy and had no indication of mastitis. The biopsy procedure was based on the method reported by Farr et al. (1996). The right rear quarter of the cow was biopsied at dry off and 2 d relative to calving, and the left rear quarter was biopsied at -20 and 20 d relative to calving. Before biopsies, cows were sedated by intravenous injection of xylazine HCL (35 µg/kg of BW, Pro Labs Ltd, St. Joseph, MO). The biopsied region in the middle of the udder was shaved and sanitized three times by scrubbing with iodine scrub followed by rinsing with 70% ethanol. Subcutaneous injection of 3 mL lidocaine HCL (Phoenix Pharmaceuticals, Burlingame, CA) was administered in a line block above the biopsy site for local anesthesia. A 3 - 4 cm incision was made through the skin and connective tissue taking care to avoid obvious blood vessels. Mammary tissue (60 × 4 mm in

diameter) was obtained using a mammary biopsy tool (Farr et al., 1996) with a cordless drill (18V, Black & Decker, Towson, MD). Incisions were closed by Michel 18-mm stainless steel woundclips and sprayed with antiseptic wound dressing (Blu-Kote, H. W. Naylor Co., Inc. Morris. NY). Biopsied tissue was rinsed with phosphate buffered saline (**PBS**) and trimmed of fat. A portion of mammary tissue (5 × 4 mm in diameter) was cut for immunohistochemistry analysis and fixed in 10% neutral formalin for 24 h at 4 ^{o}C, and then transferred to 70% ethanol for future analysis. Samples were dehydrated and embedded in paraffin (MBI Cell and Tissue Analysis Core, University of Florida, Gainesville, FL) according to the standard protocol and 4 µm paraffin tissue sections were collected onto poly-L-lysine coated slides.

Immunohistochemistry

Ki67 antigen localization

The Ki67 antigen is expressed in the nuclei of proliferating cells (Scholzen and Gerdes, 2000). Mammary tissue sections were deparaffinized in mixed xylenes and hydrated through a graded ethanol series. After quenching in 3% (v/v) H_2O_2 for 10 min, antigen retrieval was accomplished by microwaving. Slides were microwaved for 5 min in a glass slide holder containing 400 mL citrate buffer (10 mM, pH 6.0), remained without disturbing for 5 min and then heated 5 more min. After microwaving, all slides stayed in the slide holder without disturbing for additional 30 min to cool. Following washes in ddH_2O and PBS, sections were blocked with 5% (v/v) non-immune horse serum to eliminate non-specific binding. All slides except the negative control slides were incubated with primary antibody (mouse anti-Ki67, Invitrogen, Camarillo, CA) diluted 1:80 (v/v) in PBS containing 1% (v/v) non-immune horse serum overnight at 4 ^{o}C. After washing with PBS, slides were incubated with goat anti-mouse horseradish peroxidase (SuperPicTure kit, Invitrogen, Camarillo, CA) for 30 min at 25 ^{o}C followed by extensive washing in PBS. Immune complexes were visualized colorimetrically with

3,3'-diaminobenzidine (Invitrogen) and counterstained with hematoxylin (Invitrogen). Slides were dehydrated through a graded ethanol series and mixed xylenes, and mounted with Histomount (Invitrogen).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)

After deparaffinization and hydration, samples were digested with 20 µl/mL proteinase K (Ambion, Applied Biosystems, Austin, TX) for 8 min at room temperature and followed by washing with ddH_2O. Endogenous peroxidase activity was quenched by incubation with 2% (v/v) H_2O_2 in PBS for 10 min. Following incubation in equilibration buffer (ApopTag Plus Peroxidase in situ Apoptosis kit, Millipore) for 10 min, sections were incubated with terminal deoxynucleotidyl transferase (333 units/mL) and digoxigenin-conjugated nucleotides (Millipore, Billerica, MA) for 60 min at 37 ^{o}C. Reactions were stopped and slides washed extensively with PBS. Labeled DNA was detected with anti-digoxigenin-peroxidase (ApopTag Plus Peroxidase in situ Apoptosis kit, Millipore) for 30 min at 25 ^{o}C followed by colorimetric detection with 3,3'-Diaminobenzidine. Sections were counterstained with methyl green, dehydrated and mounted.

Quantification of Immunohistochemistry Slides

Tissue sections were viewed using a light microscope (Nikon, Japan) to quantify Ki67 antigen or TUNEL labeled cells. One section per cow at each time point was evaluated. For each section, 5 fields were randomly selected and quantified. At least 2000 cells were counted in each section. Cell types were classified based on the mammary gland structure. Epithelial cells represent cells inside the basement membranes of alveoli, including secretory epithelial cells, myoepithelial cells, and leukocytes. Stromal cells were defined as cells in the connective tissue, including the fibroblasts, adipocytes, endothelial cells and blood cells. Total cells represent all the cells included in a field. The percentage of labeled cells to all the cells within each cell type was calculated as proliferation or apoptotic rate.

Statistical Analysis

Pen THI, water intake and biopsy dates were calculated using PROC UNIVARIATE of SAS 9.2 (SAS Institute, Cary, NC). The means ± standard error or the mean (**SEM**) of biopsy dates and the means ± standard deviation (**SD**) of THI and water intake are reported. Parity, dry period length, gestation length and calf weight were analyzed using PROC GLM of SAS 9.2 and least squares means ± SEM are presented. The repeated measurement procedure of PROC MIXED of SAS 9.2 was used to analyze milk production, milk composition, rectal temperature, respiration rate, cumulative BW change, cumulative BCS change, DMI and PRL concentration and the least squares means ± SEM are reported; the statistical model included treatment, time and treatment by time with cow(treatment) as random effect. In addition, data from cumulative BW change, cumulative BCS change and DMI was split into prepartum and postpartum periods (i.e. treatment and post-treatment) and analyzed separately. To avoid the variation of BW on DMI, the BW at dry off and calving were included in the SAS model as covariates to analyze the DMI during the prepartum and postpartum periods, respectively. For circulating PRL analysis, the samples taken at dry off were considered as covariates and included in the SAS model. Data from immunohistochemistry assays were analyzed by PROC GLIMMIX of SAS 9.2 using a generalized linear mixed model based on the binomial distribution with a logit link function and least squares means ± SEM are reported. For mammary cell proliferation and apoptotic rate, the value at dry off were considered as baseline and included in the SAS model as covariates. Since the effect of the covariate was not significant ($P > 0.25$), the covariate was removed from the SAS model in the mammary apoptotic rate analysis.

Results

Dry Period Length, THI, Rectal Temperature, Respiration Rate and Calf Weight

Pens for HT and CL cows had similar THI (76.6, SD = 4.9) during the dry period, which was expected because the cooling system in the current experiment was designed to cool the animals and not the environment. During the dry period, cows exposed to cooling had lower ($P < 0.001$) rectal temperatures in the morning (Table 2-2) and afternoon (Table 2-2) and a lower ($P < 0.001$) respiration rate (Table 2-2) relative to cows in heat stress. Compared with CL cows, HT cows tended to have a shorter ($P < 0.15$) dry period and gestation length (Table 2-2). In addition, the calves from CL cows were heavier ($P = 0.01$) than those from HT cows at birth (Table 2-2).

Production Measurements, BW, BCS, DMI and PRL

Heat stress during the dry period decreased ($P < 0.03$) milk production compared with cooling (Figure 2-1). From 0 to 40 weeks postpartum, CL cows produced 5 kg/d more milk relative to HT cows. There were no treatment effects on milk fat and lactose concentration (Table 2-3) until 40 weeks postpartum, and, relative to HT cows, CL cows produced more ($P = 0.02$) milk lactose (Table 2-3) and tended ($P = 0.06$) to produce more milk fat (Table 2-3) as expected with the higher milk yield. Both groups had similar milk protein yield (Table 2-3), even though CL cows have lower ($P = 0.01$) milk protein concentration (Table 2-3) compared with HT cows. Interestingly, CL cows tended to have lower ($P = 0.10$) SCS compared with HT cows (Table 2-3).

Heat stress during the dry period decreased ($P < 0.02$) DMI of the cows prepartum (Figure 2-2) compared with cooling, but not postpartum (Figure 2-2). In addition, a tendency ($P < 0.08$) for a treatment by time interaction was observed (Figure 2-2), such that CL cows tended to eat more as the lactation advanced. During the dry period, HT cows consumed more water compared with CL cows (48.5 vs. 30.2 L/d/cow, SD = 10.1; respectively). During the prepartum period,

HT cows gained less ($P = 0.05$) weight than CL cows (Table 2-3), but losses were not different after calving (Table 2-3). Both groups had similar BCS change in the dry period (Table 2-3) and postpartum period (Table 2-3). Compared with cows in CL, cows exposed to HT had increased ($P < 0.01$) circulating PRL during the dry period (Figure 2-3).

Mammary Cell Proliferation and Apoptosis

Compared with cooling, heat stress tended ($P < 0.10$) to decrease mammary epithelial cell proliferation rate (Figure 2-4) but did not affect mammary stromal cell proliferation rate (Figure 2-4), as measured by Ki67 antigen labeling. A tendency ($P = 0.13$) of treatment by day interaction for mammary total cell proliferation rate (Figure 2-4) was observed, such that HT cows had lower ($P = 0.06$) total mammary cell proliferation rate at -20 d relative to calving compared with CL (Figure 2-4). In addition, relative to the postpartum period, mammary epithelial, stromal and total cells had higher ($P < 0.01$) proliferation rate at -20 d relative to calving (Figure 2-4).

Heat stress during the dry period did not affect mammary epithelial, stromal and total cell rate of apoptosis (Figure 2-5) compared with heat stress abatement. However, mammary epithelial and total cells had a higher ($P < 0.01$) rate of apoptosis at 2 d relative to calving (Figure 2-5), but no time effect was observed for mammary stromal cells (Figure 2-5).

Discussion

Observation that THI in the barn where both treatments were housed exceeded 72 indicates that cows were exposed to heat stress (Armstrong, 1994). In addition, decreased rectal temperatures and reduced respiration rate in CL cows suggests that the cooling system in the current experiment effectively alleviated the heat strain on CL cows compared with HT cows. Moreover, lower calf weight and decreased BW during the dry period of HT cows provide further evidence that heat stress was present but abated in CL cows relative to HT cows. Similar

results have been reported in these indices previously under a variety of housing conditions (Adin et al., 2009; Collier et al., 1982a; Wolfenson et al., 1988).

Cows exposed to cooling during the dry period produced more milk in the subsequent lactation compared with heat stressed cows in the postpartum period, consistent with the results from other studies (Avendaño-Reyes et al., 2006; Adin et al., 2009; do Amaral et al., 2009). Similar to other reports (Avendaño-Reyes et al., 2006; do Amaral et al., 2009), milk fat yield was greater in the CL cows compared with HT cows. Yet with regard to milk fat concentration, published results are not consistent. For example, do Amaral et al. (2009, 2011) reported that heat stress during the dry period decreased milk fat concentration in the subsequent lactation, but, Adin et al. (2009) did not observe any effect of prepartum cooling. In the current study, heat stress during the dry period did not affect the milk fat concentration in the subsequent lactation. The decrease in milk protein concentration of CL cows was not expected and in contrast with other studies (Adin et al., 2009; do Amaral et al., 2009, 2011). The biological significance of this decrease is unknown, but because of the potential economic impact to the producer it is of interest for further investigation.

Similar to other reports in the dry period (do Amaral et al., 2009) and lactation (Rhoads et al., 2009), HT cows consumed less dry matter compared with CL cows during the dry period. Even though there was no treatment effect on DMI during the postpartum period, a tendency of treatment by time interaction was observed, which indicates that CL cows have higher DMI after 6 weeks postpartum compared with HT cows. That may reflect the higher nutrient demand of CL cows relative to HT cows due to greater milk yield between treatments after the peak of lactation. During the dry period, CL cows gained more weight compared with HT cows, which is likely a result of higher DMI in CL cows. Because no difference of BCS change was observed

during the prepartum period, the increased BW of CL cows during the dry period may result from higher fetal growth. There were no significant differences between treatments for BW change and BCS change during the postpartum period, which suggests that both groups of cows had similar adipose tissue mobilization in the early lactation. This result is contrast with those of do Amaral et al. (2009) who reported that cows exposed to cooling during the dry period had greater NEFA concentrations in the early lactation relative to cows exposed to heat stress. However, the relatively small number of animals in the current experiment may have resulted in a lack of sensitivity to detect differences of BW and BCS change in the early lactation.

Mammary development during the dry period is characterized by increased DNA content (Tucker, 1987) and high cell proliferation (Capuco et al., 1997; Sorensen et al., 2006), which are consistent with the current study in which the mammary cell proliferation rate during the dry period (-20 d relative to calving) was higher than early lactation (2 and 20 d relative to calving). In addition, the CL cows had higher mammary epithelial cell proliferation rate during the transition period and had higher total cell proliferation rate at -20 d relative to calving relative to HT cows, although no difference was observed for mammary stromal cells. Adin et al. (2009) concluded that cows cooled during the dry period had higher mammary cell proliferation during the close-up period compared with non-cooled cows based on higher gene expression of hormone sensitive lipase in the mammary gland. However, this conclusion is relatively arbitrary because during the last week of pregnancy there are already increased mammary secretory cell numbers (Capuco et al., 1997) and different differentiation states between two treatments may also account for any difference of gene expression in lipid synthetic pathways. Our observation provides direct evidence that heat stress during the dry period depresses proliferation, and thus milk yield capacity.

The mechanism that underlies the reduction in mammary cell proliferation during heat stress in the dry period is still unclear. One possibility is disturbed PRL signaling under heat stress, given the critical role of PRL in mammogenesis and lactogenesis in cattle as in other mammals (Tucker, 2000). Effects of PRL are mediated through PRLR, which belongs to the cytokine receptor superfamily, and *PRLR* is expressed in multiple tissues including the mammary gland (Bole-Feysot et al., 1998; Chilton and Hewetson, 2005). There is an inverse relationship between circulating PRL and *PRLR* expression such that environmentally induced increases in circulating PRL depress *PRLR* expression in multiple tissues. For example, exposure to LDPP increases circulating PRL but *PRLR* expression in mammary gland, liver and lymphocytes is concurrently decreased (Auchtung et al., 2003). Further, the enhanced PRL signaling in the mammary gland of cows exposed to SDPP during the dry period results in increased mammary gland development in the dry period (Wall et al., 2005) and improved lactation performance in the next lactation (Auchtung et al., 2005) compared with cows exposed to LDPP. Heat stress also increases circulating PRL concentrations (Collier et al., 1982b). Moreover, the inverse relationship between blood PRL concentration and *PRLR* gene expression in multiple tissues observed under variable photoperiods (Dahl, 2008) also exists in thermally challenged cattle, at least during the dry period. Indeed, do Amaral et al. (2009, 2010) demonstrated that cows exposed to heat stress in the dry period had higher blood PRL concentration and lower *PRLR* gene expression in the liver and immune cells compared with cows receiving heat stress abatement. So it is logical to extend this inverse relationship to the mammary gland. In the current study, heat stress also increased circulating PRL compared with cooling, as shown by others (do Amaral et al., 2009, 2010; Collier et al., 1982b).

Mammary epithelial cells and total cells tended to undergo higher apoptosis at 2 d compared with 20 d relative to calving and prepartum period. The higher mammary epithelial cell apoptosis in the early lactation is consistent with previous reports (Capuco et al., 2001; Sorensen et al., 2006). However, the mechanism of increased programmed cell death in the early postpartum period is still not well understood. Possible mechanisms include greater leukocyte apoptosis and tissue edema (Capuco et al., 2001) after calving and further elimination of senescent cells from last lactation or undifferentiated cells from dry period (Sorensen et al., 2006). No treatment effect was observed for mammary epithelial, stromal, and total cell rate of apoptosis. Wall et al. (2005) reported that SDPP during the dry period tended to decrease mammary epithelial cell apoptosis compared with LDPP, which differs from our findings, even though the effects of photoperiod and heat stress during the dry period are both thought to be mediated by PRL signaling (Dahl, 2008). In addition to the pro-proliferative effect, PRL elicits anti-apoptotic effect in mammary cells of mice (Flint et al., 2001) and cattle (Accorsi et al., 2002). However, other hormonal responses involved in heat stress may also account for modulation of mammary cell apoptosis. For example, progesterone (Collier et al., 1982a; Roman-Ponce et al., 1981) and glucocorticoid (Alvarez and Johnson, 1973; Roman-Ponce et al., 1981; Wise et al., 1988) concentrations increase with heat stress in cattle, and both hormones prevent murine mammary gland involution by decreasing mammary epithelial cell apoptosis (Feng et al., 1995). Possibly, the anti-apoptotic effect of elevated progesterone and cortisol concentrations offset the pro-apoptotic effect result from decreased PRL signaling in HT cows during the dry period compared with CL cows.

Although the different response of PRL signaling in the mammary gland may be a reasonable mechanism for the observed effects on mammary cell response in the present study,

we cannot exclude other possibilities. Indeed, heat stress during the late gestation also increases circulating progesterone and decreases the blood concentration of placenta hormone estrone sulfate relative to cooling (Collier et al., 1982a). It is well known that estrogen is involved in the initiation of lactation and progesterone has the negative effect on lactogenesis (Tucker, 2000), so the increased progesterone and decreased estrone sulfate in heat stressed cows may suppress the mammary epithelial cell differentiation during the lactogenesis which in turn results in the decreased milk production in the postpartum period. However, Collier et al. (1982a) did not observe the significant differences in milk production between the cooled and non-cooled cows during the late gestation, so the altered steroid hormones and compromised placental function may not be enough to explain the significant decrease in lactation performance of HT cows in current study. The other reason for decreased milk production of HT cows may be the shorten gestation length and dry period. It is possible that HT cows missed the critical period for the mammary gland development during the dry period. However, even though 3 - 6 days shorter gestation length by induced parturition decreases the milk production in the early lactation (Beardsley et al., 1976; Bremmer et al., 1999), calving induction doesn't affect the milk yield for the whole lactation (Bremmer et al., 1999, Schmitt et al., 1975).

Manipulation of mammary cell number and secretory capacity can affect lactation performance (Capuco et al., 2003). The relative difference between rates of mammary cell proliferation and apoptosis determine the mammary cell number and thus cell secretory ability can be increased by enhanced cell renewal (Capuco et al., 2001). Because the dry period has more extensive mammary growth and cell turnover compared with lactation (Capuco et al., 1997; Capuco et al., 2001; Sorensen et al., 2006), this non-lactating period provides an opportune window to manipulate mammary gland development. In the present study, heat stress during the

dry period did not affect mammary cell apoptosis, but the higher rate of mammary epithelial cell proliferation of CL cows during the transition period indicates that CL cows have greater mammary growth when dry and enter the next lactation with more mammary epithelial cells relative to HT cows. A greater number of mammary secretory cells in CL cows during lactation are consistent with the higher milk yield of CL cows relative to HT cows. Also, the lactation curves of CL cows and HT cows were separated throughout lactation, which provides further evidence that heat stress during the dry period compromises the lactation performance in the subsequent lactation through a depression of mammary gland functional capacity.

Conclusions

Heat stress during the dry period decreased milk production in the subsequent lactation. Compared with CL cows, HT cows had lower mammary epithelial cell proliferation during the transition period. But there were no differences in mammary stromal cell proliferation and mammary cell apoptosis in the dry period. Therefore, the compromised mammary gland development induced by heat stress in the dry period decreases lactation performance in the next lactation by decreasing mammary cell proliferation.

Table 2-1. Ingredient composition of total mixed ration for cows in both prepartum and postpartum period

Ingredient (% of DM)	Prepartum	Postpartum
Corn silage	37	40.93
Sorghum silage	25	---
Alfalfa hay	---	8.05
Wet brewers grain	---	5.56
Distillers grain	5	6.98
Corn meal	11	18.28
Soybean meal	11	5.26
Citrus pulp	5	7.18
Soy plus	---	4.02
Mineral and vitamin mix[1]	6	3.73

[1]Mineral and vitamin mix prepartum included 21% CP, 2% crude fat, 13% crude fiber, 8.5% Ca, 1% P, 3% NaCl, 3.5% Mg, 4.4% S, 34 mg/kg Co, 129 mg/kg Cu, 15mg/kg I, 300 mg/kg Mn, 7 mg/kg Se, 435 mg/kg Zn, 200 mg/kg F, 220459 IU vitamin A/kg, 70000 IU vitamin D_3/kg, 5512 IU vitamin E/kg. Mineral and vitamin mix postpartum included 25% CP, 0.25% crude fat, 1% crude fiber, 3% ADF, 5.75% Ca, 1.2% P, 4.75% NaCl, 9.25 mg/kg Se, 110230 IU vitamin A/kg, 39683 IU vitamin D_3/kg, 1102 IU vitamin E/kg, 381 mg/kg Monensin.

Table 2-2. Dry period length, gestation length, rectal temperatures, respiration rate, calf weight of cows exposed to either heat stress (n = 15) or cooling (n = 14) during the dry period

Variable	Heat stress	Cooling	SEM	*P*-value
Dry period length (d)	38.93	42.21	1.47	0.13
Gestation length (d)	274.1	277.4	1.34	0.10
Rectal temperature AM (°C)	38.81	38.60	0.03	< 0.001
Rectal temperature PM (°C)	39.40	39.04	0.04	< 0.001
Respiration rate (breath/min)	78.36	45.59	2.14	< 0.001
Calf weight (kg)	41.63	46.45	1.33	0.01

Table 2-3. Milk composition, BW, BCS and DMI of cows exposed to heat stress (n = 15) or cooling (n = 14) during the dry period

Variable	Heat stress	Cooling	SEM	*P*-value
Milk fat (%)	3.58	3.52	0.07	0.57
Milk protein (%)	3.01	2.87	0.06	0.01
Milk lactose (%)	4.63	4.67	0.03	0.28
Milk fat yield (kg/d)	1.02	1.16	0.05	0.06
Milk protein yield (kg/d)	0.87	0.96	0.05	0.17
Milk lactose yield (kg/d)	1.36	1.59	0.08	0.02
Milk SCS	3.35	2.94	0.18	0.10
BW change (Prepartum, kg)[1]	-15.3	-1.9	4.60	0.05
BW change (Postpartum, kg)[2]	-37.7	-53.4	10.2	0.29
BCS change (Prepartum)[3]	-0.06	0.00	0.05	0.40
BCS change (Postpartum)[4]	-0.3	-0.5	0.10	0.15

[1]Prepartum accumulative BW change was calculated by subtracting data at -32, -18 d relative to calving and calving by data at dry off.
[2]Postpartum accumulative BW change was calculated by subtracting data at 14, 28 and 42 d relative to calving by data at calving.
[3]Prepartum accumulative BCS change was calculated by subtracting data at -32, -18 d relative to calving and calving by data at dry off.
[4]Postpartum accumulative BCS change was calculated by subtracting data at 14, 28 and 42 d relative to calving by data at calving.

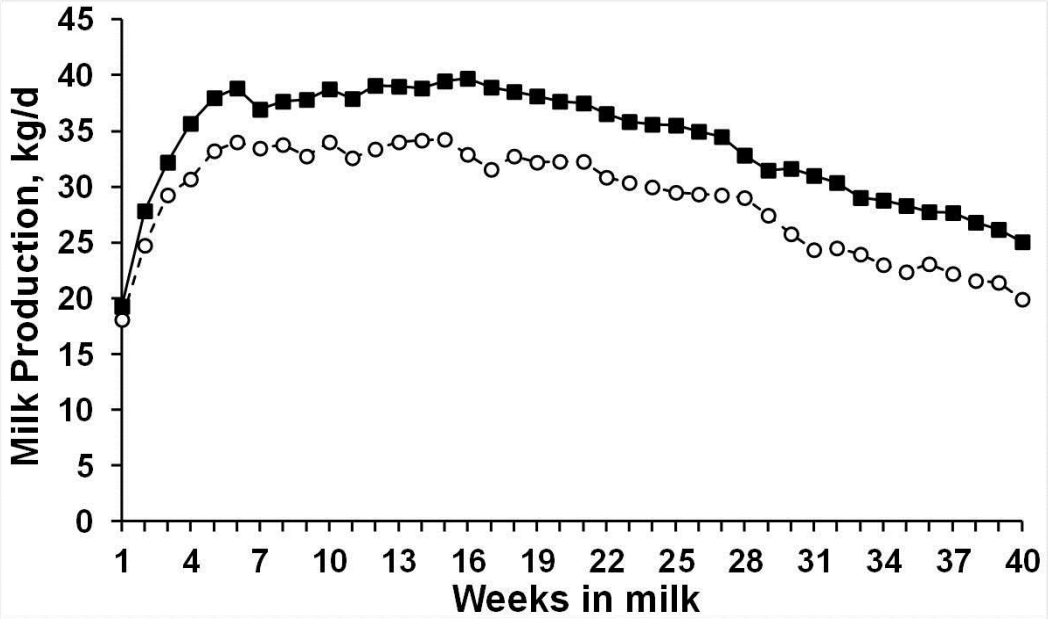

Figure 2-1. Effect of heat stress (n = 15) and cooling (n = 14) during the dry period on milk production in the subsequent lactation. Solid squares (■) and open circles (○) represent cooling and heat stress, respectively. All cows were managed and housed as one group following parturition. Cows exposed to cooling during the dry period produced more milk compared with cows in heat stress up to 40 weeks in milk (33.9 ± 1.6 vs. 28.9 ± 1.5 kg/d, respectively; $P < 0.03$).

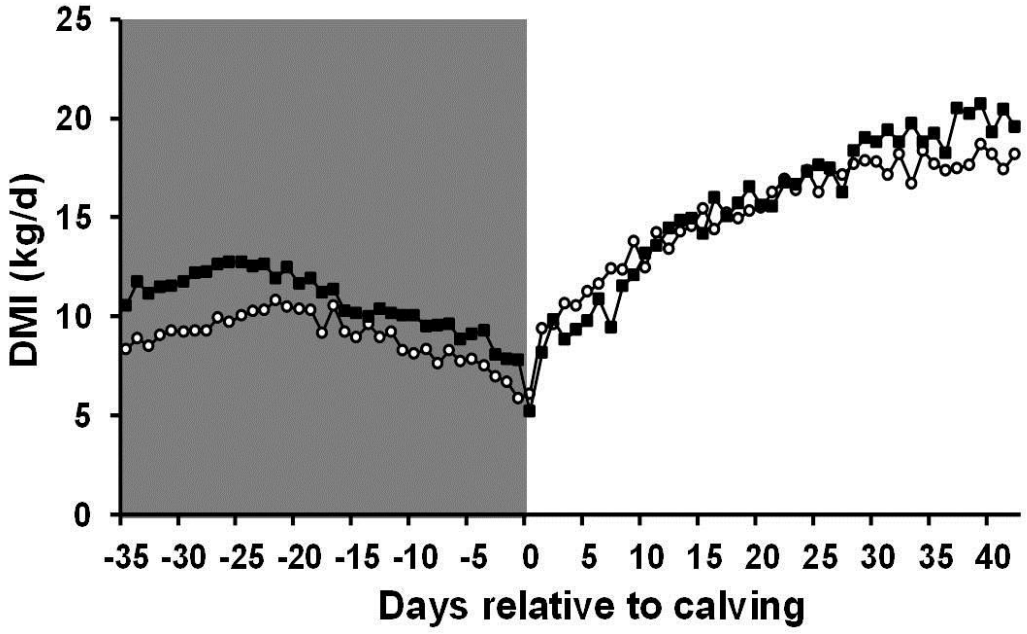

Figure 2-2. Effect of heat stress (HT, n = 15) and cooling (CL, n = 14) during the dry period on the DMI from -35 to 42 d relative to calving. Solid squares (■) and open circles (○) represent CL and HT respectively. Data were split into prepartum and postpartum periods and analyzed separately. BW at dry off and at calving were included in the model as covariate during the prepartum and postpartum period respectively. CL cows consumed more DMI during the prepartum period compared with HT cows (10.6 ± 0.5 vs. 8.9 ± 0.5 kg/d, respectively; $P < 0.02$). During the postpartum period, there was no difference between CL cows and HT cows (15.8 ± 0.7 vs. 15.4 ± 0.7 kg/d, respectively; $P = 0.7$), however, a tendency of treatment by time difference was observed ($P < 0.08$).

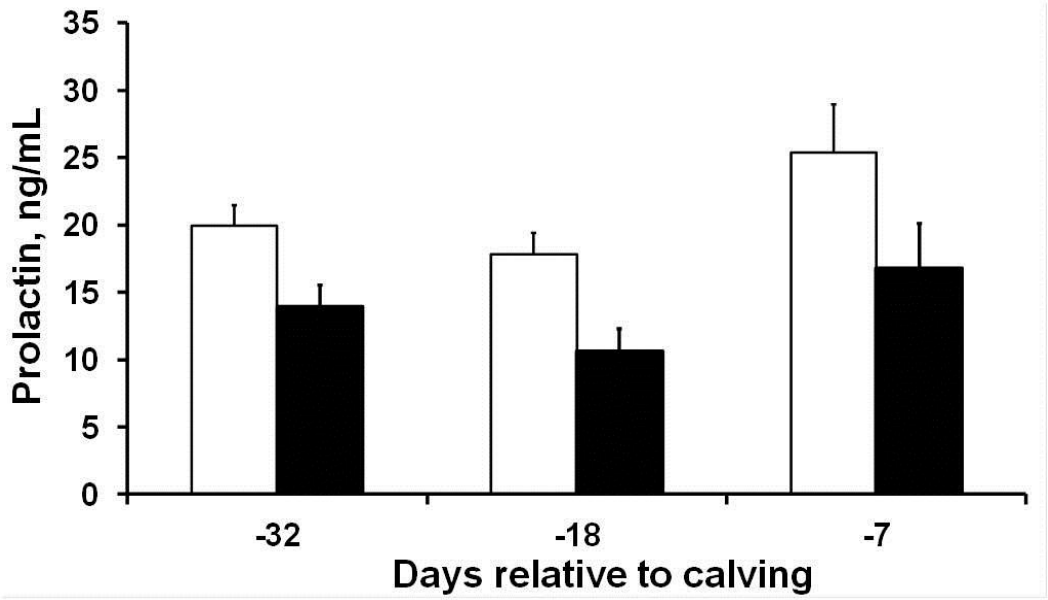

Figure 2-3. Effect of heat stress (n = 15) and cooling (n = 14) during the dry period on the prolactin concentration of plasma. Solid bars represent cows exposed to cooling and open bars represent cows in heat stress. Prolactin concentrations of plasma at dry off were included in the SAS model as covariates. Heat stressed cows had increased prolactin concentration of plasma compared with cooled cows (21.04 ± 1.6 vs. 13.78 ± 1.5 ng/mL, respectively; $P < 0.01$).

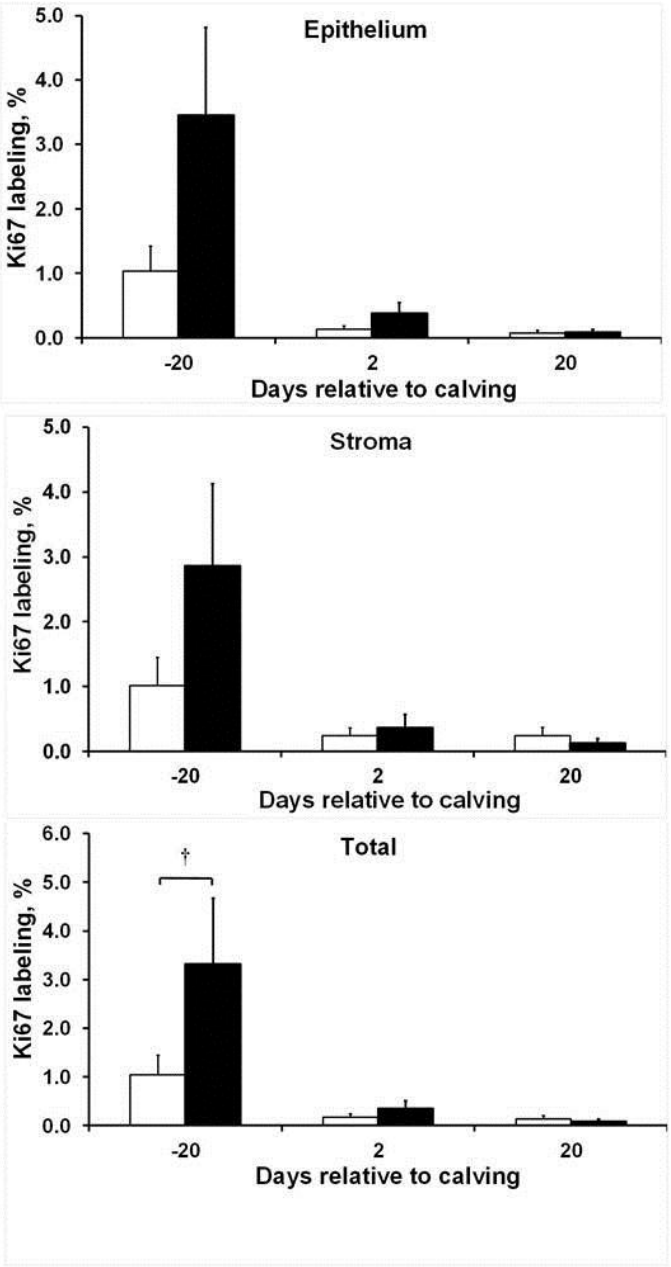

Figure 2-4. Effect of heat stress (n = 7) and cooling (n = 7) during the dry period on mammary cell proliferation rate. Solid bars represent cows exposed to cooling and open bars represent cows in heat stress. Mammary cell proliferation rate at dry off was considered as baseline and included in the SAS model as covariate. There was a tendency of treatment effect for epithelial cells ($P < 0.10$), and no treatment effects were observed for stromal and total cells ($P = 0.56$ and $P = 0.31$ respectively). A tendency of treatment by day interaction for total cells ($P = 0.13$) was observed. Time effects were observed for epithelial, stromal and total cells ($P < 0.01$, $P < 0.01$ and $P < 0.01$ respectively). †$P = 0.06$.

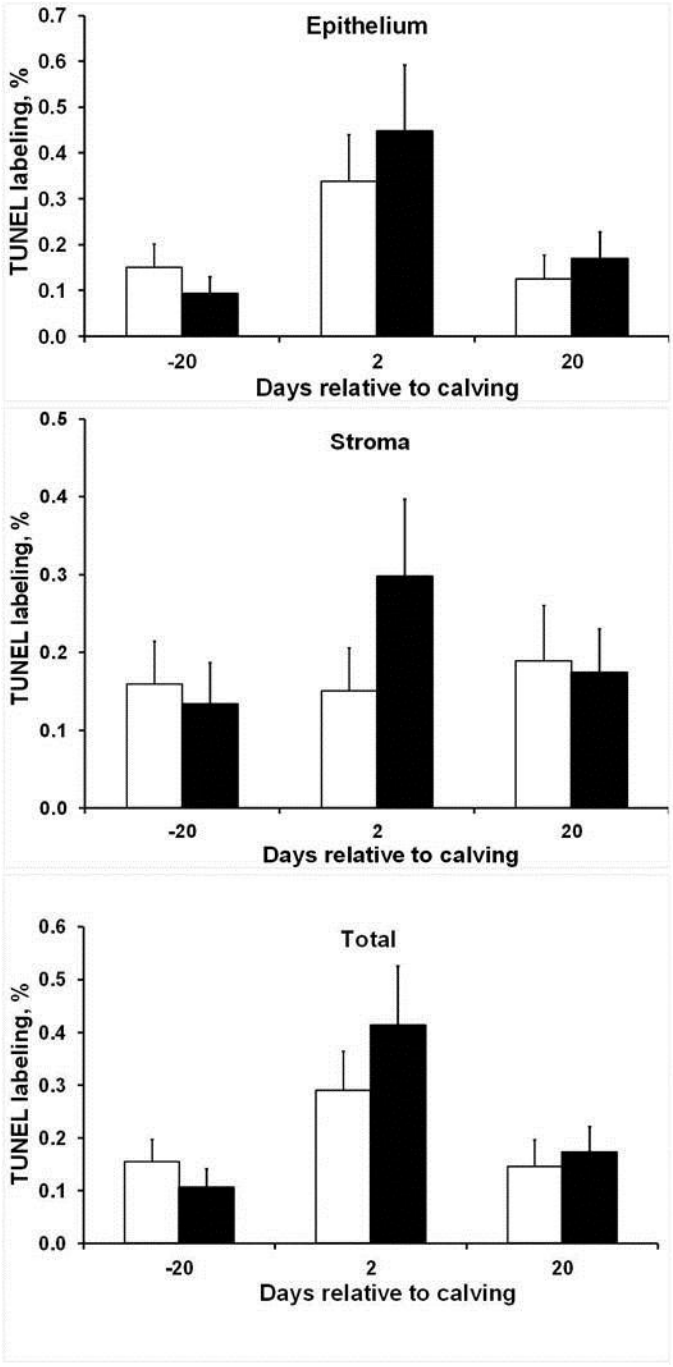

Figure 2-5. Effect of heat stress (n = 7) and cooling (n = 7) during the dry period on mammary cell apoptotic rate determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). There were no treatment effects of epithelial, stromal and total cells ($P = 0.9$, $P = 0.64$ and $P = 0.83$, respectively). Time effects were observed for both epithelial and total cell apoptotic rate ($P < 0.01$ and $P < 0.01$ respectively).

CHAPTER 3
EFFECT OF HEAT STRESS DURING THE DRY PERIOD ON GENE EXPRESSION OF MAMMARY TISSUE AND LYMPHOCYTES

Abstract

Heat stress (**HT**) during the dry period compromises mammary gland development, decreases future milk production, and impairs immune status of dairy cows. Our objective was to evaluate the effect of cooling HT cows during the dry period on gene expression of the mammary gland and lymphocytes. Cows were dried off 46 d before their expected calving and assigned to one of two treatments, HT or CL. Cows in the CL group were cooled with sprinklers and fans whereas HT cows were not. After parturition, all cows were housed in a free-stall barn with cooling. Lymphocytes were isolated at dry-off, -20, 2, and 20 d relative to calving from a subset of cows of Chapter 2 (HT, n = 9; CL, n = 10) and mammary biopsies were taken at the same intervals (HT, n = 7; CL, n = 6) for RNA extraction. Gene expression was assessed using a custom multiplex gene expression assay based on reverse transcription-PCR. Genes involved in PRL signaling (PRL receptor long form [*PRLR-L*], PRLR short form [*PRLR-S*], suppressor of cytokine signaling 2 [*SOCS2*], *SOCS3*, *IGF2*, *IGFBP5*, and *cyclin D1* [*CCND1*]), fatty acid (FA) metabolism (acetyl-CoA carboxylase alpha [*ACACA*] and lipoprotein lipase [*LPL*]), and *IGF1* were evaluated in mammary tissue, and genes related to FA metabolism (*ACACA*, *FA synthase* [*FASN*], and *LPL*), cytokine production (*IL6*, *IL8*, and *tumor necrosis factor* [*TNF*]), and *IGF1* were evaluated in lymphocytes. No differences ($P > 0.05$) were observed in PRL signaling or FA metabolism gene expression in the mammary gland. In lymphocytes, HT cows had greater ($P \leq 0.05$) *IGF1* and *TNF* mRNA expression during the transition period and up-regulated *IL8* and down-regulated *FASN* mRNA expression at 2 d relative to calving relative to CL. We conclude that cooling HT cows during the dry period alters expression of genes involved in cytokine production and lipid metabolism in lymphocytes.

Introduction

Environmental modification during the dry period, such as heat stress and photoperiod, exert dramatic effects on production of dairy cows. For example, compared to LDPP, exposure to SDPP up-regulates mammary gland remodeling before parturition (Wall et al., 2005) and increases milk yield of cows in the next lactation (Auchtung et al., 2005). HT abatement also enhances mammary growth during the dry period and improves lactation performance after parturition (Chapter 2). PRL plays important roles in bovine lactogenesis and mammogenesis (Tucker, 2000) and there is evidence that the altered PRL signaling mediates photoperiodic effects on the mammary gland development during the dry period. Specifically, SDPP decreases blood PRL concentration but increases gene expression of *PRLR* in the mammary tissue relative to cows exposed to LDPP (Auchtung et al., 2005). The cellular mechanism for compromised mammary gland development by heat stress during the dry period is still unknown but has been proposed to be associated with modified PRL signaling (Chapter 2). However, data related to gene expression in the PRL signaling of mammary gland in HT or CL dry cows are still not available. In addition, the effect of cooling heat-stressed dry cows on gene expression involved in fatty acid (**FA**) metabolism in the mammary gland has yet to be evaluated.

Heat stress also influences an animal's immune function during the dry period. Cooling cows during the dry period results in change in lymphocyte function during the transition period such that enhanced lymphocyte proliferation and TNF-α production in response to mitogen in vitro (do Amaral et al., 2010). However, the effect of heat stress during the dry period on endogenous inflammatory cytokine gene expression of lymphocytes during the transition period has never been evaluated. In addition, gene expression data for *IGF-I* and enzymes involved in the FA metabolism of PBMC in HT dry cows are also not available. We hypothesized that HT during the dry period suppresses PRL signaling and FA metabolism in the mammary gland and

alters inflammatory cytokine gene expression of lymphocytes. Therefore, our objective was to evaluate the effect of HT during the dry period on gene expression of the mammary gland and lymphocytes of dairy cows.

Materials and Methods

Cows used in the current study were from a subset of animals in a larger study and details of treatment and management of animals were reported previously (Chapter 2). The University of Florida Institute of Food and Agricultural Sciences Animal Research Committee approved all the procedures used. Briefly, cows were dried off ~46 d before the expected calving date and randomly assigned to 2 treatments, HT or CL. During the dry period, CL cows were cooled with shade, sprinklers, and fans, and HT cows were only provided with shade. After parturition, all cows were managed as 1 group. Mammary biopsies were collected from a subset of animals (HT, n = 7; CL, n = 6) at dry-off, -20, 2, and 20 d relative to calving and PBMC were isolated at the same intervals (HT, n = 9; CL, n = 10). The procedure of mammary biopsy was described previously (Chapter 2) and PBMC isolation was performed based on methods reported by do Amaral et al. (2010).

RNA Extraction and 22-Gene Multiplex Expression Assay

The RNeasey Midi kit (Qiagen, Valencia, CA) was used for total RNA extraction of the mammary tissues and PBMC, following the manufacturer's protocol with on-column DNase treatment. Quality of RNA was assessed using the Agilent 2100 Bioanalyzer with RNA 6000 Nano LabChip kits (Agilent Technologies, Palo Alto, CA) and RNA concentration was determined using the ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Gene expression was evaluated by a custom 22-gene multiplex expression assay (Table 3-1, 3-2) using the GeXP Genetic Analysis System and GeXP Start Kit (Beckman Coulter, Inc., Brea, CA), which is based on reverse transcription-PCR. Assay optimization, generation of standard

curves, and assay procedures were performed based on manufacturer's instructions, and the details were published by Connor et al. (2010). Briefly, total RNA (100 ng) was reverse transcribed with pooled chimeric primers containing a universal sequence attached at the 5′ end and gene-specific antisense sequence for each transcript of interest at the 3′ end. *KanR* RNA (2.5 pg/reaction) provided in the GeXP Start Kit was added as an internal normalization control. Negative control reactions in the absence of reverse transcriptase were also performed on each sample to confirm the absence of contaminating genomic DNA. Reaction conditions were 48°C for 1 min, 42°C for 60 min, 95°C for 5 min, and then holding at 4°C. Reactions for subsequent PCR included 25 mM MgCl2, pooled forward primers, cDNA, PCR buffer, and Thermo-Start Taq DNA polymerase (Thermo Scientific, Waltham, MA), according to manufacturer's instructions and optimized conditions. The PCR reaction conditions were 95°C for 10 min, 94°C for 30 s, 55°C for 30 s, and 70°C for 1 min for 35 cycles. The PCR products were diluted in sample loading solution provided in the GeXP Start Kit plus DNA size standard 400 and analyzed by GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Inc.). Data were exported to the eXpress Profiler Program (Beckman Coulter, Inc.) and calculated as area under the curve and normalized to *KanR* RNA. The final data were normalized to the most stably expressed reference genes as determined by the GeNorm program (http://medgen.ugent.be/~jvdesomp/genorm/) using the GeneQuant Tool (Beckman Coulter, Inc.) and reported as the value relative to the geometric mean expression of the reference genes. For lymphocyte RNA, gene expression was normalized to *beta-2 microglobulin and GAPDH* (GeNorm M-value = 0.33) and for mammary RNA, expression was normalized to *ATP synthase*, *beta-2 microglobulin*, and *hypoxanthine phosphoribosyltransferase 1* (M-value < 0.70).

Statistical Analysis

Gene expression data were analyzed using repeated measures of PROC MIXED of SAS 9.2 (SAS Institute, Cary, NC) and the LSM ± SEM are presented. The statistical model included treatment, time, and treatment by time with cow within treatment as random effect. The data from the samples taken at dry-off were considered as covariates and included in the SAS model. If a treatment by time effect was observed, the treatment effect at individual time point was obtained by the SLICE function in SAS models.

Results

The details of the physiological responses of the cows within the experiment were reported in Chapter 2. Briefly, compared with CL cows, HT cows had elevated body temperatures during the dry period, averaging 38.98 and 39.34 ^{o}C, respectively. Relative to CL cows, HT cows had lower mammary epithelial cell proliferation rate at -20 d relative to calving but not at other time points. There were no differences between treatments for the mammary cell apoptotic rate at any time point in the transition period. Heat stress during the dry period also increased the PRL concentration of the blood compared with cooling.

In mammary tissue, no treatment or treatment by time effects were observed for most of the mRNA expression of genes examined (Table 3-3). There was a trend for increased ($P = 0.15$) *IGFBP5* gene expression in HT cows during the transition period compared with CL cows. Across treatments, gene expression of *PRLR long* (*PRLR-L*) and *short forms* (*PRLR-S*), *SOCS2*, *SOCS3*, *ACACA*, and *LPL* at -20 d relative to calving was less ($P \leq 0.05$) than that in early lactation (2 and 20 d relative to calving). However, *CCND1* and *IGF1* mRNA expression at d -20 was greater (time effect: $P < 0.01$) compared with that in lactation. In lymphocytes (Table 3-3), HT cows had increased ($P \leq 0.05$) *IGF1* and *TNF* mRNA expression during the transition period

and up-regulated ($P < 0.05$) *IL8* and down-regulated ($P = 0.01$) *FASN* mRNA expression at 2 d relative to calving compared with CL.

Discussion

Regardless of treatment, increased *PRLR* gene expression in the mammary gland from the dry period into early lactation probably reflected increased epithelial cell secretory activity in lactation because of the important role of PRL in mammary cell metabolism (Akers et al., 1981). Following increased gene expression of *PRLR*, expression of *SOCS2* and *SOCS3* mRNA may be up-regulated in early lactation to act as feedback inhibitors of PRL signaling (Krebs and Hilton, 2001). It is suggested that IGF-II and IGFBP-5 are downstream effectors in the PRL signaling of the mammary gland (Accorsi et al., 2002; Brisken et al., 2002); however, patterns of *IGF2* and *IGFBP5* mRNA expression during the transition period in the current study did not follow that of PRLR. Relative to tightly-controlled, transgenic or knockout mouse mammary epithelial cell models (Brisken et al., 2002) or in vitro bovine mammary tissue explants (Accorsi et al., 2002), gene expression data of *IGF2* and *IGFBP5* observed in the current study may reflect the complexity of the endocrine events occurring around parturition. The elevated rate of mammary cell proliferation in the dry period (Capuco et al., 1997) is reflected by the greater gene expression in the current study of *CCND1*, which is the key regulator of G1/S transition during the cell cycle, and *IGF1*, which is the local modulator of mammary growth (Akers, 2006) during the dry period.

In contrast to our hypothesis, no treatment or treatment by time effects were observed for most of the genes examined in the mammary gland. Based on these trials, it seems that the enhanced mammary growth by cooling HT dry cows (Chapter 2) is not associated with PRL signaling at the level of the individual mammary epithelial cell, as no differences in mRNA expression of genes related to PRL signaling were observed between treatments. Heat stress is

related to decreased blood PRL concentrations in dry cows (do Amaral et al., 2010; Chapter 2) and the inverse relationship between circulating PRL and gene expression of *PRLR* in the mammary gland of cows exposed to different photoperiods (Dahl et al., 2012) led us to hypothesize that enhanced PRL signaling mediates up-regulated mammary growth observed during the dry period in response to HT abatement. Additionally, increased blood estrone-sulfate and decreased progesterone concentrations in CL cows compared with HT cows (Collier et al., 1982) should enhanced PRL signaling in the mammary gland (Tucker, 2000). Data in the current study indicate that heat stress may disrupt normal connections among the endocrine regulators in the mammary gland. Mammary cell apoptosis is influenced in part by IGFBP-5 and the addition of PRL negatively affects gene expression of *IGFBP-5* in bovine mammary tissue explants in vitro (Accorsi et al., 2002). Thus, the increase in PRL concentration (Chapter 2) of HT cows is inconsistent with the expected mechanism for the slightly increased *IGFBP-5* gene expression observed in HT cows the current study. Therefore, the mechanism of the compromised mammary growth by HT during the dry period is still unknown and deserves further investigation. Additionally, similar gene expression of *ACACA* and *LPL* between treatments indicates that mammary cells of both HT and CL cows may have a similar secretory potential, it is just that CL cows have greater mammary growth in the dry period (Chapter 2) and thus greater numbers of secretory cells and productive capacity.

To my knowledge, this is the first study to report the effects of HT during the dry period on inflammatory cytokine gene expression in lymphocytes. Relative to CL cows, HT cows had increased *TNF* mRNA expression during the transition period. Tumor necrosis factor-α is a potent stimulator of IL8 gene expression (Hoffman et al., 2002). Therefore, the increased *TNF* mRNA expression may provide explanation of up-regulated *IL8* mRNA expression in early

lactation of HT cows compared with CL cows. Under stressed conditions, cattle have an increased acute-phase response (Lomborg et al., 2008), which is mediated by enhanced inflammatory cytokine production (Petersen et al., 2004). Thus, the greater PBMC inflammatory cytokine mRNA expression of HT cows may reflect an adaptive response to environmental HT compared with CL cows. Additionally, the increased *IGF-I* mRNA expression in the PBMC of HT cows may enhance gene expression of inflammatory cytokines (Renier et al., 1996) through a paracrine or autocrine pathway. In vitro responses of PBMC isolated from HT cows to mitogen included a reduced proliferation rate and reduced TNF-α production during the transition period compared with CL cows (do Amaral et al., 2010). Combined with gene expression results, the impaired in vitro immune responses of HT cows probably indicate that, despite greater endogenous PBMC inflammatory cytokine mRNA expression, HT cows actually may have compromised immunological responses when encountering a pathogen compared with CL cows. The physiological explanation for increased *FASN* mRNA expression in CL cows relative to HT cows is unknown, but may be related to different immunological responses, as FA composition in immune cells dramatically influences their function (Calder, 2008).

Conclusions

In conclusion, cooling HT dry cows does not appear to affect mammary cell FA synthesis capacity during the transition period. The enhanced mammary growth observed through HT abatement before parturition does not appear to be mediated by PRL signaling in the mammary gland. Additionally, relative to CL cows, HT cows have increased PBMC inflammatory cytokine gene expression, which may be due to the effects of IGF-I.

Table 3-1. Genes evaluated using a multiplex gene expression assay, their amplicon sizes and standard curve fit (R^2)

Gene symbol	Gene name	Amplicon size (bp)	Linearity of standard curve
ACACA	Acetyl-CoA carboxylase alpha	200	> 0.993
ATP5B	ATP synthase	221	≥ 0.998
B2M	Beta-2 microglobulin	193	> 0.999
CCND1	Cyclin D1	300	> 0.998
FASN	Fatty acid synthase	340	> 0.997
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	172	> 9.997
HPRT1	Hypoxanthine phosphoribosyltransferase 1	242	> 0.999
IGFBP5	Insulin-like growth factor binding protein 5	142	> 0.999
IGF1	Insulin-like growth factor 1	179	≥ 0.999
IGF2	Insulin-like growth factor 2	214	> 0.999
IL6	Interleukin 6	358	≥ 0.995
IL8	Interleukin 8	307	> 0.999
LPL	Lipoprotein lipase	365	> 0.995
PRLR-L	Prolactin receptor, long form	379	> 0.999
PRLR-S	Prolactin receptor, short form	386	≥ 0.995
SOCS2	Suppressor of cytokine signaling 2	260	≥ 0.994
SOCS3	Suppressor of cytokine signaling 3	158	> 0.994
TNF	Tumor necrosis factor	207	> 0.999

Table 3-2. Genes evaluated using a multiplex gene expression assay, their primer sequences and GenBank accession numbers used in primer design

Gene symbol	Primer	Sequence (5' to 3') with universal sequence	GenBank accession no.
ACACA	Forward	AGGTGACACTATAGAATATCTCCTCCAACCTCAACCAC	NM_174224
	Reverse	GTACGACTCACTATAGGGAAAGCAGCCCATCACTTCATC	
ATP5B	Forward	AGGTGACACTATAGAATACCCTCAAGGAGACCATCAAA	NM_175796
	Reverse	GTACGACTCACTATAGGGAGGACACCATGGAGGATGAGT	
B2M	Forward	AGGTGACACTATAGAATAAGCGTCCTCCAAAGATTCAA	NM_173893
	Reverse	GTACGACTCACTATAGGGAACAGGTCTGACTGCTCCGAT	
CCND1	Forward	AGGTGACACTATAGAATACATGAACTACCTGGACCGCT	NM_001046273
	Reverse	GTACGACTCACTATAGGGAGCATCTTGGAGAGGAAGTGC	
FASN	Forward	AGGTGACACTATAGAATAGTCTTCTCCTCCGTGAGCTG	NM_001012669
	Reverse	GTACGACTCACTATAGGGAACGGGATGTAGCCTTCTCTG	
GAPDH	Forward	AGGTGACACTATAGAATAGATTGTCAGCAATGCCTCCT	NM_001034034
	Reverse	GTACGACTCACTATAGGGACCATCCACAGTCTTCTGGGT	
HPRT1	Forward	AGGTGACACTATAGAATAGCCGACCTGTTGGATTACAT	NM_001034035
	Reverse	GTACGACTCACTATAGGGAGCATTGTCTTCCCAGTGTCA	
IGFBP5	Forward	AGGTGACACTATAGAATAGGTTTGCCTGAACGAAAAGA	NM_001105327
	Reverse	GTACGACTCACTATAGGGAGAGTAGGTCTCCTCTGCCATCT	
IGF1	Forward	AGGTGACACTATAGAATAGTTGGTGGATGCTCTCCAGT	NM_001077828
	Reverse	GTACGACTCACTATAGGGACCTCCTCAGATCACAGCTCC	
IGF2	Forward	AGGTGACACTATAGAATAATCAATTTGCTCCCTACCCC	NM_174087
	Reverse	GTACGACTCACTATAGGGAAGGCTCCACTCTCCACTCAA	
IL6	Forward	AGGTGACACTATAGAATATGCTTGATCAGAACCACTGC	NM_173923
	Reverse	GTACGACTCACTATAGGGACTACCACAATCATGGGAGCC	
IL8	Forward	AGGTGACACTATAGAATATGAATTTGGAGAAATGGGAAA	NM_173925
	Reverse	GTACGACTCACTATAGGGAGGCCAGGCATCTCAAAAGTA	
LPL	Forward	AGGTGACACTATAGAATACGGAGGTGGATATTGGAGAA	NM_001075120
	Reverse	GTACGACTCACTATAGGGAAAGCACTGGGCATCTTTTGT	
PRLR-L	Forward	AGGTGACACTATAGAATAAGCAACTGATTGGGAGACTCA	NM_001039726
	Reverse	GTACGACTCACTATAGGGATCGGACTTGCCCTTCTCC	
PRLR-S	Forward	AGGTGACACTATAGAATAAGCAACTGATTGGGAGACTCA	NM_174155
	Reverse	GTACGACTCACTATAGGGAAAGGCGAGAAGGCTGTGATA	
SOCS2	Forward	AGGTGACACTATAGAATAGAGGCACCAGAAGGAACTTT	NM_177523
	Reverse	GTACGACTCACTATAGGGATCCGCTTATCCTTGCACATC	
SOCS3	Forward	AGGTGACACTATAGAATAGGCCACTCTCCAACATCTCT	NM_174466
	Reverse	GTACGACTCACTATAGGGACTAAAGCGGGGCATCGTACT	
TNF	Forward	AGGTGACACTATAGAATAAACTCTCCCTTCCTGCCAAT	NM_173966
	Reverse	GTACGACTCACTATAGGGAAGGACACCTTGACCTCCTGA	

Table 3-3. Gene expression of the mammary tissue and lymphocyte in cows exposed to either heat stress (HT) or cooling (CL) during the dry period

	-20[1]		2		20			*P*-value		
	HT	CL	HT	CL	HT	CL	SEM	TRT[2]	Day	T × D[3]
Mammary										
PRLR-L	0.94	0.68	1.28	1.05	1.71	1.17	0.26	0.23	< 0.01	0.87
PRLR-S	1.39	1.37	1.87	1.89	2.21	2.00	0.35	0.84	0.09	0.92
SOCS2	0.48	0.47	0.77	0.47	0.70	0.75	0.12	0.48	0.05	0.29
SOCS3	1.17	2.22	3.87	6.49	10.38	9.26	1.94	0.48	< 0.01	0.84
IGF2	0.95	1.23	1.21	1.12	0.99	0.85	0.15	0.93	0.15	0.26
IGFBP5	0.59	0.39	2.17	0.48	0.88	0.64	0.63	0.15	0.35	0.90
CCND1	1.43	1.08	0.50	0.38	0.45	0.40	0.20	0.70	< 0.01	0.88
ACACA	0.15	0.35	0.63	0.99	0.96	0.71	0.18	0.94	< 0.01	0.50
LPL	0.18	0.30	0.64	1.01	1.32	1.23	0.57	0.50	< 0.01	0.97
IGF1	0.63	0.48	0.44	0.36	0.17	0.28	0.16	0.84	< 0.01	0.48
Lymphocyte										
IL6	1.45	1.18	1.40	2.36	2.33	1.05	0.90	0.40	0.97	0.80
IL8	2.83	1.40	4.82	1.57*	2.99	2.88	0.78	0.35	0.11	0.10
TNF	1.16	1.04	1.04	0.31	1.53	1.39	0.41	0.05	0.11	0.20
ACACA	0.70	0.88	0.56	0.71	0.80	0.82	0.12	0.41	< 0.01	0.35
FASN	1.53	2.06	1.07	3.35**	2.05	3.31	0.70	0.07	0.19	0.01
LPL	2.39	0.86	7.70	2.75	5.48	2.95	1.41	0.57	< 0.01	0.34
IGF1	1.33	1.06	1.29	1.09	1.37	1.16	0.09	0.02	0.72	0.89

[1]Days relative to calving.
[2]TRT = treatment.
[3]T × D = treatment by day
*$P < 0.05$, **$P = 0.01$ within a day.

CHAPTER 4
EFFECT OF COOLING HEAT-STRESSED DAIRY COWS DURING THE DRY PERIOD ON INSULIN RESPONSE

Abstract

Heat stress (**HT**) during the dry period affects hepatic gene expression and adipose tissue mobilization during the transition period. In addition, it is postulated that HT may alter insulin action at peripheral tissues. Our objective was to evaluate the effect of cooling heat-stressed cows during the dry period on insulin effects on peripheral tissues during the transition period. Cows were dried off 46 d before expected calving and assigned to one of two treatments: HT (n=16) or cooling (CL, n=16). During the dry period, the average temperature-humidity index was 78, but CL cows were cooled with sprinklers and fans whereas HT cows were not. After calving, all cows were housed and managed under same conditions. Rectal temperatures were measured twice daily (0730 and 1430 h) and respiration rate recorded thrice weekly during the dry period. Dry matter intake was recorded daily from dry-off to 42 d relative to calving (DRC). Body weight and body condition score were measured weekly from dry-off to 42 DRC. Milk yield and composition were recorded daily to 42 wk postpartum. Glucose tolerance tests (GTT) and insulin challenges (IC) were performed at dry-off, -14, 7 and 28 DRC in a subset of cows (HT, n=8; CL, n=8). Relative to HT, CL cows had lower rectal temperatures (39.3 vs. 39.0 ^{o}C) in the afternoon and respiration rate (69 vs. 48 breath/min). CL cows tended to consume more feed than HT cows prepartum and postpartum. Compared with HT, CL cows gained more weight before calving but lost more weight and body condition in early lactation. CL cows produced more milk than HT cows (34.0 vs. 27.7 kg/d), but treatment did not affect milk composition. Treatments did not affect concentration of circulating insulin and metabolites prepartum, but CL cows had decreased glucose, increased non-esterified fatty acid and tended to have lower insulin concentrations in plasma postpartum compared with HT cows. Cooling prepartum HT cows did

not affect the insulin responses to GTT and IC during the transition period and glucose responses to GTT and IC at -14 and 28 DRC were not affected by treatments. At 7 DRC, CL cows tended to have slower glucose clearance to GTT and weaker glucose response to IC relative to HT cows. CL cows had stronger non-esterified fatty acid responses to IC postpartum but not prepartum compared with HT. In conclusion, cooling heat-stressed dairy cows in the dry period reduced insulin effects on peripheral tissues in early lactation but not the dry period.

Introduction

One of the consequences of exposure to environmental heat stress is a reduction in milk yield. Heat stress compromises lactational performance not only when occurring during lactation (Collier et al., 2006) but also during the non-lactating period before parturition. In particular, exposure to heat stress during the dry period impairs mammary gland development (Chapter 2) which in turn decreases milk yield in the subsequent lactation (Wolfenson et al., 1988, Chapter 2).

In addition to the decrease in DMI, metabolism of lactating dairy cows is also altered by heat stress. Heat-stressed cows in mid-lactation have faster glucose clearance and increased insulin response to a glucose tolerance test (**GTT**) compared with pair-fed contemporaries under thermo-neutral conditions (Wheelock et al., 2010), which indicates that peripheral tissues of heat-stressed mid-lactation cows have increased sensitivity to insulin. In addition, the ability to mobilize adipose tissue is also compromised by heat stress during mid-lactation (Wheelock et al., 2010). Even though the physiological mechanisms are unclear, it is believed that these metabolic modifications under heat stress during mid-lactation account for ~50% of the milk production loss observed (Rhoads et al., 2009; Wheelock et al., 2010), whereas decreased DMI explains the remainder of the depressed milk yield. However, it is unknown if the responses of peripheral tissues to heat stress are similar during early and late lactation to those observed in mid-lactation.

During the dry period, cooled cows have increased DMI compared with heat-stressed cows (Chapter 2). Relative to heat-stressed cows, those under cooling during the dry period have higher circulating NEFA in early lactation (do Amaral et al., 2009). Corresponding to the higher adipose tissue mobilization, cooled cows have higher hepatic fatty acid uptake and incorporate more fatty acids into milk fat in early lactation compared with non-cooled herdmates (do Amaral et al., 2009). However, the impact of cooling heat-stressed dry cows on the insulin effects on peripheral tissues during the transition period has never been evaluated. It was hypothesized that cooling heat-stressed dairy cows during the prepartum period decreased the insulin responsiveness of peripheral tissues during the transition period. Thus, the objective of the current study was to study the effect of cooling heat-stressed dairy cows during the dry period on the insulin effects on peripheral tissues during late gestation and early lactation.

Materials and Methods

Animals, Experimental Design and Sampling

The experiment was conducted at University of Florida Dairy Unit between June and November, 2010. Institutional Animal Care and Use Committee of University of Florida approved all the experimental procedures. The experimental design and cooling setup were based on Chapter 2. A total of 32 multiparous Holstein cows were included in this experiment and randomly assigned to each treatment (n=16/treatment), based on mature equivalent milk production (heat stress [**HT**]: 12045 kg; cooling [**CL**]: 12100 kg) of the just completed lactation. The average parity of HT and CL cows was 1.4 ± 0.2 and 1.7 ± 0.2, respectively and did not differ between treatments (P = 0.68). All the calving events occurred from July to September and evenly distributed amongst treatments.

Air temperature and relative humidity were measured using Hobo Pro Series Temp probes (Onset Computer Corporation, Pocasset, MA) every 30 min. THI was calculated based on

following equation: THI = (1.8×T+32) - ((0.55-0.0055×RH) × (1.8×T - 26)), where T = air temperature (^{o}C) and RH = relative humidity (%) (Dikmen et al., 2008). Rectal temperature was measured using a GLA M700 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA) twice daily (0730 and 1430 h) and respiratory rate was counted thrice weekly (1500 h, Mon-Wed-Fri) during the prepartum period. During the dry period and the first 6 wk postpartum, BW was recorded and BCS was scored for each cow once weekly. Additionally, starting at 7 wk postpartum, daily BW was recorded by the Afifarm system (Kibbutz Afikim, Israel) at each milking until 42 wk postpartum. Lactating cows were milked twice daily (0800 and 2000 h) and daily milk production was recorded up to 42 wk postpartum. Milk composition was measured by the AfiLab^{TM} milk analyzer (Kibbutz Afikim, Israel) at each milking until 42 wk postpartum. Dry cows were fed once a day (0800 h) and lactating cows were fed twice daily (0730 and 1300 h). Daily DMI was measured from dry-off until 42 d relative to calving. The composition of diets for dry and milking cows are presented in Table 4-1.

Blood samples were collected from coccygeal vessels into sodium-heparinized Vacutainer^{TM} (Becton Dickinson, Franklin Lakes, NJ) for all the cows before morning feeding (0700 h) at dry-off, -32, -18, -7, -3, calving, 2, 14, 28, 42 d relative to calving. Samples were immediately put in the ice and centrifuged at 2,619 × g at 4 ^{o}C for 30 min within 1 h after collection. After centrifugation, the plasma samples were aliquoted and frozen until insulin and metabolite analyses.

Metabolic Tests

At dry-off, -14, 7 and 28 d relative to calving, a subset of cows were subjected to metabolic tests (n = 16, 8 cows per treatment). A catheter (14 gauge × 14 cm Abbocath-T, Hospira, Finisklin Business Park, Sligo, Ireland) was inserted into the jugular vein of each cow the day before the metabolic tests. Intravenous GTT was conducted on the first day of the

metabolic tests and insulin challenge (**IC**) was performed the following day. The actual dates for GTT were -43 ± 1.3, -12 ± 1.1, 8 ± 0.4 and 29 ± 0.5 d relative to calving; and the actual dates for IC were -42 ± 1.3, -11 ± 1.1, 9 ± 0.4 and 30 ± 0.5 d relative to calving. All metabolic tests were performed at noon following the morning milking and feeding. Additionally, all the cows were fasted for one hour before each test. The procedures of metabolic tests were based on Pires et al. (2008).

For the GTT, 0.25 g/kg BW of glucose (dextrose 50%, wt/vol; Phoenix Scientific Inc., St. Joseph, MO) was infused into the jugular vein through the catheter followed by 50 mL of sterile saline solution to flush the catheter. The duration of glucose infusion was 5.6 ± 0.2 min. Blood samples were drawn through the catheter at –15, –5, and 0 min relative to the starting point of glucose infusion and 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 min relative to the ending point of glucose infusion into Vacutainer^{TM} tubes containing sodium fluoride and potassium oxalate (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed in the ice and centrifuged at 2,619 × g at 4 ^{o}C for 15 min within 1 h after collection. The catheter was flushed with sterile saline containing sodium heparin between samplings to avoid clotting and the first 3 mL of blood collection was discarded before each subsequent sample.

The IC was performed by administering 0.1 IU of insulin/kg of BW (100 IU/mL, human insulin, rDNA origin, Eli Lilly and Company, Indianapolis, IN) through the jugular catheter followed by 50 mL of sterile saline solution. The duration of insulin infusion was 2.7 ± 0.1 min. Blood samples were collected at –15, –5, and 0 min relative to the starting point of insulin administration and 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, and 120 min relative to the ending point of insulin administration through the catheter.

Insulin and Metabolite Analyses

The concentration of insulin in the plasma samples was determined by radioimmunoassay (Malven et al., 1987), and the inter- and intra-assays coefficient of variation (**CV**) were 12.8 and 7.3% respectively. Plasma glucose (Autokit Glucose; Wako Chemicals USA, Inc., Richmond, VA) and NEFA (HR Series NEFA-HR(2), Wako Chemicals USA, Inc., Richmond, VA) concentrations were measured by enzymatic methods. The inter- and intra-assays CV were 10 and 7.1% respectively for glucose assays, and 5.1 and 3.2% respectively for NEFA assays.

Calculations and Statistical Analysis

The observed concentrations of insulin and metabolites from GTT and IC to 180 and 120 min, respectively, were used to create response curves. The area under each these curves (**AUC**) was calculated by the trapezoidal method in which the insulin or metabolite concentration value was calculated by subtracting the baseline value from the actual value. The mean value of the insulin or metabolite concentration of the samples collected at -15, -5 and 0 min relative to glucose or insulin infusion was considered as the baseline value. The accumulated AUC of insulin and metabolites at 30, 60 and 180 min in the GTT and at 30, 60 and 120 min in the IC was calculated and analyzed.

PROC UNIVARIATE of SAS 9.2 (SAS Institute, Cary, NC) was used to calculate the dates of metabolic tests and the means ± SEM are reported. The temperature-humidity index was calculated by the PROC UNIVARIATE procedure and the means ± SD are reported. Parity, dry period and gestation length were analyzed by the PROC GLM procedure of SAS 9.2 and data are reported as least squares means ± SEM. Repeated measures data (rectal temperature, respiration rate, milk production, milk composition, DMI, cumulative BW change, cumulative BCS change, BW after 6 wk postpartum, feed efficiency, blood insulin and metabolites) were analyzed using the PROC MIXED procedure of SAS 9.2. The SAS model included fixed effects of treatment,

time and treatment by time with cow (treatment) as the random effect and the least squares means ± SEM are reported. In order to separate the treatment and post-treatment effects, data from cumulative BW change, cumulative BCS change, DMI, blood insulin and metabolites were split into prepartum and postpartum periods and analyzed separately. The BW at dry-off and calving were used as covariates and included in the SAS models of prepartum and postpartum DMI analyses, respectively, to minimize the impact of BW on DMI. In addition, the samples collected at dry-off were included in the SAS model as covariates for statistical analyses of blood insulin and metabolites during the prepartum period. The accumulated AUC at 30, 60 and 180 min in the GTT and at 30, 60 and 120 min in the IC were analyzed individually using the PROC MIXED procedure. The SAS models included fixed effects of treatment, day relative to calving and treatment by day relative to calving with cow (treatment) as the random effect. Least squares means ± SEM are reported. In addition, the data of accumulated AUC at dry-off were considered as covariates and included in the analyses. The insulin and metabolites concentration in plasma of each metabolic test within each test day was analyzed using PROC MIXED procedure. The SAS models included fixed effects of treatment, time relative to infusion and treatment by time relative to infusion with cow (treatment) as the random effect and the baseline value of insulin and metabolites concentrations of plasma was also included in the SAS model as covariates. Least squares means ± SEM are reported. For all the covariate analysis, if the effect of covariate was not significant ($P > 0.3$), the covariate was removed from the model.

Results

Thermal Environment, Rectal Temperature, Respiration Rate and Dry Period and Gestation Length

The stall areas for both HT and CL cows had similar temperature-humidity index (78.3, SD = 4.2) during the dry period, which indicates that both groups of cows were exposed to

similar heat stress. Compared with HT cows, CL cows tended ($P = 0.12$) to have decreased rectal temperature in the morning and had lower ($P < 0.01$) rectal temperature and respiration rate in the afternoon (Table 4-2). Additionally, the dry period length of HT cows tended ($P = 0.08$) to be 4 days shorter which was caused by a 4 d reduction ($P = 0.02$) in gestation length relative to CL cows (Table 4-2).

Milk Production and Composition

Compared with HT cows, CL cows produced 6.3 kg/d more milk ($P < 0.01$; Figure 4-1) until 42 wk postpartum. No treatment effect was observed for concentrations of milk fat, protein or lactose or for SCS (Table 4-3). However, with the higher milk production, cows cooled during the dry period had greater ($P \leq 0.01$) yields of milk fat, protein and lactose compared with HT cows (Table 4-3).

DMI, BW, BCS and Feed Efficiency

Cooling heat-stressed cows during the dry period tended ($P \leq 0.10$) to increase DMI during the prepartum and postpartum periods (Figure 4-2). Additionally, there was no difference between treatments of the DMI during the first 14 days after calving (15.2 vs. 14.3 kg/d for CL and HT cows, respectively), however, CL cows consumed more ($P = 0.04$) DM compared with HT cows after 2 wk postpartum (19.7 vs. 17.6 kg/d for CL and HT cows, respectively). Relative to HT, CL cows gained more ($P = 0.01$) weight before calving and lost more ($P = 0.02$) weight in early lactation (Table 4-3). Nevertheless, there was no treatment effect on BW after 6 wk postpartum and the mean BW were 654.7 ± 12.2 and 662.9 ± 11.6 kg for HT and CL, respectively. During the dry period, treatment did not affect BCS change, however, CL cows tended ($P = 0.14$) to lose more body condition compared with HT cows in early lactation (Table 4-3). After calving, CL cows had higher ($P < 0.01$) feed efficiency relative to HT cows within 6 wk postpartum (Table 4-3).

Insulin and Blood Metabolites

Plasma insulin and glucose concentrations were similar between treatments during the prepartum period (Figure 4-3), but cooling heat-stressed cows during the dry period tended (P = 0.12) to decrease circulating insulin and lowered (P = 0.01) plasma glucose concentration during the postpartum period (Figure 4-3). CL cows had increased (P = 0.03) circulating NEFA in early lactation compared with HT cows (Figure 4-3). Additionally, there was a tendency (P = 0.06) for a treatment by time effect for circulating NEFA during the prepartum period, such that CL cows had higher circulating NEFA at parturition compared with HT (Figure 4-3).

Metabolic Tests

There were no differences of effects of treatments or treatment by day interaction for insulin AUC during the GTT during the transition period (Table 4-4). Regardless of treatments, cows during the dry period had greater AUC for insulin in response to the GTT compared with measurements taken in early lactation. No overall treatment effects were observed for the glucose AUC to GTT during the transition period (Table 4-4). However, the glucose AUC tended ($P \leq 0.15$) to be larger for CL cows at 7 d relative to calving compared with HT cows (Table 4-4, Figure 4-4). In addition, the glucose AUC was greater ($P \leq 0.01$) for cows during the dry period compared with early lactation (Table 4-4).

Cooling heat-stressed prepartum cows did not affect the insulin clearance after IC pre- and postpartum (Table 4-5). There were day effects for insulin AUC to IC at 30 and 60 min as cows in late gestation had greater ($P < 0.01$ and P = 0.02, respectively) insulin AUC relative to early lactation (Table 4-5). A tendency (P = 0.09) for a treatment by day effect of glucose AUC to IC at 60 min was observed, such that CL cows tended to have weaker glucose response to IC compared with HT cows at 7 d relative to calving (Table 4-5, Figure 4-5). The NEFA response to IC was affected by the day ($P < 0.01$) as the cows in early lactation had an increase in NEFA

AUC compared with those values in the dry period and cows at 7 d relative to calving had the strongest response (Table 4-5). In addition, a treatment by day interaction ($P = 0.02$) was observed for the NEFA AUC to IC at 30 min as the CL cows had stronger NEFA response to IC during the postpartum period compared with HT (Table 4-5, Figure 4-6).

Discussion

All the cows in the present study were exposed to similar thermal stress during the dry period, but the cooling system effectively minimized the heat load on CL cows compared with HT cows as indicated by the decreased rectal temperature and respiration rate. Similar to other reports (do Amaral et al., 2009, 2011), the longer gestation length and dry period length of CL cows also provide further evidence that the CL cows were less heat stressed than HT cows during the dry period. Therefore, the heat stress model in the present experiment was appropriate to evaluate the hypothesis that cooling heat-stressed cows during the dry period decreases the insulin responsiveness of peripheral tissues during the transition period.

The increase in milk production of cooled cows relative to heat-stressed cows was expected and consistent with previous reports (Wolfenson et al., 1988). The higher milk synthesis in the subsequent lactation results from the increased mammary gland development during the dry period due to heat stress abatement (Chapter 2). Specifically, CL cows have higher mammary epithelial cell proliferation during the dry period relative to HT cows.

The lack of effect of cooling heat-stressed cows during the dry period on the concentration of milk fat is not consistent with earlier reports and may be from differences in the duration of sample collection. When milk samples were collected until only 6 - 8 wk postpartum, do Amaral et al. (2009, 2011) and Avendaño-Reyes et al. (2006) reported that CL cows had increased milk fat concentration compared with HT cows. However, when sample collection extends beyond early lactation (Adin et al., 2009) and into late lactation (Chapter 2), no difference in fat

concentration is observed. In other words, the increased milk fat concentration that results from cooling heat-stressed dry cows is limited to early lactation and the effect is lost if the entire lactation is considered. Consistent with that interpretation, in the present study CL cows had higher (P = 0.07) milk fat concentration in the first 4 wk postpartum (3.92 vs. 3.75 %, respectively) compared with HT cows, but no difference was observed between treatments when analyzed for the full 42 wk postpartum.

As previously reported (do Amaral et al., 2009; Chapter 2), cooling heat-stressed cows increases DMI during the dry period. Additionally, CL cows tended to consume more DM relative to HT cows during the postpartum period. This increased nutrient intake may reflect the higher milk production of CL cows compared with HT cows. do Amaral et al. (2009, 2011) reported that cooling heat-stressed dry cows did not affect the DMI during the first 6 weeks of the postpartum period, however, in those experiments, the pronounced milk production difference only appeared after 7 weeks of lactation. In the present study, CL cows produced more milk compared with HT cows from the beginning of the lactation. The higher gain of BW during the dry period for CL cows relative to HT cows is consistent with other reports (do Amaral et al., 2009; Chapter 2) and may reflect the higher DMI. The fact that CL cows lost more BW and body condition in the postpartum period compared with HT cows suggests that CL cows have more body fat to mobilize in early lactation relative to HT cows.

Similar to earlier studies (Collier et al., 1982b; Urdaz et al., 2006, do Amaral et al., 2009), the glucose, NEFA and insulin concentrations during late gestation were not affected by treatments. Under same level of nutrition, heat-stressed mid-lactation cows have compromised adipose tissue mobilization (Baumgard et al., 2011) and lower circulating NEFA (Wheelock et al., 2010) compared with those under thermo-neutral conditions, even though both groups of

cows have a similar negative energy balance. In the current study, the similar body condition change and NEFA concentration between HT and CL cows during the dry period indicate that the decreased fat mobilization of heat-stressed cows in mid-lactation did not occur in the late gestation. The discrepancy between heat-stressed mid-lactation and dry cows is still not clear and likely reflects differences of energetic status at different points in the production cycle. In mid-lactation, the heat-stressed cows are in the negative energy balance because of a decrease in DMI (Wheelock et al., 2010). In contrast, heat-stressed dry cows may remain in positive energy balance because the energy cost of pregnancy is much less than lactation (NRC, 2001). Indeed, in late lactation, the heat-stressed cow stays in positive energy balance (Kim., et al., 2010) and has similar circulating NEFA compared with cooled cows (Tarazón-Herrera et al., 1999). Thus, the difference of the energetic status of mid-lactation versus dry cows may be responsible to the variable NEFA responses to heat stress.

After parturition, CL cows had lower circulating glucose compared with HT cows. Although CL cows consumed more DM in early lactation, the higher milk lactose output due to the higher milk production may explain the decreased circulating glucose of CL cows relative to HT cows. The increased circulating NEFA in the CL cows relative to HT cows at parturition and in early lactation indicates up-regulated body fat mobilization and is consistent with previous work (do Amaral et al., 2009). At the onset of lactation, adipose tissue mobilization is an important adaptation of nutrient partitioning to support milk synthesis (Bauman and Currie, 1980; Vernon and Pond, 1997). Thus, the increased body fat mobilization may reflect the higher nutrient demand for milk production in CL cows. Moreover, the higher DMI during the dry period may be another reason for the extensive fat mobilization of CL cows because the magnitude of the adipose tissue mobilization in early lactation is related to the level of nutrient

intake in the dry period (Holtenius et al., 2003). Physiologically, the lower circulating insulin of CL cows relative to HT cows in early lactation is consistent with higher NEFA because insulin is an anti-lipolytic factor and enhances lipogenesis (Bell, 1995; Hayirli, 2006).

In mid-lactation, heat-stressed cows appear to have a preference to use glucose as an oxidative substrate and the increased insulin response shunts glucose into other peripheral tissues at the expense of the mammary gland (Wheelock et al., 2010, Baumgard et al., 2011). Whether this altered glucose sparing mechanism observed in mid-lactation cows under heat stress occurs at all the lactation stages is unknown. In the current study, the glucose clearance rate of GTT was not affected by treatments during the dry period. Glucose disposal rate in the GTT is dependent on the decreased glucose production and increased utilization (Hayirli et al., 2001). With the similar insulin response to GTT, both groups of cows may have similar decreases in hepatic glucose output because insulin is a potent inhibitor of hepatic gluconeogenesis and glycogenolysis (Hayirli, 2006). Therefore, both groups of cows likely have similar glucose utilization following GTT during the dry period. Additionally, the similar glucose responses to IC between treatments during the dry period indicates similar glucose utilization at peripheral tissues for HT and CL cows which is in contrast with the increased glucose utilization in the heat-stressed mid-lactation cows. The differences of insulin action on the peripheral tissue and glucose metabolism between heat-stressed cows in mid-lactation and dry period may result from variable glucose balance in different lactation stages. The glucose demand for milk lactose synthesis in lactation is much higher compared with the gravid uterus glucose requirement for the fetal growth in late gestation (Bell, 1995; Drackley et al., 2001). Thus, the heat-stressed mid-lactation cows may be under negative glucose balance due to the decrease in DMI relative to thermo-neutral cows. On the other hand, even though DMI decreases during heat stress in dry

cows, they likely remain in positive glucose balance because of the less glucose demand by the gravid uterus compared with lactose synthesis. The increased insulin action on peripheral tissues observed in the heat-stressed mid-lactation cows may therefore not be activated in dry cows.

Insulin resistance at peripheral tissues is one of the homeorhetic mechanisms active in early lactation to spare glucose from adipose and muscle tissues to the mammary gland in order to satisfy the enormous glucose demand for milk synthesis (Bell, 1995; Bell and Bauman, 1997). In the present experiment, the similar insulin response to GTT, coupled with the slower glucose clearance during the GTT of CL cows compared with HT cows probably indicates that the CL cows had decreased glucose utilization compared with HT cows at 7 d relative to calving. Major routes for glucose flow after GTT in early lactation cows include peripheral tissues and the mammary gland. Whether or not mammary blood flow or glucose transporter distribution in the mammary gland in early lactation are altered by late gestation heat stress is unknown, however, the higher lactose production suggests that the mammary glands of CL cows have higher ability to sequester glucose compared with HT cows. Alternatively, CL cows may have decreased glucose utilization in peripheral tissue compared with HT cows in early lactation. The reduced glucose response to IC at 7 d relative to calving is also consistent with lower insulin sensitivity of peripheral tissues of CL cows relative to HT cows. The weaker insulin effects on the peripheral tissues in CL cows compared with HT cows may reflect the higher glucose demand for the milk lactose synthesis in CL cows. Even though CL cows consumed more feed compared with HT cows in the postpartum period, the differences of DMI did not appear until 14 days after calving. In the first week of lactation, with similar nutrient intake, the reduced insulin effects on peripheral tissues of CL cows may be an important homeorhetic adaptation to further spare glucose for mammary lactose synthesis compared with HT cows. However, after 14 d relative to

calving, the greater DMI of CL cows provide more dietary glucose substrate for the hepatic gluconeogenesis relative to HT cows. The similar insulin effects on peripheral tissues between treatments on the 28 d relative to calving suggest that the insulin resistance of CL cows was overcome by increased DMI relative to HT cows as lactation advanced.

The observation that CL cows had stronger NEFA response to IC compared with HT cows in early lactation indicates that CL cows have higher adipose tissue responsiveness to insulin relative to HT cows, at least with regard to lipolytic processes. This result is surprising considering the fact that CL cows had higher basal circulating NEFA and reduced insulin effects on peripheral tissues in terms of glucose metabolism compared with HT cows in early lactation. The insulin response during a GTT results from insulin release and turnover and the insulin clearance during an IC reflects the insulin degradation rate. In the present study, cooling heat-stressed cows during the dry period did not affect the insulin response to GTT or clearance to IC during either the dry period or early lactation. These findings suggest that both groups of cows had similar pancreatic sensitivity to glucose and insulin degradation during the transition period. This may also indicate that the variable tissue responsiveness to insulin of HT and CL cows in early lactation is due to an alteration at the post-receptor level (Kahn, 1978).

The altered insulin effects on peripheral tissues in HT vs. CL cows in early lactation likely result from differences in homeorhetic adaptation to the different milk synthetic capacity between HT and CL cows. As a result of higher mammary gland development during the dry period (Chapter 2), the CL cows produce more milk and require higher total nutrients for milk synthesis relative to HT cows in the subsequent lactation. Based on DMI, the cows had similar nutrient intake in early lactation, but it is clear that more nutrients partition to mammary gland in CL cows relative to HT cows. In the present study, at least part of the enhanced homeorhetic

regulation in CL cows was mediated by lower basal circulating insulin and decreased insulin effects on peripheral tissue. This allows the greater mammary capacity for milk synthesis (Chapter 2) to be realized and CL cows produce more milk.

The physiological reason for the altered metabolic adaptations and insulin effects on peripheral tissue in early lactation between treatments is unknown, but may partly due to the GH effects. In the current study, the higher circulating NEFA in early lactation likely indicates that the CL cows have higher homeorhetic control by GH compared with HT cows because GH is correlated with the fatty acid mobilization and circulating NEFA (Etherton and Bauman, 1998). The observation of do Amaral et al. (2009) of increased hepatic suppressors of cytokine signaling 3 gene expression 2 days after calving in cows cooled during the prepartum period compared with non-cooled cows provides additional evidence that the CL cows have increased GH in early lactation compared with HT cows because somatotropin administration during lactation dramatically increases the hepatic suppressors of cytokine signaling 3 mRNA abundance (Rhoads et al., 2010). GH increases fatty acid mobilization by increasing catecholamine-stimulated lipolysis and decreasing insulin-mediated lipogenesis in adipose tissue (Sechen et al., 1990; Etherton and Bauman, 1998). Thus, an increase in GH coupled with a decrease in circulating insulin in early lactation provides a physiological explanation for the greater fatty acid mobilization of CL cows compared with HT cows. In addition to an inhibitory effect on insulin-stimulated lipogenesis in adipose tissue, GH enhances the anti-lipolytic effect of insulin (Sechen et al., 1990; Etherton and Bauman, 1998) which may explain the stronger NEFA response to IC of CL cows compared with HT cows in early lactation. GH alters nutrient partitioning by increasing insulin resistance and decreasing whole body glucose oxidation (Etherton and Bauman, 1998) which leads to a slower glucose clearance rate during GTT and

weaker glucose response to IC of CL cows compared with HT cows. Thus, the increased GH in CL cows relative to HT cows in early lactation can explain the observed effects in the present experiment. Other endocrine regulators, such as PRL and glucocorticoids (Bauman, 2000) are affected by heat stress in cattle (Collier et al., 1982a; Wise et al., 1988) and may also be involved in the enhanced homeorhetic regulation in CL cows compared with HT cows and therefore cannot be excluded. Indeed, cooling heat-stressed cows during the dry period decreases the circulating prolactin during the prepartum period (do Amaral et al., 2009, 2011; Chapter 2) and increases hepatic *PRLR* gene expression during the dry period and early lactation (do Amaral et al., 2011). Thus, increased hepatic prolactin signaling in CL cows relative to HT cows during the transition period is suggested to be involved in the enhanced hepatic lipid metabolism to cope with extensive adipose mobilization in early lactation (do Amaral., 2009, 2011).

Conclusions

Cooling heat-stressed cows during the dry period increased milk production in the subsequent lactation. There were no differences in blood insulin and metabolite concentrations or insulin effects on peripheral tissues during late gestation when cows experienced heat stress. But, compared with HT cows, CL cows had higher adipose tissue mobilization, lower basal glucose and insulin concentrations and reduced insulin effects on the peripheral tissues in the first week of lactation. These metabolic adaptations are consistent with the greater milk yield observed in CL cows, and follow the observation of HT cows having reduced mammary capacity for milk synthesis. Therefore, heat stress abatement during the dry period did not affect the metabolism in the late gestation but the increased milk yield of CL cows was consistent with known homeorhetic responses in early lactation.

Table 4-1. Ingredient composition of TMR fed to cows on both treatments in prepartum and postpartum periods

Ingredient (% of DM)	Prepartum	Postpartum
Corn silage	42.55	31.65
Alfalfa hay	---	10.55
Wet brewers grain	12.77	10.55
Rye silage	21.28	---
Corn meal	4.26	16.87
Solvent extracted soybean meal	6.38	5.91
Whole cotton seed	---	6.33
Citrus pulp	8.5	12.66
Expeller soybean meal (Soy Plus)	---	2.11
Mineral and vitamin mix[1]	4.26	3.38

[1]Mineral and vitamin mix prepartum included 21% CP, 2% crude fat, 13% crude fiber, 8.5% Ca, 1% P, 3% NaCl, 3.5% Mg, 4.4% S, 34 mg/kg Co, 129 mg/kg Cu, 15mg/kg I, 300 mg/kg Mn, 7 mg/kg Se, 435 mg/kg Zn, 200 mg/kg F, 220459 IU vitamin A/kg, 70000 IU vitamin D_3/kg, 5512 IU vitamin E/kg. Mineral and vitamin mix postpartum included 25% CP, 0.25% crude fat, 1% crude fiber, 3% ADF, 5.75% Ca, 1.2% P, 4.75% NaCl, 9.25 mg/kg Se, 110230 IU vitamin A/kg, 39683 IU vitamin D_3/kg, 1102 IU vitamin E/kg, 381 mg/kg Monensin.

Table 4-2. Dry period length, gestation length, rectal temperatures and respiration rate of cows exposed to either heat stress (n = 16) or cooling (n = 16) during the dry period

Variable	Heat stress	Cooling	SEM	*P*-value
Dry period length (d)	40.4	44.3	1.5	0.08
Gestation length (d)	272	276	1.2	0.02
Rectal temperature AM (°C)	38.64	38.55	0.04	0.12
Rectal temperature PM (°C)	39.34	38.98	0.05	< 0.01
Respiration rate (breath/min)	69.2	48.3	2.8	< 0.01

Table 4-3. Milk composition, BW and BCS change and feed efficiency of cows exposed to heat stress (n = 16) or cooling (n = 16) during the dry period

Variable	Heat stress	Cooling	SEM	*P*-value
Milk fat (%)	3.69	3.61	0.07	0.43
Milk protein (%)	3.05	3.08	0.05	0.68
Milk lactose (%)	4.67	4.69	0.02	0.54
Milk fat yield (kg/d)	0.98	1.21	0.06	0.01
Milk protein yield (kg/d)	0.82	1.04	0.05	< 0.01
Milk lactose yield (kg/d)	1.27	1.61	0.09	0.01
Milk SCS	3.16	2.89	0.18	0.67
BW change prepartum, kg[1]	9.6	26.0	4.5	0.01
BW change postpartum, kg[2]	-46.9	-72.4	7.5	0.02
BCS change prepartum[3]	-0.05	0.02	0.05	0.42
BCS change postpartum[4]	-0.21	-0.34	0.07	0.14
Feed efficiency[5]	1.90	2.23	0.08	< 0.01

[1]Prepartum accumulative BW change was calculated by subtracting data at -5, -4, -3, -2 and -1 week relative to calving and calving by data at dry-off.
[2]Postpartum accumulative BW change was calculated by subtracting data at 1, 2, 3, 4, 5 and 6 week relative to calving by data at calving.
[3]Prepartum accumulative BCS change was calculated by subtracting data at -5, -4, -3, -2 and -1 week relative to calving and calving by data at dry-off.
[4]Postpartum accumulative BCS change was calculated by subtracting data at 1, 2, 3, 4, 5 and 6 week relative to calving by data at calving.
[5]Feed efficiency = kg of 3.5% FCM/kg of DMI. Feed efficiency was calculated from calving until 6 wk postpartum.

Table 4-4. Insulin and glucose responses to glucose tolerance tests of cows exposed to heat stress (HT, n = 8) or cooling (CL, n = 8) during the dry period

	-14[1]		7		28				*P*-value	
	HT	CL	HT	CL	HT	CL	SEM	TRT[2]	Day	TRT×Day
Insulin AUC[3] (ng×min/mL)										
30 min	158	148	87	90	79	95	20	0.90	< 0.01	0.62
60 min	225	218	110	115	102	117	22	0.86	< 0.01	0.82
180 min	218	196	112	99	89	113	22	0.88	< 0.01	0.37
Glucose AUC (mg×min/dL)										
30 min	2025	1910	1480	1790	1521	1545	128	0.64	< 0.01	0.09
60 min	3177	2550	1942	2570	1901	1895	296	0.97	< 0.01	0.03
180 min	3611	2508	1758	3256	1567	1864	609	0.75	0.01	0.05

[1]Days relative to calving.
[2]TRT = treatment.
[3]AUC = area under the curve.

Table 4-5. Insulin, glucose and NEFA responses to insulin challenges of cows exposed to heat stress (HT, n = 8) or cooling (CL, n = 8) during the dry period

	-14[1]		7		28				*P*-value	
	HT	CL	HT	CL	HT	CL	SEM	TRT[2]	Day	TRT×Day
Insulin AUC[3] (ng×min/mL)										
30 min	141	151	119	124	121	125	7	0.47	< 0.01	0.86
60 min	160	175	149	149	144	149	10	0.54	0.02	0.65
120 min	130	152	149	154	136	145	14	0.29	0.72	0.68
Glucose AUC (mg×min/dL)										
30 min	-455	-496	-474	-390	-444	-460	38	0.82	0.51	0.26
60 min	-1348	-1440	-1313	-1075	-1178	-1180	79	0.52	0.02	0.09
120 min	-2357	-2415	-2297	-2097	-1775	-1991	146	0.84	0.01	0.30
NEFA AUC (µEq×min/dL)										
30 min	-576	-528	-2670	-4655	-1285	-2973	435	0.01	< 0.01	0.02
60 min	-835	284	-6194	-12247	390	-4289	1494	0.01	< 0.01	0.06
120 min	8476	9850	-2834	-19417	8534	1203	4312	0.05	0.01	0.22

[1]Days relative to calving.
[2]TRT = treatment.
[3]AUC = area under the curve.

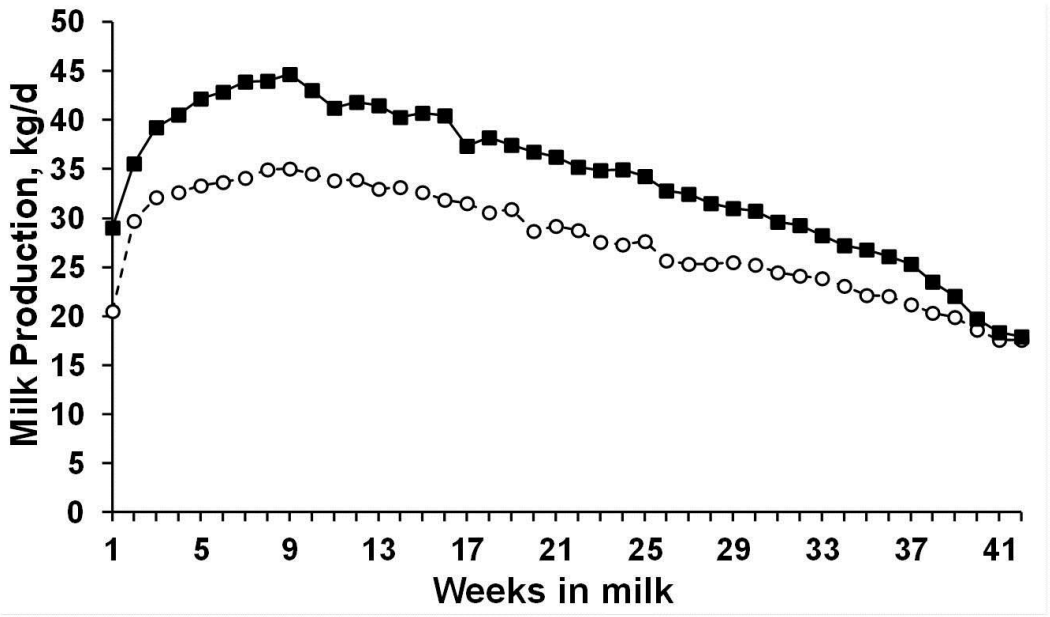

Figure 4-1. Effect of cooling heat-stressed cows (n = 16/treatment) during the dry period on milk production up to 42 wk postpartum in the subsequent lactation. Solid squares (■) and open circles (○) represent cooled cows and heat-stressed cows, respectively. After calving, all cows were managed and housed under same conditions. Effect of treatment ($P < 0.01$), time ($P < 0.01$) and treatment by time interaction ($P = 0.11$).

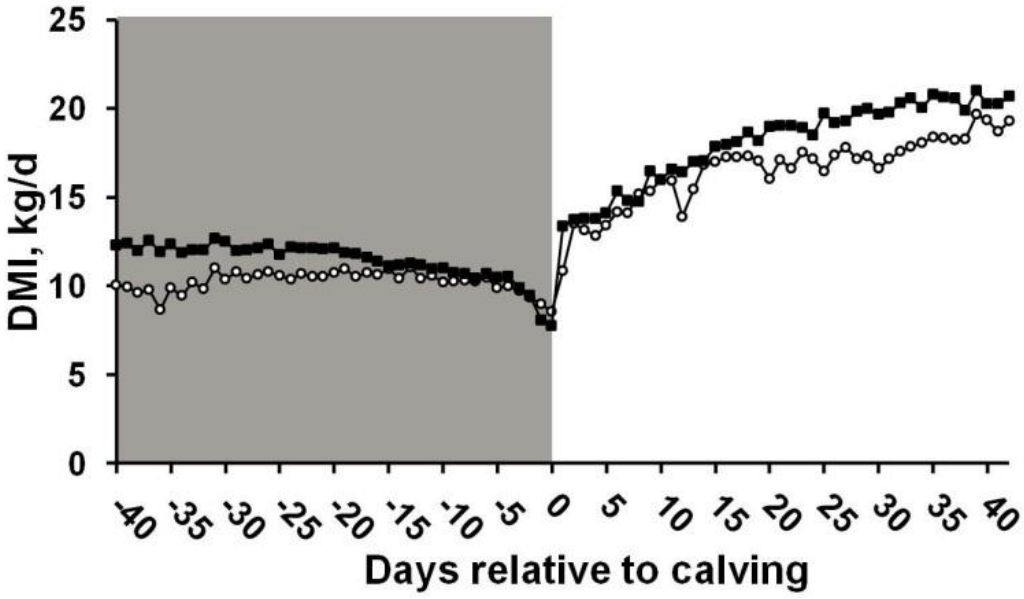

Figure 4-2. Effect of cooling heat-stressed cows (n = 16/treatment) during the dry period on the DMI from -40 to 42 d relative to calving. Solid squares (■) and open circles (○) represent cooled cows and heat-stressed cows, respectively. Data was split into prepartum and postpartum and analyzed separately. During the prepartum period, effect of treatment ($P = 0.06$), time ($P < 0.01$) and treatment by time interaction ($P = 0.60$); during the postpartum period, effect of treatment ($P = 0.10$), time ($P < 0.01$) and treatment by time interaction ($P = 0.38$). Shade represents the prepartum period.

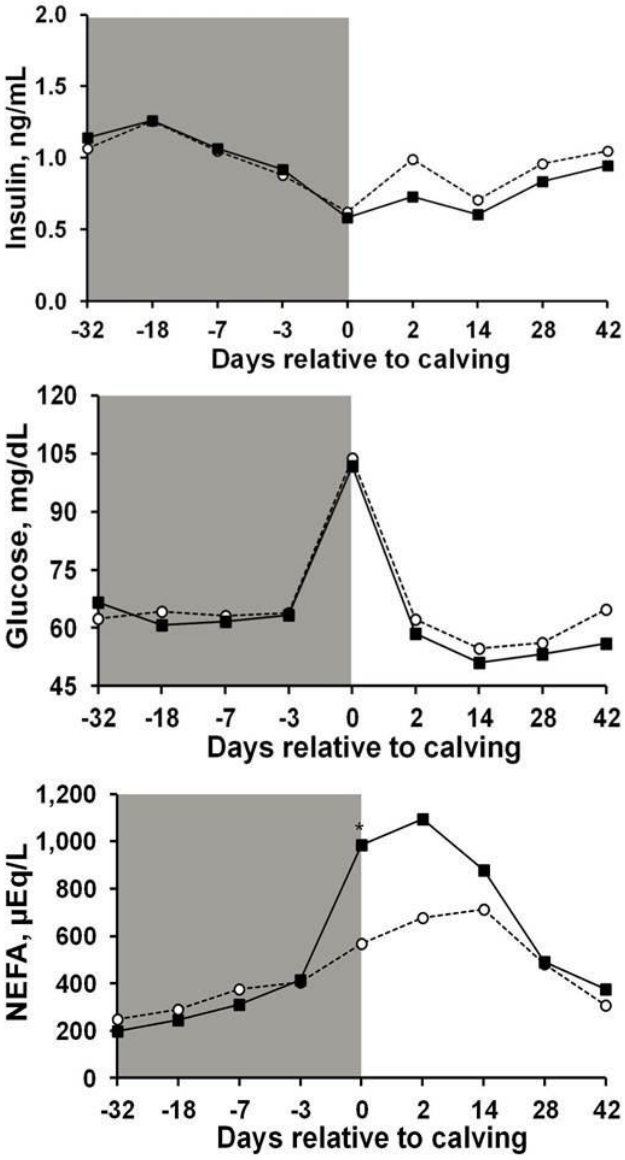

Figure 4-3. Effect of cooling heat-stressed cows (n = 16/treatment) during the dry period on the insulin, glucose and NEFA concentrations of plasma. Solid squares (■) and open circles (○) represent cooled cows and heat-stressed cows, respectively. Data was split into prepartum and postpartum and analyzed separately. In the insulin analysis, during the prepartum period, effect of treatment ($P = 0.94$), time ($P < 0.01$) and treatment by time interaction ($P = 0.99$); during the postpartum period, effect of treatment ($P = 0.12$), time ($P < 0.01$) and treatment by time interaction ($P = 0.50$). In the glucose analysis, during the prepartum period, effect of treatment ($P = 0.99$), time ($P < 0.01$) and treatment by time interaction ($P = 0.58$); during the postpartum period, effect of treatment ($P = 0.01$), time ($P < 0.01$) and treatment by time interaction ($P = 0.28$). In the NEFA analysis, during the prepartum period, effect of treatment ($P = 0.70$), time ($P < 0.01$) and treatment by time interaction ($P = 0.06$); during the postpartum period, effect of treatment ($P = 0.03$), time ($P < 0.01$) and treatment by time interaction ($P = 0.15$). Shade represents the prepartum period. *$P < 0.05$.

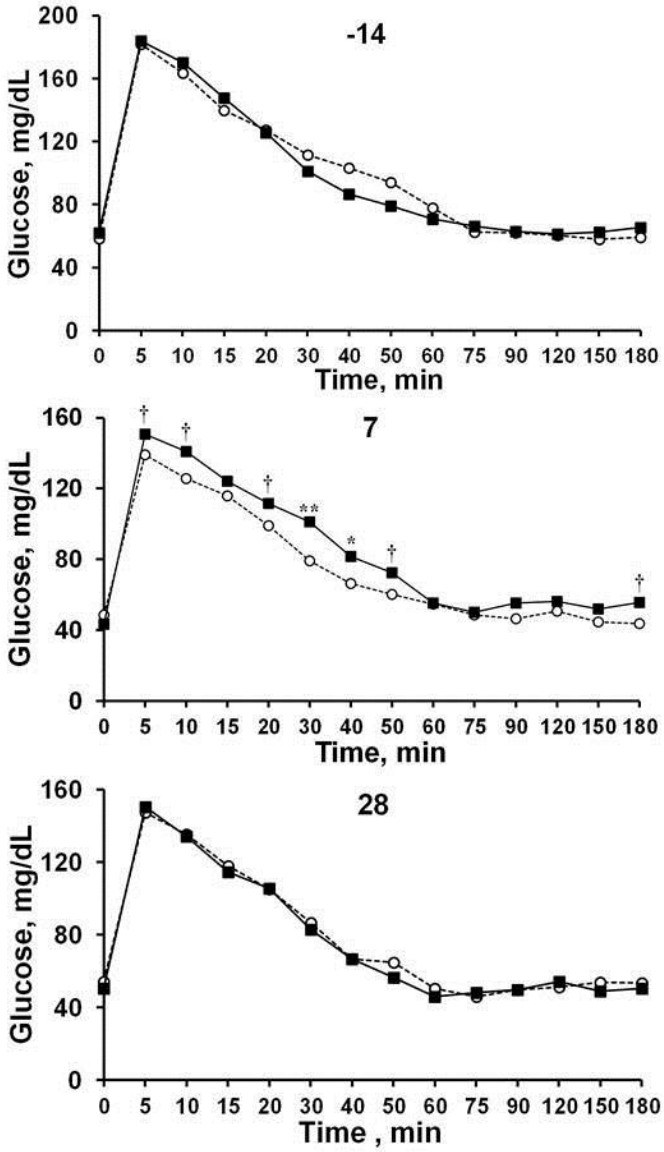

Figure 4-4. Effect of cooling heat-stressed cows (n = 8/treatment) during the dry period on plasma glucose response to glucose tolerance test at -14, 7 and 28 d relative to calving. Solid squares (■) and open circles (○) represent cooled cows and heat-stressed cows, respectively. After calving, all cows were managed and housed under same conditions. At -14 d relative to calving, effect of treatment ($P = 0.81$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.50$). At 7 d relative to calving, effect of treatment ($P = 0.15$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.05$). At 28 d relative to calving, effect of treatment ($P = 0.56$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.44$). $^{**}P < 0.01$, $^{*}P < 0.05$, †$P < 0.15$.

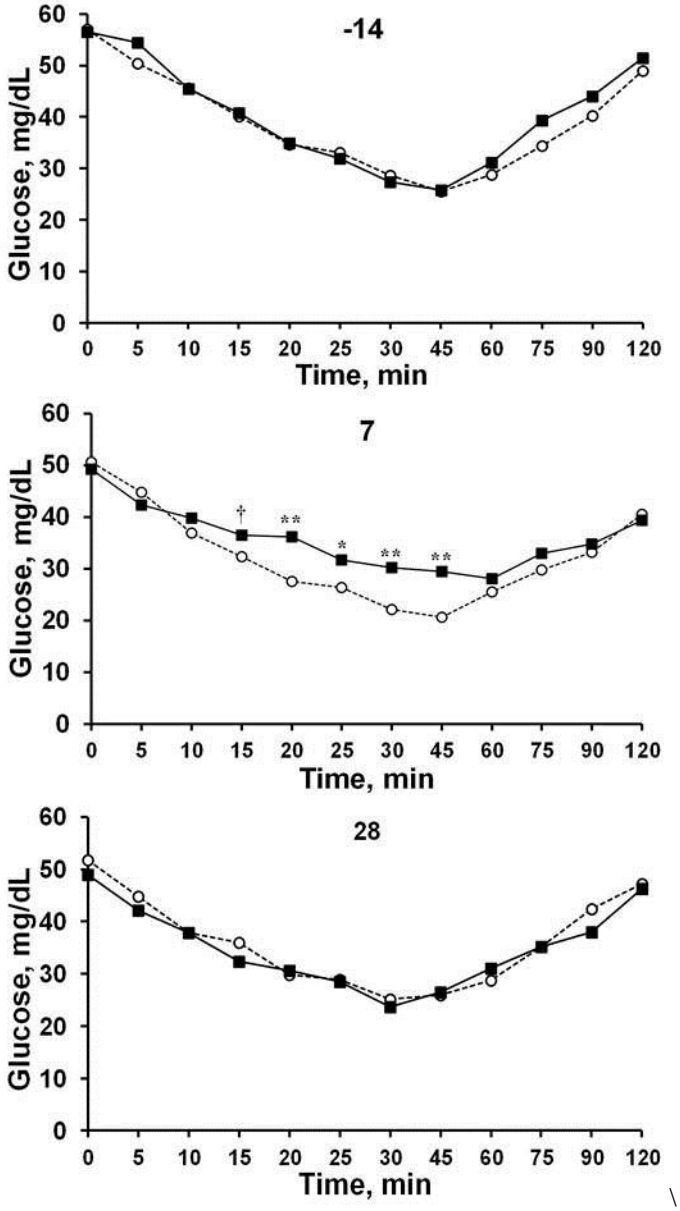

Figure 4-5. Effect of cooling heat-stressed cows (n = 8/treatment) during the dry period on plasma glucose response to insulin challenge at -14, 7 and 28 d relative to calving. Solid squares (■) and open circles (○) represent cooled cows and heat-stressed cows, respectively. After calving, all cows were managed and housed under same conditions. At -14 d relative to calving, effect of treatment ($P = 0.41$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.46$). At 7 d relative to calving, effect of treatment ($P = 0.01$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.01$). At 28 d relative to calving, effect of treatment ($P = 0.36$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.54$). **$P < 0.01$, *$P < 0.05$, †$P < 0.15$.

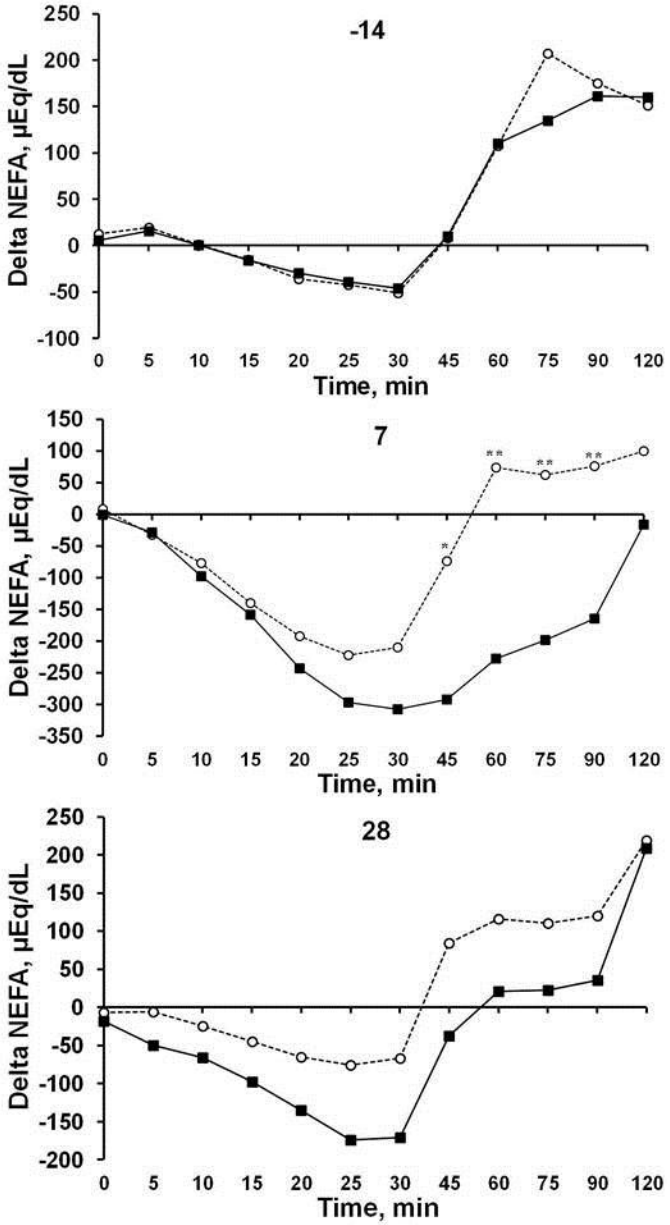

Figure 4-6. Effect of cooling heat-stressed cows (n = 8/treatment) during the dry period on plasma NEFA response to insulin challenge at -14, 7 and 28 d relative to calving. Solid squares (■) and open circles (○) represent cooled cows and heat-stressed cows, respectively. After calving, all cows were managed and housed under same conditions. Delta NEFA was calculated by subtracting data from samples collected at 0 - 120 min relative to insulin infusion by the average of data from samples taken at -15, -5 and 0 relative to insulin infusion. At -14 d relative to calving, effect of treatment ($P = 0.96$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.95$). At 7 d relative to calving, effect of treatment ($P = 0.11$), minute ($P < 0.01$) and treatment by minute interaction ($P < 0.01$). At 28 d relative to calving, effect of treatment ($P = 0.09$), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.36$). $^{**}P < 0.01$, $^{*}P < 0.05$.

CHAPTER 5
EFFECT OF LATE GESTATION MATERNAL HEAT STRESS ON GROWTH AND IMMUNE FUNCTION OF DAIRY CALVES

Abstract

Heat stress during the dry period affects the cow's mammary gland development, metabolism, and immunity during the transition period. However, the impact of late gestation heat stress on calf performance and immune status is unknown. The objective was to evaluate the effect of heat stress during the final ~45 d of gestation on growth and immune function of calves. Calves (17/treatment) were born to cows that were exposed to cooling (CL) or heat stress (HT) during the dry period. Only heifer calves (CL, n=12; HT, n=9) were used in measurements of growth and immune status after birth. Heifer calves were managed under identical conditions. All were fed 3.78 L of colostrum from their respective dams within 4 hours of birth and were weaned at 2 months of age (MOA). Body weight (BW) was obtained at weaning and then monthly until 7 MOA. Withers height (WH) was measured monthly from 3 to 7 MOA. Hematocrit and plasma total protein was assessed at birth, 1, 4, 7, 11, 14, 18, 21, 25 and 28 days of age (DOA). Total serum IgG was evaluated at 1, 4, 7, 11, 14, 18, 21, 25 and 28 DOA and apparent efficiency of absorption was calculated. Peripheral blood mononuclear cells were isolated at 7, 28, 42 and 56 DOA and proliferation rate was measured by 3H-thymidine incorporation in vitro. Blood cortisol concentration was measured in the dams during the dry period and in calves in the pre-weaning period. HT cows had 4 days shorter gestation length than CL cows. Calves from CL cows had greater BW than those from HT cows at birth (42.5 vs. 36.5 kg). Compared with CL heifers, HT heifers had decreased weaning BW (78.5 vs. 65.9 kg) but similar BW (154.6 vs. 146.4 kg) and WH (104.8 vs. 103.4 cm) from 3 to 7 MOA. Compared with CL, heifers from HT cows had less total plasma protein (6.3 vs. 5.9 g/dL), total serum IgG (1577.3 vs. 1057.8 mg/dL) and apparent efficiency of absorption (33.6 vs. 19.2%) and tended to

have decreased hematocrit (33 vs. 30%). Additionally, CL heifers had greater peripheral blood mononuclear cell proliferation relative to HT heifers (23.8 vs. 14.1 fold). Compared with CL, late gestation HT did not affect the blood cortisol concentration of dams during the dry period or that of the calves in the pre-weaning period, but CL calves tended to have increased circulating cortisol at birth (7.6 vs. 5.7 μg/dL). We conclude that heat stress of the dam during the dry period compromises the fetal growth and immune function of offspring from birth through weaning.

Introduction

Maternal manipulations during gestation affect fetal growth and immunity of neonates. Depending on the stage of gestation, nutrition has profound effects on fetal growth and immune function of the neonate. During late gestation, malnutrition is related to the lower birth weight of offspring, increased incidence of dystocia and high mortality and morbidity of neonates (Wu et al., 2006). However, ewes with restricted nutrition in early pregnancy birth heavier lambs compared with those with unrestricted nutrition (Funston et al., 2010). In addition to nutrition, other prenatal stressors, such as environmental stress, psychological stress and social stress, also lead to compromised fetal development and postnatal immunity (Merlot et al., 2008; Reynolds et al., 2010).

Heat stress is one of the environmental stressors that dramatically impacts the dairy industry. In lactating dairy cattle, heat stress is associated with decreased lactational and reproductive performance and increased disease incidence (Kadzere et al., 2002; Collier et al., 2006). In the dry period, cows exposed to heat stress have decreased milk production in the subsequent lactation and compromised immune function in the transition period (do Amaral et al., 2010, 2011; Chapter 2). In addition to the adverse effects on the dams, heat stress during late gestation is also related to lower birth weight of calves, which suggests compromised fetal

growth (Collier et al., 1982b). However, the effect of prenatal heat stress during the dry period on the postnatal growth of the calves has never been evaluated.

Ambient temperature during late gestation also affects the transfer of passive immunity. During late gestation, piglets from heat stressed sows have lower circulating IgG compared with those from sows under thermo-neutral conditions (Machado-Neto et al., 1987). In contrast, cold stress in late pregnancy increases the IgG absorption of piglets relative to their counterparts from sows in thermo-neutrality (Bate and Hacker, 1985). It remains unknown if maternal heat stress during the dry period has similar detrimental effect on passive immunity and cellular immune function of neonatal calves. Our hypothesis was that heat stress during the dry period compromises postnatal growth and immune function of dairy calves compared with cooling. The objective of current study was to evaluate the effect of heat stress during the dry period on the postnatal growth and immune function of dairy calves.

Materials and Methods

Animals and Experimental Design

This study was conducted at the Dairy Unit and Calf Unit of University of Florida (Hague, Florida) from July to November, 2010. All the treatments and procedures were approved by Institutional Animal Care and Use Committee of the University of Florida. The dams included in this experiment were from Chapter 4. The treatments of calves were reflected by the treatments of their dams during the dry period. Except for birth weight, only heifer calves (CL: n = 12; HT: n = 9) were used in the current experiment. Heifer calves were housed and managed in the same manner after birth. Two heifers from HT dams died due to navel infection at 2 wk of age and the data before the infection from these two calves were still incorporated into the statistical analyses.

Colostrum was collected from HT and CL cows within 2 hours after calving and 40 mL colostrum samples were frozen immediately after collection and stored at -20 ^{o}C. Each calf received 3.78 L of colostrum from its respective dam within 4 hours of birth by esophageal feeder. After one day of age, calves were fed pasteurized milk. All calves were weaned at 2 mo. of age.

Growth Measures and Sample Collection

All of the calves (n = 34; 17 per treatment) were weighed at birth. Additionally, BW of the heifer calves was obtained at weaning and then monthly until 7 months of age. Withers height (**WH**) of heifer calves was measured monthly from 3 to 7 months of age.

Blood samples were collected via jugular venipuncture from the heifer calves into sodium-heparinized Vacutainer^{TM} tubes (Becton Dickinson, Franklin Lakes, NJ) at birth (before colostrum feeding), and at 1, 4, 7, 11, 14, 18, 21, 25 and 28 days of age. Samples were immediately put in an ice bath and hematocrit and plasma total protein were accessed. Additionally, serum samples were collected at 1, 4, 7, 11, 14, 18, 21, 25 and 28 days of age for the total IgG analysis.

Ovalbumin Challenge

Heifer calves were injected s.c. with 1 mL of ovalbumin solution containing albumin from chicken egg white (0.5 mg/mL, Sigma-Aldrich, Saint Louis, MO) and adjuvant Quil A (0.5 mg/mL, Accurate Chemical & Scientific Corp., Westbury, NY) at 28 and 42 days of age (Magalhães et al., 2008). Serum samples were collected at 35, 42, 49, 56 days of age for anti-ovalbumin IgG analysis.

PBMC Isolation and Proliferation

Blood samples were collected from heifer calves at 7, 28, 42 and 56 days of age into sodium-heparinized Vacutainer^{TM} tubes (Becton Dickinson) and immediately transported to the

lab at ambient temperature. The procedure of PBMC isolation and proliferation assessment is based on do Amaral et al. (2010). Briefly, samples were centrifuged at 1000 × g for 30 min at room temperature. The buffy coat between the red blood cell and plasma was transferred to the tubes containing 2 mL TCM-199 media (Sigma-Aldrich) and mixed completely. Cell suspension was transferred into tubes containing 2 ml Fico/Lite LymphoH (Atlanta Biologicals, Lawrenceville, GA) and followed by centrifugation at 1000 × g for 30 min at room temperature. Mononuclear cells were collected and washed before the proliferation assay.

To examine PBMC proliferation, concentrations of PBMC were determined by hemacytometer and adjusted to 1×10^6 cells/mL in modified TCM-199 media supplemented with horse serum (5%, Atlanta Biologicals), penicillin (200 IU/mL, MP Biomedicals, Solon, OH), streptomycin (0.2 mg/mL, MP Biomedicals), glutamine (2 mM, Sigma-Aldrich) and β-mercaptoethanol (10-5 M, Sigma-Aldrich). A volume of 100 µL diluted PBMC suspension was added to each well of a 96 well, flat-bottom sterile plate. Twenty µL of Concanavalin A (**ConA**, 100 µg/mL, Sigma-Aldrich) or Dulbecco's phosphate buffered saline (Sigma-Aldrich) was added to the corresponding ConA wells or Control wells in triplicate. All the wells were filled with 80 µL modified TCM-199 to reach a final volume of 200 µL. Plates were incubated 72 h at 37 ^{o}C with 5% CO_2. At 48 hours of incubation, 2 µL of ^{3}[H]-Thymidine (0.2 µCi/µL, MP Biomedicals) was added to each well. At the end of the incubation, cells were harvested on a cell harvester (Brandel, Gaithersburg, MD). Stimulation index (**SI**) was calculated as the ratio of the average value of count per minute (**CPM**) of the ConA wells to the average CPM value of the Control wells.

IgG Analysis

Total IgG concentration of colostrum and serum samples collected from 1 to 28 d of age was measured by the radial immunodiffusion assay (Triple J Farms, Bellingham, WA) according

to the manufacturer's protocol. Briefly, 5 µL colostrum or serum samples were added into the wells in the gel containing anti-bovine IgG antibody and incubated 27 h at room temperature and in the absence of light exposure. After incubation, the diameter of the precipitin rings was measured and the total IgG concentration was calculated based on the linear relationship between diameters squared and total IgG concentration. The inter-assay CV of the radial immunodiffusion assay was 3.7%. The apparent efficiency of absorption (**AEA**) was calculated based on Quigley and Drewry (1998). The total IgG concentration of serum samples collected at 1 d of age was used to calculate AEA and the serum volume was considered as 9% of the birth weight.

The anti-ovalbumin IgG were measured by ELISA based on do Amaral et al. (2011). Briefly, flat-bottom, 96 well high binding affinity plates (Immulon 2 HB, Fisher, Pittsburgh, PA) were coated with sodium carbonate-bicarbonate buffer containing 1.4 mg/mL ovalbumin and incubated at 4 ^{o}C for 48 hours. Following incubation, plates were washed 4 times with PBS containing 0.05% Tween-20 solution (Fisher, Pittsburgh, PA) and blocked with 1% (w/v) BSA (Sigma-Aldrich) solution for one hour at room temperature. After washing, the control sera and samples were added to the plate based on a quadrant layout and incubated at room temperature for 2 hours. At the end of incubation, the plates were washed 4 times and 100 µL alkaline phosphatase conjugated rabbit anti-bovine IgG solution (Sigma-Aldrich, Saint Louis, MO) diluted in the Tris-buffer solution was added to each well. Following 1 h of further incubation, the plates were washed 4 times and 80 µL p-nitrophenyl phosphate liquid substrate (Sigma-Aldrich) were added to each well and the plates were incubated for 30 min at room temperature and in the absence of light. Subsequently, the plates were read on an automatic ELISA plate reader (MRX Revelation, Dynex Technologies Inc.) at 450 nm wavelength. The number of

animals in each treatment was balanced in each plate. The initial mean and SD were calculated based on the positive controls in the first 3 plates. If the mean of the positive controls in the following plates was outside the range of initial mean ± 1.5 SD of the first 3 plates, the whole plate was repeated. The optical density of the respective sample is reported. The inter- and intra-assay CV of the ELISA were 5.8 and 8.1% respectively.

Cortisol Analysis

Blood samples from the dams were collected from coccygeal vessel puncture into sodium-heparinized Vacutainer^{TM} (Becton Dickinson) at dry off and then once daily (1500 h) at -32, -18, -7 d relative to calving and at calving. Upon collection, samples were immediately placed in ice and harvested at 2,619 × *g* at 4 ^{o}C for 30 min within 1 h after collection. After centrifugation, plasma samples were frozen at -20 ^{o}C until analysis. The circulating cortisol concentration in the serum samples collected from the heifer calves at birth, 1, 4, 7, 14, 21, 28, 35, 42, 49 and 56 d of age were also determined. The concentration of cortisol in plasma and serum was analyzed by RIA using a commercial kit (Coat-A-Count Cortisol kit, Siemens Healthcare Diagnostics, Deerfield, IL). The inter- and intra-assay CV of the RIA assay of the plasma from the dams were 10.1 and 9.2% respectively; the CV's of the serum samples from the calves were 13.8 and 5.0% respectively.

Statistical Analysis

Birth weight, weaning weight, weaning to BW gain, colostrum IgG concentration, apparent efficiency of absorption and the serum cortisol concentration at birth were analyzed by PROC GLM procedure of SAS 9.2 (SAS Institute, Cary, NC) and least squares means ± SEM are reported. The PROC MIXED procedure of SAS 9.2 was used to analyze the repeated measurements (BW and WH from 3 to 7 months of age, hematocrit, plasma total protein, total serum IgG, anti-ovalbumin IgG concentration of serum, PBMC proliferation, plasma cortisol

concentration of the dams and serum cortisol concentration of the calves) and least squares means ± SEM are presented. The SAS model included fixed effects of treatment, time and treatment by time with calf or cow within the treatment as random effect.

Results

Data of the Dams

The descriptive data of the dams during the dry period was reported at Chapter 4. Briefly, all the cows were exposed to similar thermal stress during the dry period, but the cows exposed to heat stress had greater rectal temperature and respiration rate compared with those under cooling. Cows exposed to HT consumed less DM and gained less BW during the dry period and produced 6.3 kg/d less milk until 42 weeks postpartum compared with CL cows. Additionally, HT cows had 4 days shorter dry period length and gestation length compared with CL cows.

BW and WH

Calves born from the HT cows had less birth weight and weaning weight ($P < 0.01$ and $P = 0.04$, respectively) compared with those from CL cows (Table 5-1). However, there was no treatment effect on the BW gain from birth to weaning for the heifer calves (Table 5-1). After weaning, heifers from both groups of cows had similar BW and WH (Table 5-1).

Hematocrit, Plasma Total Protein, IgG Contents and PBMC Proliferation

Heifer calves from the HT cows tended ($P = 0.15$) to have lower hematocrit (Figure 5-1) and had decreased ($P < 0.01$) plasma total protein (Figure 5-1) from birth to 28 days of age compared with those from CL cows. Heat stress during the dry period did not affect the colostral IgG content (Table 5-1) but the calves exposed to HT in utero had decreased ($P < 0.01$) AEA (Figure 5-2) and decreased ($P = 0.03$) total IgG concentration in serum (Figure 5-2) during the first 28 days of age compared with those cooled in utero. In response to ovalbumin challenge, HT calves had similar ($P = 0.60$) IgG production relative to CL calves (0.41 ± 0.03 vs. 0.43 ±

0.01 optical density, respectively). Additionally, the heifers from the HT cows had decreased (P = 0.05) PBMC proliferation during the pre-weaning period compared with those from CL cows (Figure 5-3). Independent of treatment, a time effect ($P < 0.01$) was also observed as calves had greater PBMC proliferation at 7 d after birth compared with other time points.

Plasma and Serum Cortisol

During the dry period and at calving, the HT cows had similar (0.62 ± 0.08 vs. 0.73 ± 0.07 µg/dL, respectively; P = 0.66) circulating cortisol compared with CL cows. There was no overall difference of the serum cortisol concentration between HT and CL heifer calves during the pre-weaning period (Figure 5-4), however, CL calves tended (P = 0.08) to have up-regulated circulating cortisol at birth compared with HT calves (Figure 5-4).

Discussion

It is important to consider the effectiveness of the heat stress model utilized in current study, as the examination of neonatal calf growth and performance was predicated on maternal responses during the dry period. All the dams were exposed to similar environmental heat stress during the dry period, but the lower rectal temperature and respiration rate of CL cows indicate that they carried less heat load compared with HT cows (Chapter 4). Additionally, as previously reported (do Amaral et al., 2009), the reduced DMI and BW gain during the dry period, along with compromised lactational performance in the next lactation of HT cows compared with CL cows provide solid evidence that the heat stress model during the dry period in the present experiment was successful.

Similar to other heat stress studies in late gestation in different species (Brown et al., 1977; Collier et al., 1982b), the newborns from the HT dams had decreased birth weight (~6 kg) compared with those from the CL dams. Several factors may contribute to the compromised fetal growth in late gestation under heat stress. One possible factor is the shorter gestation length of

heat stressed cows considering the fact that the last two months of gestation is critical to bovine fetal development and accounts for 60% of the body weight gain before birth (Bauman and Currie, 1980). If we assume that the fetus of a Holstein dairy cow has 0.5 kg average daily gain in the uterus in the last week of gestation (Muller et al., 1975), the 4 day shorter gestation length in HT cows relative to CL cows in the current study accounts for approximately 33% (2 kg) of the decreased birth weight of the newborn HT calves. Malnutrition in late gestation may also reduce calf birth weight (Wu et al., 2006) but whether or not the small decrease in DMI (10-15%, do Amaral et al., 2009; Chapter 2 and 4) in heat stressed dry cows could affect fetal growth so dramatically is questionable. Tudor (1972) reported that feed restriction in the last trimester of pregnancy dramatically decreased the calf birth weight of beef cattle, however, in that experiment, the energy intake of cows in the low plane of nutrition group was severely restricted such that animals were only fed 3.5 kg/d/head of a low energy density diet whereas cows in the high nutrition group were fed 7 kg/d/head of a high energy density diet. On the other hand, the moderate decrease in energy intake in late gestation does not affect calf birth weight in dairy or beef cattle (Hough et al., 1990; Janovick and Drackley, 2010). Indeed, IUGR caused by late gestation heat stress in pregnant ewes is independent of restricted nutrition (Brown et al., 1977). In ruminants, heat stress during gestation is associated with decreased uterine blood flow (Reynolds, et al., 1985; 2006) and reduced placental size (Collier et al., 1982a) and function (Collier et al., 1982b; Bell et al., 1989; Early et al., 1991) which in turn impair oxygen diffusion into the fetal circulation (Dreiling et al., 1991) and maternal-to-fetal exchange of glucose and amino acids (Reynolds et al., 1985; Regnault et al., 2005). In order to cope with heat stress related hypoxemia and malnutrition, the ruminant fetus develops a series of endocrine and metabolic adaptations to promote survival at the expense of somatic growth (Yates et al., 2011).

In addition to the maternal and placental effects, environmental heat stress may also have direct effect on fetal growth. In heat-stressed pregnant ewes and goats, the fetal body temperature rises in parallel to the maternal temperature (Laburn et al., 1992; Faurie et al., 2001), and the maternal heat stress driven fetal hyperthermia may more directly affect fetal development beyond placental insufficiency (Bell et al., 1989). Thus, the heat stress related IUGR and the fetal hyperthermia may account for the other 67% (4 kg) of decreased fetal growth in HT animals relative to those from CL cows.

Calves from CL cows were heavier relative to those from HT cows at weaning; however, the greater weaning weight of CL calves seems to be due to the greater birth weight rather than additional body weight gain during the pre-weaning period. After weaning, the fact that both groups of calves had similar WH and BW suggests a similar rate of growth during the pre-pubertal period. However, we did not examine if body composition of calves is changed by prenatal heat stress in the current experiment. Small-for-gestational-age infants and IUGR lambs have accelerated fat deposition and develop obesity in early life relative to normothermic controls (Morrison et al., 2010; Yates et al., 2011). If a similar response occurs in the HT calves remains a question that requires more research because body composition during the pre-pubertal period can alter mammary gland development of dairy heifers, and thus is closely related to future milk production.

During the first month after birth, the HT calves had slightly lower hematocrit level compared with CL calves. The reason for this phenomenon is unknown, but possibly results from the postnatal adaptation to the lower oxygen consumption during in utero development, similar to IUGR fetus (Dreiling et al., 1991; Yates et al., 2011). In contrast to other reports (Nardone et al., 1997; Adin et al., 2009), the colostral IgG content was not affected by heat stress during the

dry period. Bovine colostrogenesis is under endocrine control and PRL is suggested to be involved in the cessation of colostrogenesis (Barrington et al., 2001) and to inhibit bovine mammary gland IgG_1 receptor expression in vitro and in vivo (Barrington et al., 1997; 1999). Heat stress results in an increase in PRL concentration during the dry period (do Amaral et al., 2009; 2010; Chapter 2). The similar colostral IgG content between HT and CL cows in the current study does not support the concept of an inhibitory effect of PRL on IgG1 receptor expression in the mammary gland. In contrast to studies conducted by Barrington et al., (1997; 1999), in which large amounts of PRL were used to mimic the PRL surge at parturition, the hyperthermia related basal PRL elevation during the dry period is modest and may not be harmful to the IgG transfer. It is possible that there is a threshold of the PRL concentration during the dry period below which the colostrogenesis will not be influenced. Supporting our theory, different photoperiodic regimes that also influence basal PRL concentration during the dry period (Auchtung et al., 2005) have no effect on mammary gland immunoglobulin transfer (Morin et al., 2010).

Results of the present study suggest that passive immunity is compromised in calves from HT dams relative to CL. The higher total plasma protein and total serum IgG during the first 28 days of age suggests that IgG transfer from colostrum was greater into the circulation of CL calves compared with HT. After adjustment of birth weight and total colostral IgG intake at birth, the lower AEA of HT calves relative to CL indicates a compromised passive immune transfer of HT calves that is due to an impaired capacity for IgG absorption in the intestine. Similar to our results, the offspring from sows (Machado-Neto et al., 1987) exposed to heat stress in late gestation had lower circulating IgG compared with those from the dams under thermo-neutrality. Late gestation heat stress also slightly decreased the IgG concentration in the

neonatal calves after ingestion of pooled colostrum (Stott, 1980); however, that author attributed the lower circulating IgG of heat stressed calves to the direct effect of thermal stress on the neonates at calving and short period after calving rather than the prenatal heat stress of the dams. The underlying mechanisms of the compromised passive immunity by prenatal heat stress are unclear. Glucocorticoid induces the gut closure in rats and is suggested as the mediator of enterocyte pre-maturation in the fetal intestine caused by maternal stress including hyperthermia in the pig (Machado-Neto et al., 1987; Merlot et al., 2008). However, piglets from cold stressed sows in late pregnancy had higher IgG absorption compared with those from the sows in the thermo-neutrality, despite the observation of increased cortisol levels in cold stressed sows (Bate and Hacker, 1985). The conflicting results with regard to the relationship between maternal blood cortisol concentration and neonatal IgG absorption are also observed in dairy cows (Stott, 1980). In the current study, the fact that no difference of circulating cortisol was observed between treatments in the dams during the dry period also suggests that maternal glucocorticoid level may not be a valid explanation for the compromised passive immunity in calves from heat stressed dams. Moreover, the higher cortisol level of CL calves relative to HT also indicates that glucocorticoid may not be the primary mediator of gut closure of newborn dairy calves as in other species. Another possibility for the decreased IgG transfer in HT calves compared with CL may be the direct effect of colostrum. Besides the Ig, bovine colostrum contains other immune components, growth factors and hormones that also affect gut closure (Sangild, 2003). For example, in heavily exercised athletes, bovine colostrum ingestion improves intestinal integrity and inhibits gut permeability (Marchbank, et al., 2011). Further, colostrum from dams with different levels of maternal nutrition has different effects on the passive immunity of neonatal calves (Hough, et al., 1990) and lambs (Hodgson et al., 1997). Possibly, a difference in

colostrum composition from HT and CL cows results in the altered IgG transfer to the HT calves.

To our knowledge, this is the first experiment to evaluate prenatal heat stress on the cell-mediated immune response of neonatal calves. During the postnatal period, the lower PBMC proliferation of HT calves indicates a compromised T lymphocyte response compared with CL calves. Maternal stress during the gestation period decreases thymus size and depresses T cell proliferative response to mitogens of neonatal rats (Merlot et al., 2008) and pigs (Tuchscherer et al., 2002). The PBMC proliferation data in the present experiment suggests that the function of lymphocytes is also reduced in the bovine neonate by the prenatal stressors, such as thermal stress. Regardless of treatment, the higher PBMC proliferation at 7 days after birth probably results from the effect of maternal leukocytes transferred from the colostrum (Reber et al., 2006) that are capable of full function relative to neonatal lymphocytes. Additionally, maternal leukocytes in colostrum play important roles in the establishment of the innate and adaptive immune systems of neonatal calves (Reber et al., 2008a, b). Thus, the different functionality of maternal leukocytes transferred from colostrum of HT or CL cows may also contribute to the different development of the immune system of the offspring. The fact that both groups of calves have similar antibody production after ovalbumin challenges suggests that prenatal heat stress has no effect on the humoral response of calves during the pre-weaning period. However, this conclusion is relatively limited because the humoral response in the current study was only evaluated at 4 weeks after birth and altered B cell function of the offspring in response to prenatal heat stress may have occurred before our measurements.

The similar serum cortisol concentrations of the heifer calves during the pre-weaning period may be expected because all the calves were exposed to the similar management and

handling. The higher cortisol concentrations of CL calves at birth relative to HT calves is intriguing and may suggest that the calves cooled in utero have a more sensitive hypothalamic-pituitary-adrenal (**HPA**) axis compared with HT calves in response to stress, such as parturition. In fetal sheep, the plasma estradiol concentration is positively related to the basal circulating ACTH and cortisol in the plasma and HPA axis sensitivity (Wood, 2005). Heat stress in late gestation of dairy cows markedly decreases the placental production of estrone-sulfate (Collier et al., 1982b) which may provide the physiological mechanism for the compromised fetal HPA axis development in HT calves relative to CL calves as observed in the current study.

Genomic imprinting, such as epigenetic regulation, may be involved in the IUGR and long-term effect in modulating immune function of the offspring from the prenatal heat stressed dams. An abnormal epigenetic profile due to the prenatal stress in the human and rat significantly changes the placental gene expression profiles which results in disturbed placental cell survival and function (Gheorghe et al., 2010; Nelissen et al., 2011), and also affect the gene expression profiles of neonatal tissues, such as brain and liver (Joss-Moore et al., 2010). Similar phenotypic outcomes including lower birth weight occur in humans and rats following maternal stress such as fetal hypoxia and malnutrition (Gheorghe et al., 2010), and thus it may be expected that epigenetic modification also plays a role in the IUGR of the dairy calves caused by late gestation heat stress observed in the current study. Immune cells are also the targets of epigenetic regulation (Sanders, 2006; Janson and Winqvist, 2011). In infants, the maternal environment during the prenatal period has dramatic influence on the fetal development of immune cells through epigenetic modification that in turn alters the disease risk in postnatal life (Martino and Prescott, 2011). Thus, it is not unreasonable to postulate that epigenetic regulation also plays some role in the altered lymphocyte response of neonatal calves to prenatal heat stress. Further

studies in this area are required in order to elucidate the cellular mechanisms involved in the prenatal heat stress related IUGR and immune function modulation in neonatal dairy calves.

Conclusions

Heat stress during the dry period of dairy cows decreased calf birth weight and compromised the passive IgG transfer from colostrum and cell-mediated immune function of the calves during the pre-weaning period. Additionally, prenatal heat stress may affect the HPA axis of the bovine fetus and alter the response to stress in the newborn calf. Genomic imprinting, i.e. epigenetic regulation, may play a role in the IUGR and immuno-modulation of fetus and neonate from prenatal heat stressed dams, but requires more research to determine the mechanisms at the cellular level.

Table 5-1. Birth weight, weaning weight, BW gain from birth to weaning, BW after weaning and withers height after weaning of calves and colostrum IgG content from dams exposed to either heat stress or cooling during the dry period

Variable	Heat stress	Cooling	SEM	*P*-value
Birth weight (kg)	36.5	42.5	1.2	< 0.01
Weaning weight (kg)	65.9	78.5	4.0	0.04
Weaning BW gain (kg)[1]	29.4	35.9	3.8	0.25
BW after weaning(kg)[2]	146.4	154.6	4.5	0.22
Withers height after weaning (cm)[3]	103.4	104.8	1.0	0.33
Colostrum IgG (mg/dL)	8681	7727	726	0.36

[1]Weaning BW gain was calculated by subtracting data at weaning by data at birth.
[2]BW at 3, 4, 5, 6 and 7 months of age.
[3]Withers height at 3, 4, 5, 6 and 7 months of age.

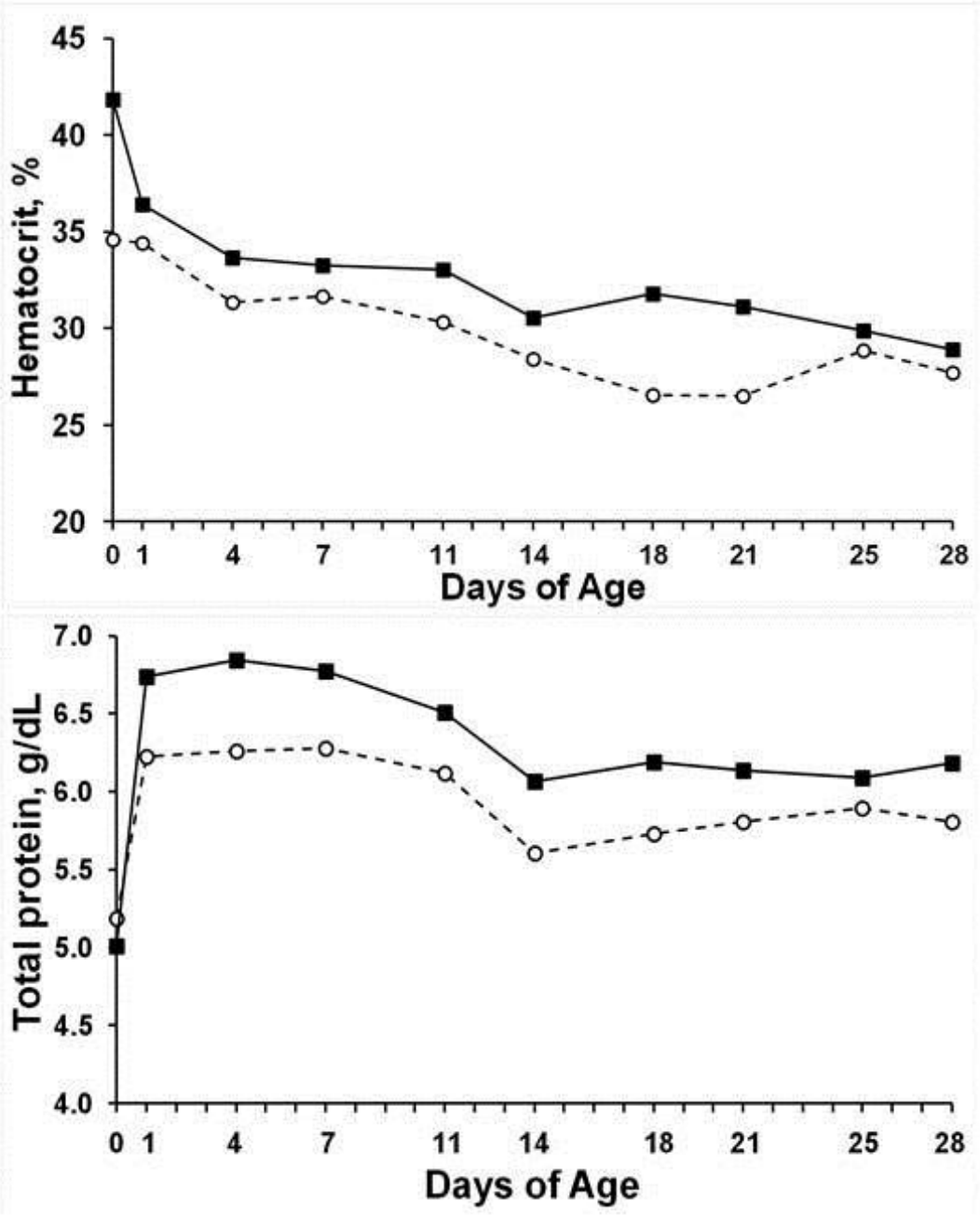

Figure 5-1. Effect of heat stress and cooling during the dry period on the hematocrit and plasma total protein of neonatal calves during the first 28 days of age. Solid squares (■) represent calves from cows exposed to cooling in the late gestation and open circles (○) represent those from cows in heat stress. Heat stress during the dry period tended ($P = 0.15$) to decrease the hematocrit (30.0 ± 1.6 vs. 33.0 ± 1.4%, respectively) and decreased ($P < 0.01$) plasma total protein (5.89 ± 0.1 vs. 6.25 ± 0.1 g/dL, respectively) of calves compared with cooling.

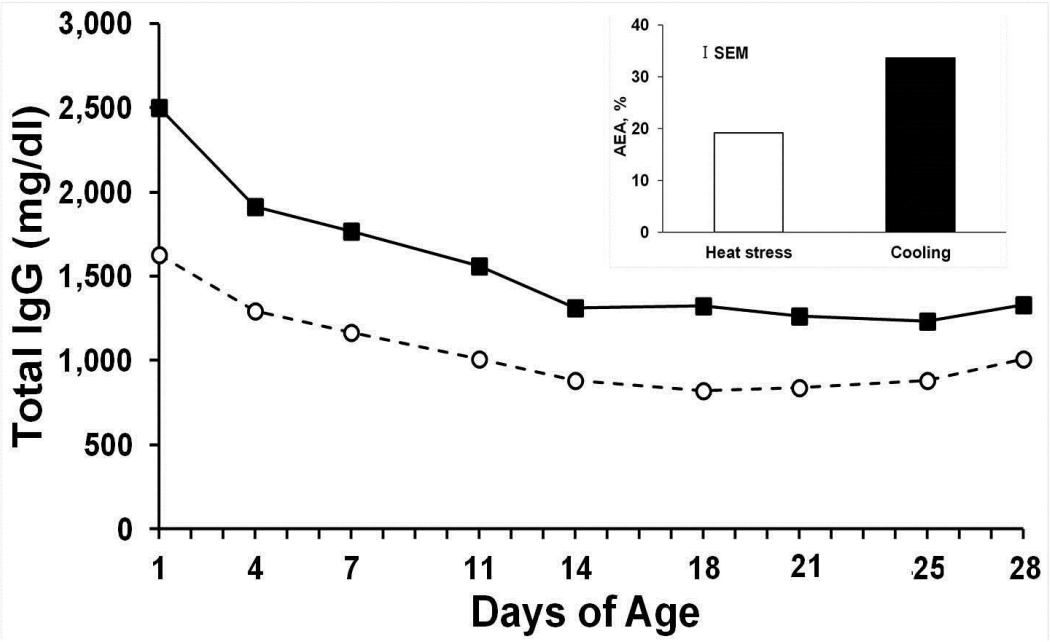

Figure 5-2. Effect of heat stress and cooling during the dry period on the total serum IgG concentration during the first 28 days of life and the apparent efficiency of absorption (AEA; Inset). Solid squares (■) and bar represent calves from cows exposed to cooling during the dry period and open circles (○) and bar represent those from cows in heat stress. Heat stress during the dry period decreased ($P = 0.03$) the total serum IgG (1057.8 ± 173.3 vs. 1577.3 ± 149.3 mg/dL, respectively) of calves during the first 28 days of age compared with cooling. Additionally, calves exposed to heat stress in utero had lower ($P < 0.01$) AEA (19.2 ± 2.4 vs. 33.6 ± 2.0%, respectively) compared with those cooled in utero.

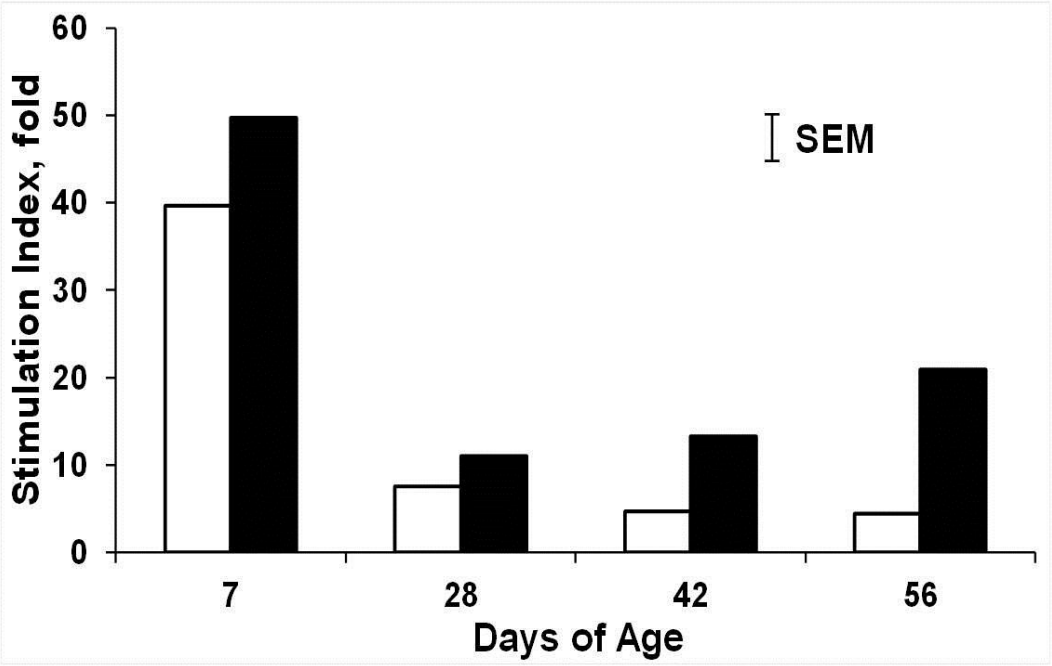

Figure 5-3. Effect of heat stress and cooling during the dry period on the peripheral blood mononuclear cell proliferation of neonatal calves. Solid bars represent calves from cows exposed to cooling during the dry period and open bars represent those from cows in heat stress. Calves from heat stressed cows during the dry period had decreased ($P = 0.05$) stimulation index of peripheral blood mononuclear cells proliferation (14.1 ± 6.0 vs. 23.8 ± 4.5 fold, respectively) compared with those from cooled cows in the neonatal period. Additionally, calves at 7 days after birth had higher ($P < 0.01$) stimulation index compared with other time points.

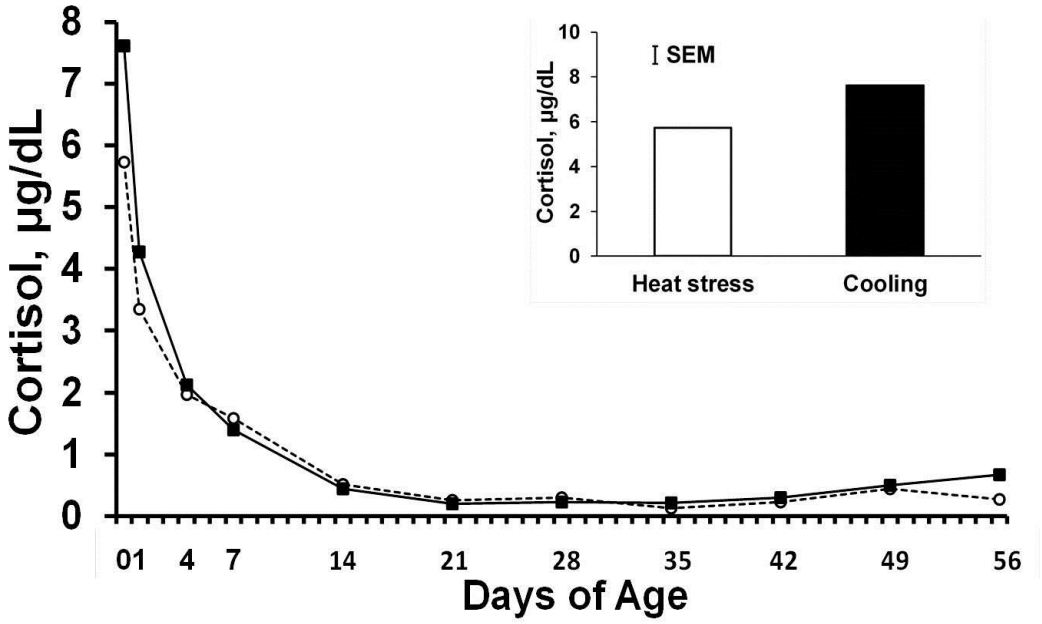

Figure 5-4. Effect of heat stress and cooling during the dry period on the serum cortisol concentration during pre-weaning period and at birth (Inset). Solid squares (■) and bar represent calves from cows exposed to cooling during the dry period and open circles (○) and bar represent those from cows in heat stress. Heat stress during the dry period did not affect (P = 0.73) the cortisol concentration (1.34 ± 0.18 vs. 1.63 ± 0.15 µg/dL, respectively) of calves during the pre-weaning period compared with cooling. However, the calves exposed to heat stress in utero tended to have lower (P = 0.08) circulating cortisol (5.73 ± 0.86 vs. 7.61 ± 0.75 µg/dL, respectively) compared with those cooled in utero.

CHAPTER 6
GENERAL DISCUSSION AND SUMMARY

Although 30 years have passed since the first publication which studied positive effects of shade cooling in late gestation on fetal growth and placental function compared with non-shade (Collier et al., 1982b), only a few studies have continued to examine the late gestation heat stress effects on dairy cattle in the past 25 years (Lewis et al., 1984; Wolfenson et al., 1988; Moore et al., 1992). More recently, several studies conducted around the US (California, Urdaz et al., 2006; Florida, do Amaral et al., 2009, 2010, 2011; Thompson et al., 2011; Mississippi, Avendano-Reyes et al., 2010), Mexico (Avendano-Reyes, et al., 2006) and Israel (Adin et al., 2009) reinforced the positive effects of heat stress abatement for dairy cattle during late gestation, especially for the increase in milk production in the subsequent lactation. Additionally, other benefits are observed with heat stress abatement of dry cows such as enhanced innate and acquired immunity during the transition period (do Amaral et al., 2010; 2011). The aim of this dissertation was to elucidate the cellular and physiological mechanisms of the dry period heat stress effect on the mammary gland and further study the effect of late gestation heat stress on metabolic responses at peripheral tissues of transition cows and growth and immune function of their offspring.

The experiment described in Chapter 2 indicates that relative to heat stress abatement, heat stress during the dry period decreases the mammary cell proliferation before parturition. This result suggests that non-cooled heat-stressed dry cows have fewer functional mammary secretory cells at calving compared with cooled cows, which provides a cellular mechanism to explain the decrease in milk production in the next lactation. The fact that enhanced PRL signaling has a positive role in mammary gland growth in the dry period (Dahl, 2008; Dahl et al., 2012) led to the hypothesis that depressed PRL signaling might mediate impaired mammary gland

development during the dry period of heat-stressed cows. However, the data in Chapter 3 did not support that hypothesis. Currently, the mechanism of the effect of heat stress to depress epithelial cell accumulation in the mammary gland remains unclear.

Heat stress results in a decrease in mammary blood flow of cattle relative to those under a thermo-neutral environment (Lough et al., 1990), which may be another possible explanation for the reduced mammary growth. Mammary perfusion is critical to support maximum milk production in lactating dairy cows. During the dry period, the mammary gland undergoes extensive growth (Capuco et al., 1997) and is also highly metabolic, thus the reduction in mammary blood flow with environmental heat stress may also impede mammary growth. Accelerated mammary cell apoptosis occurs at the beginning of the dry period, but it is still unknown how the enhanced mammary involution affects subsequent lactation. The HSPs have cytoprotective effects on stressed cells partly through their anti-apoptotic properties (Lanneau et al., 2007). In vitro, elevated incubation temperatures induce *HSP70* mRNA expression in bovine mammary epithelial cells. Thus, it is likely that mammary cells of heat-stressed dry cows have less apoptotic capacity at the beginning of the dry period. However, if the possible decline in mammary involution by heat stress affects subsequent milk production is not clear. One possible approach to investigate the effect of heat stress on involution would involve frequent mammary biopsies during the initial stages of milk stasis and the dry period.

Cooling heat-stressed dry cows rescues mammary growth and results in greater subsequent milk production. In order to support the greater milk synthesis, cooled cows need to consume more feed during lactation; however, the increase in feed intake of cooled cows relative to non-cooled cows only occurs after several weeks of lactation. In early lactation, prepartum cooled cows develop series of homeorrhetic adaptations to compensate the deficit between energy input,

such as DMI, and output, in the form of milk production. For example, compared with non-cooled heat-stressed cows, prepartum cooled cows have lower insulin responsiveness at peripheral tissues and greater adipose tissue mobilization in early lactation (Chapter 4). These metabolic adaptations of prepartum cooled cows appear to be mediated through the increased effects of GH on peripheral tissues in early lactation. However, the question still remains as to whether muscle tissue has a similar adaptive response as adipose tissue. For example, it is unclear if both muscle and adipose tissue are all insulin insensitive or just one of them is responsible for the greater peripheral tissue insulin resistance observed in prepartum cooled cows in early lactation.

It is well known that maternal heat stress in late gestation is associated with compromised placental function and fetal growth retardation. However, heat stress during the dry period in dairy cows has any carryover effect on the offspring has never been evaluated. Data in Chapter 5 suggests that, in addition to the dramatic effects on the dam, late gestation heat stress also compromises the passive and cell-mediated immune function of offspring, but the overall growth rate during the pre-pubertal period is not affected. But one has to be cautious in interpreting the growth variables of calves presented in Chapter 5 because of the small number of animals used in that experiment that may limit detection of a significant difference. A larger scale study with more animals is warranted in order to confirm or eliminate any carryover effect of maternal heat stress on the growth of offspring.

Cellular mechanisms of altered immune function of offspring by maternal heat stress are still unknown. Preliminary data indicates that relative to newborn calves from non-cooled cows, those from cooled cows during the dry period tend to have lower thymus weight after adjustment body weight (Tao et al., unpublished data). These results suggest that maternal hyperthermia

during late gestation affects development of a primary lymphoid organ in utero. In addition to organ weight, the immune cell population of the thymus of calves may also be altered by maternal hyperthermia in utero, which probably contributes to the impaired PBMC proliferation observed in Chapter 5. Ileal Peyer's patch is the primary lymphoid organ for B-lymphocyte development in cattle from late gestation to the neonatal period (Yasuda et al., 2006) and may also be influenced by hyperthermia in utero. Thus, further studies with regard to morphology, function and immune cell population of primary lymphoid organs in the fetal and neonatal calves from cooled or non-cooled heat-stressed cows during the dry period is also needed to better understand the fetal immune organ reprograming by environmental insults in utero.

From previous studies and the current dissertation, heat stress during the dry period impairs the mammary gland and placental development in late gestation. Indeed, both mammary gland and placenta present similarities of the compromised functions. For example, late gestation heat stress decreases the total uterine (Dreiling et al., 1991; Reynolds et al., 2006) and mammary (Lough et al., 1990) blood flow, which in turn limit fetal and mammary gland development. With the decreased mammary cell proliferation, the mammary gland of heat-stressed dry cows has a lower number of functional cells at parturition (Chapter 2) which is consistent with a smaller number of placental cells from hyperthermic animals (Early et al., 1991). Additionally, the linear relationship between calf birth weight and milk production of the dam (Collier et al., 1982b) also suggests the analogy between mammary gland and placental development in the late gestation. Thus, in future studies with regard to effects of other management tools, such as photoperiod, and nutrition during the dry period, it is important to consider the effect on both mammary gland and placental development.

In summary, the results presented in this dissertation indicate that, in addition to immune function and lactation performance, heat stress during the dry period also profoundly affect mammary gland development before parturition and exerts a residual effect on the metabolism in early lactation. Additionally, maternal heat stress in late gestation also negatively impacts immune competency of the calf.

LIST OF REFERENCES

Accorsi, P. A., B. Pacioni, C. Pezzi, M. Forni, D. J. Flint, and E. Seren. 2002. Role of prolactin, growth hormone and insulin-like growth factor 1 in mammary gland involution in the dairy cow. J. Dairy Sci. 85:507-513.

Adin, G., A. Gelman, R. Solomon, I. Flamenbaum, M. Nikbachat, E. Yosef, A. Zenou, A. Shamay, Y. Feuermann, S. J. Mabjeesh, and J. Miron. 2009. Effects of cooling dry cows under heat load conditions on mammary gland enzymatic activity, intake of food water, and performance during the dry period and after parturition. Livest Sci. 124:189-195.

Agnew, L. L., and I. G. Colditz. 2008. Development of a method of measuring cellular stress in cattle and sheep. Vet. Immunol. Immunopathol. 123:197-204.

Akers, R. M. 2006. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. J. Dairy Sci. 89:1222-1234.

Akers, R. M., D. E. Bauman, A. V. Capuco, G. T. Goodman, and H. A. Tucker. 1981. Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. J. Endocrinol. 109:23-30.

Alvarez, M. B., and H. D. Johnson. 1973. Environmental heat exposure on cattle plasma catecholamine and glucocorticoids. J. Dairy Sci. 56:189-194.

Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. J. Dairy Sci. 87:3746-3761.

Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77:2044-2050.

Asakura, H. 2004. Fetal and neonatal thermoregulation. J Nippon Med Sch. 71:360-370.

Auchtung, T. L., and G. E. Dahl. 2004a. 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., P. E. Kendall, J. L. 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. 2004b. Effects of photoperiod during the dry period on cellular immune function of Dairy cows. J. Dairy Sci. 87:3683-3689.

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., J. W. Fuquay, R. B. Moore, Z. Liu, B. L. Clark and C. Vierhout. 2010. Relationship between accumulated heat stress during the dry period, body condition score, and reproduction parameters of Holstein cows in tropical condition. Trop Anim Health Prod. 42:265-273.

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. Livest Sci. 281:2535-2547.

Bachman, K. C., and M. L. Schairer. 2003. Bovine studies on optimal lengths of dry period. J. Dairy Sci. 86:3027-3037.

Barrington, G. M., T. E. Besser, C. C. Gay, W. C. Davis, J. J. Reeves, and T. B. McFadden. 1997. Effect of prolactin on in vitro expression of the bovine mammary immunoglobulin G1 receptor. J. Dairy Sci. 80:94-100.

Barrington, G. M., T. E. Besser, C. C. Gay, W. C. Davis, J. J. Reeves, T. B. McFadden, and R. M. Akers. 1999. Regulation of the immunoglobulin G1 receptor: effect of prolactin on in vivo expression of the bovine mammary immunoglobulin G1 receptor. J. Endocrinol. 163:25-31.

Barrington, G. M., T. B. McFadden, M. T. Huyler, and T. E. Besser. 2001. Regulation of colostrogenesis in cattle. Livest. Prod. Sci. 70:95-104.

Bate, L. A., and R. R. Hacker. 1985. Influence of environmental temperature during the late gestation and soon after birth on IgG absorption by newborn piglets. Can. J. Anim. Sci. 65:87-93.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and hemeorhesis revisited. Pages 311-328 in Ruminant physiology: Digestion, metabolism, growth and reproduction. P. B. Cronjé, ed. CABI Publishing, Wallingford, UK.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Baumgard, L. H., and R. P. Rhoads. 2011. Ruminant production and metabolic responses to heat stress. J. Anim. Sci. doi: 10.2527/jas.2011-4675.

Baumgard, L. H., J. B. Wheelock, S. R. Sanders, C. E. Moore, H. B. Green, M. R. Waldron, and R. P. Rhoads. 2011. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. J. Dairy Sci. 94:5620-5633.

Beardsley, G. L., L. D. Muller, H. A. Garverick, F. C. Ludens, and W. L. Tucker. 1976. Initiation of parturition in dairy cows with dexamethasone. II. Response to dexamethasone in combination with estradiol benzoate. J. Dairy Sci. 59:241-247.

Bell. A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bell. A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J Mammary Gland Biol Neoplasia. 2:265-278.

Bell, A. W., and R. A. Ehrhardt. 2002. Regulation of placental nutrient transport and implications for fetal growth. Nutr Res Rev. 15:211-230.

Bell, A. W., B. W. McBride, R. Slepetis, R. J. Early, and W. B. Currie. 1989. Chronic heat stress and prenatal development in sheep: I. Conceptus growth and maternal plasma hormones and metabolites. J. Anim. Sci. 67:3289-3299.

Berman. A. 2005. Estimates of heat stress relief needs for Holstein dairy cows. J. Anim. Sci. 83:1377-1384.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal. 4:1167-1183.

Bole-Feysot, C., V. Goffin, M. Edery, N. Binart, and P. A. Kelly. 1998. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr. Rev. 19:225-268.

Brisken, C., A. Ayyannan, C. Nguyen, A. Heineman, F. Reinhardt, J. Tian, S. K. Dey, G. P. Dotto, and R. A. Weingerg. 2002. IGF-2 is a mediator of prolactin-induced morphogenesis in the breast. Dev. Cell. 3:877-887.

Bremmer, D. R., J. O. Christensen, R. R. Grummer, F. E. Rasmussen, and M. C. Wiltbank. 1999. Effects of induced parturition and estradiol on feed intake, liver triglyceride concentration, and plasma metabolites of transition dairy cows. J. Dairy Sci. 82:1440-1448.

Brown, D. E., P. C. Harrison, F. C. Hinds, J. A. Lewis, and M. H. Wallace. 1977. Heat stress effects on fetal development during late gestation in the ewe. J. Anim. Sci. 44:442-446.

Calder, P. C. 2008. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins Leukot. Essent. Fatty Acids. 79:101-108.

Capuco, A. V., R. M. Akers, and J. J. Smith.1997. Mammary growth in Holstein cows during the dry period: quantification of nucleic acids and histology. J. Dairy Sci. 80:477-487.

Capuco, A. V., S. E. Ellis, S. A. Hale, E. Long, R. A. Erdman, X. Zhao and M. J. Paape. 2003. Lactation persistency: Insights from mammary cell proliferation studies. J. Anim. Sci. 81:18-31.

Capuco, A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad Jr. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725.

Capuco, A. V., D. L. Wood, R. Baldwin, K. Mcleod, and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: Relation to milk production and effect of bST. J. Dairy Sci. 84:2177-2187.

Chen, X., A. L. Fahy, A. S. Green, M. J. Anderson, R. P. Rhoads, and S. W. Limesand. 2010. β2-Adrenergic receptor desensitization in perirenal adipose tissue in fetuses and lambs with placental insufficiency-induced intrauterine growth restriction. J. Physiol. 588:3539-3549.

Collier, R. J., D. K. Beede, W. W. Thatcher, L. A. Isreal, and C. J. Wilcox. 1982a. Influences of environment and its modification on dairy animal health and production. J. Dairy Sci. 65:2213-2227.

Collier, R. J., J. L. Collier, R. P. Rhoads, and L. H. Baumgard. 2008. Invited Review: Genes involved in the bovine heat stress response. J. Dairy Sci. 91:445-454.

Collier, R. J., G. E. Dahl, and M. J. Vanbaale. 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 89:1244-1253.

Collier, R. J., S. G. Doelger, H. H. Head, W. W. Thatcher, and C. J. Wilcox. 1982b. 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.

Connor, E. E., R. L. Baldwin, A. V. Capuco, C. M. Evock-Clover, S. E. Ellis, and K. S. Sciabica. 2010. Characterization of glucagon-like peptide 2 pathway member expression in bovine gastrointestinal tract. J. Dairy Sci. 93:5167-5178.

Couret, D., A. Jamin, G. Kuntz-Simon, A. Prunier, and E. Merlot. 2009. Maternal stress during late gestation has moderate but long-lasting effects on the immune system of the piglets. Vet. Immunol. Immunopathol. 131:17-24.

Chilton, B. S., and A. Hewetson. 2005. Prolactin and growth hormone signaling. Curr. Top. Dev. Biol. 68:1-23.

Choshniak, I., D. M. Jenkinson, D. R. Blatchford and M. Peaker. 1982. Blood flow and catecholamine concentration in bovine and caprine skin during thermal sweating. Comp. Biochem. Physiol. 71C:37-42.

Dahl, G. E. 2008. Effects of short day photoperiod on prolactin signaling in dry cows: A common mechanism among tissues and environments. J. Anim. Sci. 86:10-14.

Dahl, G. E., S. Tao, and I. M. Thompson. 2012. Lactation biology symposium: Effects of photoperiod on mammary gland development and lactation. J. Anim. Sci. 90:755-760.

de Vrijer, B., T. R. H. Regnault, R. B. Wilkening, G. Meschia, and F. C. Battaglia. 2004. Placental uptake and transport of ACP, a neutral nonmetabolizable amino acid, in an ovine model of fetal growth restriction. Am. J. Physiol. Endocrinol. Metab. 287:E1114-E1124.

de Vries, A., M. J. Hayen, E. J. Diepersloot, A. H. Sanders, D. W. Webb, and D. R. Bray. 2009. Agreement between fat and protein measurements from DHIA and the AfiLab™ real time milk analyzer. J. Dairy Sci. 92(Suppl. 1):273. (Abstr.)

Dikmen, 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.

Dikmen, S., and P. J. Hansen. 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J. Dairy Sci. 92:109-116.

do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. 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.

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

do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. Bubolz, and G. E. Dahl. 2011. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. J. Dairy Sci. 94:86-96.

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

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84(E. Suppl.):E100-E112.

Dreiling, C. E., F. S. Carman III, D. E. Brown. 1991. Maternal endocrine and fetal metabolic responses to heat stress. J. Dairy Sci. 74:312-327.

Early, R. J., B. W. McBride, I. Vatnick, and A. W. Bell. 1991. Chronic heat stress and prenatal development in sheep: II. Placental cellularity and metabolism. J. Anim. Sci. 69:3610-3616.

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

Elvinger, F., R. P. Natzke, and P. J. Hansen. 1992. Interactions of heat stress and bovine somatotropin affecting physiology and immunology of lactating cows. J. Dairy Sci. 75:449-462.

Etherton, T. D., and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiol. Rev. 78:745-761.

Faurie, A. S., D. Mitchell and H. P. Laburn. 2001. Feto-maternal relationships in goats during heat and cold exposure. Exp. Physiol. 86:199-204.

Farr, V. C., K. Stelwagen, L. R. Cate, A. J. Molenaar, T. B. McFadden, and S. R. Davis. 1996. An improved method for the routine biopsy of bovine mammary tissue. J. Dairy Sci. 79:543-549.

Feng, Z., A. Marti, B. Jehn, H. J. Altermatt, G. Chicaiza, and R. Jaggi. 1995. Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. J. Cell Biol. 131:1095-1103.

Flint, D. J., E. Tonnor, C. H. Knight, C. B. A. Whitelaw, J. Webster, M. Barber, and G. Allan. 2001. Control of mammary involution by insulin-like growth factor binding proteins: role of prolactin. Livest. Prod. Sci. 70:115-120.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. J. Anim. Sci. 88:E205-E215.

Fuquay, J. W. 1981. Heat stress as it affects animal production. J. Anim. Sci. 51:164-174.

Gheorghe, C. P., R. Goyal, A. Mittal, and L. D. Longo. 2010. Gene expression in the placenta: maternal stress and epigenetic responses. Int. J. Dev. Biol. 54:507-523.

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

Grummer, R. R., and R. R. Rastani. 2004. Why reevaluate dry period length? J. Dairy Sci. 87:(E. Suppl.):E77-E85.

Guerriero Jr, V., and D. A. Raynes. 1992. Synthesis of heat stress proteins in lymphocytes from livestock. J. Anim. Sci. 68:2779-2783.

Gutierrez, J. A., and V. Guerriero Jr. 1995. Relative abundance of bovine Hsp70 mRNA and protein. Biochim. Biophys. Acta. 1260:239-242.

Hahn, G. L. 1997. Dynamic responses of cattle to thermal heat loads. J. Anim. Sci. 77:10-20.

Hayirli, A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet. Res. Commun. 30:749-774.

Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. J. Dairy Sci. 84:1218-1230.

Hodgson, J. C., S. M. Rhind, and D. J. Flint. 1997. Influence of maternal nutrition and stress on gut permeability to immunoglobulin in newborn lambs. Biochem. Soc. Trans. 25:339S.

Hoffmann, E., O. Dittrich-Breiholz, H. Holtmann, and M. Kracht. 2002. Multiple control of interleukin-8 gene expression. J. Leukoc. Biol. 72:847-855.

Holtenius, S., S. Agenas, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal response. J. Dairy Sci. 86:883-891.

Hough, R. L., F. D. McCarthy, H. D. Kent, D. E. Eversole, and M. L. Wahlberg. 1990. Influence of nutritional restriction during late gestation on production measures and passive immunity in beef cattle. J. Anim. Sci. 68:2622-2627.

Janovick, N. A., and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086-3102.

Janson, P. C, and O. Winqvist. 2011. Epigenetics - the key to understand immune responses in health and disease. Am J Reprod Immunol. 66(Suppl. 1):72-74.

Joss-Moore, L. A., D. B. Metcalfe, K. H. Albertine, R. A. McKnight, and R. H. Lane. 2010. Epigenetics and fetal adaptation to perinatal events: Diversity through fidelity. J. Anim. Sci. 88(Suppl.):E216-E222.

Kadzere, C. T., M. R. Murphy, N. Silaninove, and E. Maltz. 2002. Heat stress in lactating dairy cows: a review. Livest. Prod. Sci. 77:59-91.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. Metab. Clin. Exp. 27:1893-1902.

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

Kim, K. H., D. H. Kim, Y. K. OH, S. S. Lee, H. J. Lee, D. W. Kim, Y. J. Seol, and N. Kimura. 2010. Productivity and energy partition of late lactation dairy cows during heat exposure. Anim. Sci. J. 81:58-62.

Krebs, D. L., and D. J. Hilton. 2001. SOCS proteins: negative regulators of cytokine signaling. Stem Cells. 19:378-387.

Kregel, K. C. 2002. Invited review: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol. 92:2177-2186.

Laburn, H. P., D. Mitchell, and K. Goelst. 1992. Fetal and maternal body temperatures measured by radiotelemetry in near-term sheep during thermal stress. J. Appl. Physiol. 72:894-900.

Laburn, H., A. Faurie, and D. Mitchell. 2000. The thermal physiology of the ruminant fetus. Pages 295-310 in Ruminant physiology: Digestion, metabolism, growth and reproduction. P. B. Cronjé, ed. CABI Publishing, Wallingford, UK.

Laburn, H. P., A. Faurie, K. Goelst, and D. Mitchell. 2002. Effects on fetal and maternal body temperatures of exposure of pregnant ewes to heat, cold, and exercise. J. Appl. Physiol. 92:802-808.

Lacetera, N., U. Bernabucci, D. Scalia, L. Basiricò, 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.

Leos, R. A., M. J. Anderson, X. Chen, J. Pugmire, K. A. Anderson, and S. W. Limesand. 2010. Chronic exposure to elevated nor-epinephrine suppresses insulin secretion in fetal sheep with placental insufficiency and intrauterine growth restriction. Am. J. Physiol. Endocrinol. Metab. 298:E770-E778.

Lewis, G. S., W. W. Thatcher, E. L. Bliss, M. Drost, and R. J. Collier. 1984. Effects of heat stress during pregnancy on postpartum reproductive changes in Holstein cows. J. Anim. Sci. 58:174-186.

Limesand, S. W., J. Jensen, J. C. Hutton, and W. W. Hay Jr. 2005. Diminished β-cell replication contributes to reduced β-cell mass in fetal sheep with intrauterine growth restriction. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288:R1297-R1305.

Limesand, S. W., P. J. Rozance, D. Smith and W. W. Hay Jr. 2007. Increased insulin sensitivity and maintenance of glucose utilization rates in fetal sheep with placental insufficiency and intrauterine growth restriction. Am. J. Physiol. Endocrinol. Metab. 293:E1716-E1725.

Limesand, S. W., P. J. Rozance, G. O. Zerbe, J. C. Hutton, and W. W. Hay Jr. 2006. Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. Endocrinology. 147:1488-1497.

Lomborg, S. R., L. R. Nielsen, P. M. H. Heegaard, and S. Jacobsen. 2008. Acute phase protein in cattle after exposure to complex stress. Vet. Res. Commun. 32:575-582.

López-Meza, J. E., L. Lara-Zárate, and A. Ochoa-Zarzosa. 2010. Effects of prolactin on innate immunity of infectious disease. Open Neuroendocrinol J. 3:175-179.

Lough, D. S., D. L. Beede, and C. J. Wilcox. 1990. Effects of feed intake and thermal stress on mammary blood flow and other physiological measurements in lactating dairy cows. J. Dairy Sci. 73:325-332.

Machado-Neto, R., C. N. Graves, and S. E. Curtis. 1987. Immunoglobulins in piglets from sows heat-stressed prepartum. J. Anim. Sci. 65:445-455.

Magalhães, V. J. A., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci. 91:1497-1509.

Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J. Dairy Sci. 81:585-595.

Malven, P. V., H. H. Head, R. J. Collier, and F. C. Buonomo. 1987. Periparturient changes in secretion and mammary uptake of insulin and in concentrations of insulin and insulin-like growth factors in milk of dairy cows. J. Dairy Sci. 70:2254-2265.

Marchbank, T., G. Davison, J. R. Oakes, M. A. Ghatei, M. Patterson, M. P. Moyer, and R. J. Playford. 2011. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am. J. Physiol. Gastrointest. Liver Physiol. 300:G477-G484.

Martino, D., and S. Prescott. 2011. Epigenetics and prenatal influences on asthma and allergic airways disease. Chest. 139:640-647.

Maust, L. E., R. E. McDowell, and N. W. Hooven. 1972. Effect of summer weather on performance of Holstein cows in three stages of lactation. J. Dairy Sci. 55:1133-1139.

McDowell, R. E., E. G. Moody, P. J. Van Soest, R. P. Lehmann, and G. L. Ford. 1969. Effect of heat stress on energy and water utilization of lactating cows. J. Dairy Sci. 52:188-194.

Merlot, E., D. Couret, and W. Otten. 2008. Prenatal stress, fetal imprinting and immunity. Brain Behav. Immun. 22:42-51.

Miller, A. R. E., R. A. Erdman, L. W. Douglass, and G. E. Dahl. 2000. Effects of photoperiodic manipulation during the dry period of dairy cows. J. Dairy Sci. 83:967-962.

Moore, R. B., J. W. Fuquay and W. J. Drapala. 1992. Effects of late gestation heat stress on postpartum milk production and reproduction in dairy cattle. J. Dairy Sci. 75:1877-1882.

Morin, D. E., S. V. Nelson, E. D. Reid, D. W. Nagy, G. E. Dahl, and P. D. Constable. 2010. Effect of colostral volume, interval between calving and first milking, and photoperiod on colostral IgG concentrations in dairy cows. J. Am. Vet. Med. Assoc. 237:420-428.

Morrison, J. L. 2008. Sheep models of intrauterine growth restriction: fetal adaptations and consequences. Clin. Exp. Pharmacol. Physiol. 35:730-743.

Morrison, J. L., J. A. Duffield, B. S. Muhlhausler, S. Gentili, I. C. McMillen. 2010. Fetal growth restriction, catch-up growth and the early origins of insulin resistance and visceral obesity. Pediatr. Nephrol. 25:669-677.

Muller, L. D., G. L. Beardsley, R. P. Ellis, D. E. Reed and M. J. Owens. 1975. Calf response to the initiation of parturition in dairy cows with dexamethasone or dexamethasone with estradiol benzoate. J. Anim. Sci. 41:1711-1716.

Nardone, A., N. Lacetera, U. Bernabucci and B. Ronchi. 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. J. Dairy Sci. 80:836-844.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle, 7th rev. ed. Nat. Acad. Press, Washington, DC.

Nelissen, E. C. M., A. P. A. van Montfoort, J. C. M. Dumoulin, and J. L. H. Evers. 2011. Epigenetics and the placenta. Hum. Reprod. Update. 17:397-417.

Ocfemia, G. O., A. Sharun, H. M. Miller and J. H. G. Holmes. 1993. Reduced foetal growth and lactation by does heat-stressed from mid-pregnancy. Small Rumin. Res. 11:33-43.

Orbach, H. and Y. Shoenfeld. 2007. Hyperprolactinemia and autoimmune diseases. Autoimmun Rev. 6:537-542.

Petersen, H. H., J. P. Nielsen, and P. M. H. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35:163-187.

Pezeshki, A., J. Mehrzad, G. R. Ghorbani, H. R. Rahmani, R. J. Collier, and C. Burvenich. 2007. Effects of short dry periods on performance and metabolic status in Holstein dairy cows. J. Dairy Sci. 90:5531-5541.

Pires, J. A. A., J. B. Pescara, A. E. Brickner, N. Silva del Rio, A. P. Cunha, and R. R. Grummer. 2008. Effects of abomasal infusion of linseed oil on responses to glucose and insulin in Holstein cows. J. Dairy Sci. 91:1378-1390.

Purwanto, B. P., M. Harada, and S. Yamamoto. 1994. Effect of environmental temperature on heat production and its energy cost for thermoregulation in dairy heifers. Asian-australas. J. Anim. Sci. 7:179-182.

Quigley, III, J. D., and J. J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre- and postcalving. J. Dairy Sci. 81:2779-2790.

Regnault, T. R. H., J. E. Friedman, R. B. Wilkening, R. V. Anthony, and W. W. Hay Jr. 2005. Fetoplacental transport and utilization of amino acids in IUGR - A review. Placenta. 26(Suppl. A):S52-S62.

Reber, A. J., A. Lockwood, A. R. Hippen, and D. J. Hurley. 2006. Colostrum induced phenotypic and trafficking changes in maternal mononuclear cells in peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. Vet. Immunol. Immunopathol. 109:139-150.

Reber, A. J., D. C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K. A. Holbert, L. Marshall, and D. J. Hurley. 2008a. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system I. Effects on monocyte lineage cells. Vet. Immunol. Immunopathol. 123:186-196.

Reber, A. J., D. C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K. A. Holbert, L. Marshall, and D. J. Hurley. 2008b. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system II. Effects on neonatal lymphocytes. Vet. Immunol. Immunopathol. 123:186-196.

Regnault, T. R. H., B. de Vrijer, H. L. Galan, M. L. Davidsen, K. A. Trembler, F. C. Battaglia, R. B. Wilkening, and R. V. Anthony. 2003. The relationship between transplacental O2 diffusion and placental expression of PIGF, VEGF and their receptors in a placental insufficiency model of fetal growth restriction. J. Physiol. 550:641-656.

Regnault, T. R. H., J. E. Friedman, R. B. Wilkening, R. V. Anthony and W. W. Hay Jr. 2005. Fetoplacental transport and utilization of amino acids in IUGR – A review. Placenta. 26(Suppl. A):S52-S62.

Renier, G., I. Clement, A. Desfaits, and A. Lambert. 1996. Direct stimulatory effect of insulin-like growth factor-I on monocyte and macrophage tumor necrosis factor-α production. J. Endocrinol. 137:4611-4618.

Reynolds, L. P., P. P. Borowicz, J. S. Caton, K. A. Vonnahme, J. S. Luther, C. J. Hammer, K. R. Maddock Carlin, A. T. Grazul-Bilska, and D. A. Redmer. 2010. Developmental programming: The concept, large animal models, and the key role of uteroplacental vascular development. J. Anim. Sci. 88:E61-E72.

Reynolds, L. P., J. S. Caton, D. A. Redmer, A. T. Grazul-Bilska, K. A. Vonnahme, P. P. Borowicz, J. S. Luther, J. M. Wallace, G. Wu, and T. E. Spencer. 2006. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J. Physiol. 572:51-58.

Reynolds, L. P., C. L. Ferrell, J. A. Nienaber, and S. P. Ford. 1985. Effects of chronic environmental heat stress on blood flow and nutrient uptake of the gravid bovine uterus and foetus. J Agric Sci. 104:289-297.

Rhoads, M. L., J. W. Kim, R. J. Collier, B. A. Crooker, Y. R. Boisclair, L. H. Baumgard, and R. P. Rhoads. 2010. Effects of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness. J. Dairy Sci. 93:170-179.

Rhoads M. L., R. P. Rhoads, M. J. VanBaale, R. J. Collier, S. R. Sanders, W. J. Weber, B. A. Crooker, and L. H. Baumgard. 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: 1. Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92:1986-1997.

Roman-Ponce, H., W. W. Thatcher, and C. J. Wilcox. 1981. Hormonal interrelationships and physiological responses of lactating dairy cows to a shade management system in a subtropical environment. Theriogenology. 16:139-154.

Sanders, V. M. 2006. Epigenetic regulation of Th1 and Th2 cell development. Brain Behav. Immun. 20:317-324.

Sangild, P. T. 2003. Uptake of colostral immunoglobulins by the compromised newborn farm animals. Acta Vet Scand Suppl. 98:105-122.

Schmitt, D., H. A. Garverick, E. C. Mather, J. D. Sikes, B. N. Day and R. E. Erb. 1975. Induction of parturition in dairy cattle with dexamethasone and estradiol benzoate. J. Anim. Sci. 40:261-268.

Scholzen, T., and J. Gerdes. 2000. The Ki-67 protein: From the known and the unknown. J. Cell. Physiol. 182:311-322.

Schröder, H. J., and G. G. Power. 1997. Engine and radiator: Fetal and placental interactions for heat dissipation. Exp. Physiol. 82:403-414.

Sechen, S. J., F. R. Dunshea, and D. E. Bauman. 1990. Somatotropin in lactating cows: effect on response to epinephrine and insulin. Am. J. Physiol. Endocrinol. Metab. 258:E582-E588.

Silva, L. F. P., M. J. Vandehaar, B. K. Whitlock, R. P. Radcliff, and H. A. Tucker. 2002. Short communication: Relationship between body growth and mammary development in dairy heifers. J. Dairy Sci. 85:2600-2602.

Sorensen, M. T., J. V. NØrgaard, P. K. Theil, M. Vestergaard, and K. Sejrsen. 2006. Cell turnover and activity in mammary tissue during lactation and dry period in dairy cows. J. Dairy Sci. 89:4632-4639.

Stott, G. H. 1980. Immunoglobulin absorption in calf neonates with special considerations of stress. J. Dairy Sci. 63:681-688.

Tarazón-Herrera, M., J. T. Huber, J. Santos, H. Mena, L. Nusso, and C. Nussio. 1999. Effects of bovine somatotropin and evaporative cooling plus shade on lactation performance of cows during summer heat stress. J. Dairy Sci. 82:2352-2357.

Thorn, S. R., T. R. H. Regnault, L. D. Brown, P. J. Rozance, J. Keng, M. Roper, R. B. Wilkening, W. W. Hay Jr, and J. E. Friedman. 2009. Intrauterine growth restriction increases fetal hepatic gluconeogenic capacity and reduces messenger ribonucleic acid translation initiation and nutrient sensing in fetal liver and skeletal muscle. Endocrinology. 150:3021-3030.

Thompson, M., A. P. Monteiro, and G. E. Dahl. 2011. Dry period seasonal effects on the subsequent lactation. J. Dairy Sci. 94(Suppl. 1):752. (Abstr.)

Tuchscherer, M., E. Kanitz, W. Otten, and A. Tuchscherer. 2002. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet. Immunol. Immunopathol. 86:195-203.

Tucker, H. A. 1987. Quantitative estimates of mammary growth during various physiological states: a review. J. Dairy Sci. 70:1958-1966.

Tucker, H. A. 2000. Hormones, mammary growth, and lactation: a 41-year perspective. J. Dairy Sci. 83:874-884.

Tudor, G. D. 1972. The effect of pre- and post-natal nutrition on the growth of beef cattle. 1. The effect of nutrition and parity of the dam on calf birth weight. Aust. J. Agric. Res. 23:389-395.

Urdaz, J. H., M. W. Overton, D. A. Moore, and J. E. P. Santos. 2006. Technical note: Effects of adding shade and fans to a feedbunk sprinkler system for preparturient cows on health and performance. J. Dairy Sci. 89:2000-2006.

Vermorel, M., C. Dardillat, J. Vernet, Saido, and C. Demigne. 1983. Energy metabolism and thermoregulation in the newborn calf. Ann. Rech. Vet. 14:382-389.

Vernon, R. G., G. M. Pond. 1997. Adaptations of maternal adipose tissue to lactation. J Mammary Gland Biol Neoplasia. 2:231-241.

Wall, E. H., T. L. Auchtung, G. E. Dahl, S. E. Ellis, and T. B. McFadden. 2005. Exposure to short day photoperiod during the dry period enhances mammary growth in dairy cows. J. Dairy Sci. 88:1994-2003.

Wayman, O., H. D. Johnson, C. P. Merian, and I. L. Berry. 1962. Effect of ad libitum or force-feeding of two rations on lactating dairy cows subject to temperature stress. J. Dairy Sci. 45:1472-1478.

Wegner, T. N., J. D. Schuh, F. E. Nelson, and G. H. Stott. 1976. Effect of stress on blood leucocyte and milk somatic cell counts in dairy cows. J. Dairy Sci. 59:949-956.

West, J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86:2131-2144.

Wheelock, J. B., R. P. Rhoads, M. J. VanBaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644-655.

Wilde, C. J., V. P. C. Addey, P. Li, and D. G. Fernig. 1997. Programmed cell death in bovine mammary tissue during lactation and involution. Exp. Physiol. 82:943-953.

Wise, M. E., D. V. Armstrong, J. T. Huber, R. Hunter, and F. Wiersma. 1988. Hormonal alterations in the lactating dairy cow in response to thermal stress. J. Dairy Sci. 71:2480-2485.

Wolfenson, D., I. Flamenbaum, and A. Berman. 1988. Dry period heat stress relief effects on prepartum progesterone, calf birth weight, and milk production. J. Dairy Sci. 71:809-818.

Wood, C. E. 2005. Estrogen/hypothalamus-pituitary-adrenal axis interactions in the fetus: the interplay between placenta and fetal brain. J. Soc. Gynecol. Investig. 12:67-76.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316-2337.

Yasuda, M., C. N. Jenne, L. J. Kennedy, and J. D. Reynolds. 2006. The sheep and cattle Peyer's patch as a site of B-cell development. Vet. Res. 37:401-415.

Yates, D. T., A. S. Green, and S. W. Limesand. 2011. Catecholamines mediate multiple fetal adaptations during placental insufficiency that contribute to intrauterine growth restriction: Lessons from hyperthermic sheep. J Pregnancy. 2011:1-9.

Yousef, M. K., and H. D. Johnson. 1966. Calorigenesis of dairy cattle as influenced by thyroxine and environmental temperature. J. Anim. Sci. 25:150-156.

Zimbleman, R. B., R. P. Rhoads, L. H. Baumgard, and R. J. Collier. 2009. Revised temperature humidity index (THI) for high producing dairy cows. J. Dairy Sci. 92(Suppl. 1):347. (Abstr.)

BIOGRAPHICAL SKETCH

Sha Tao was born in Kaifeng, Henan, China. In 2000, he entered the Henan University of Technology and obtained his B.S. in agriculture and M.S. in animal sciences at the same university. In 2007, he moved to Gainesville, Florida, USA with his wife and joined the Animal Molecular and Cellular Biology program of University of Florida as a Ph.D. student in 2008. His Ph. D. work focuses on the effect of heat stress during the dry period on dairy cattle.

Sha has been married to his beautiful wife, Yinping Guo, for 5 years and they just had a lovely baby boy, Ye-han Tao, in February 2012.