Periconceptional Heat Stress of Holstein Cows Affects Subsequent Production Parameters Measured During Adulthood.

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ABSTRACT

Periconceptional heat stress is known to reduce the likelihood of establishing pregnancy; however, some conceptuses will survive. Of the pregnancies that continue to term, a proportion will be heifers which are retained as replacement animals. Alterations that occur as a result of exposure to thermal stress during such critical stages in development likely result in differential performance between the heat stress-conceieved (HSC) cows and thermoneutral-conceived (TNC) cows. National Dairy Herd Improvement Association data was obtained from Dairy Records Management Systems. Records (n =14,189,891) included cows born between 1977 and 2010 in FL, GA, SC, MS, LA, AL, and TX. Records were edited to include only Holsteins born between 2000 and 2010 (n = 704,419). Conception dates were calculated by subtracting 276 d from the recorded birth date. Records for cows conceived within the months of June, July, and August were retained as HSC cows; cows conceived within the months of December, January, and February were retained as TNC contemporaries. Significant differences (P<0.01) in mature-equivalent milk yield were observed in all first lactation cows, and in cows that were retained within one herd for three lactations. In the latter group alterations in milk compositions were statistically significant (P<0.01), but not biologically so. Furthermore, significant differences (P<0.01) in days open were observed in cows retained within one herd for three lactations. The effects of periconceptional heat stress were particularly noticeable during seasonal comparisons, with HSC cows seemingly having an advantage in subsequent episodes of heat stress.

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Dedication

Believing in yourself can be one of the hardest things to do. Trusting that things will work out when everything you've ever planned for changes can be immensely trying. Working through pain can feel impossible …but staying strong; accepting what will be, will be; and knowing you can accomplish what you put your mind to can get you through anything.

This is dedicated to years of hard work, to realizing that doing what makes you happy is the only way to get through life, and to finally accepting that life doesn't always take you where you thought you would go- but it is so much more satisfying to enjoy the journey than to worry about where you'll end up.

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List of Abbreviations

DIM – Days in milk

DMI – Dry matter intake

FSH – Follicle-stimulating hormone

HSC – Heat stress conceived

HSCONCP – Heat stress at the time of conception-variable

HSP70 – 70 kilodalton heat shock protein IGF-I – insulin-like growth factor I

LACTNO –Lactation number -variable

LH – Luteinizing hormone

ME –Mature-equivalent

NEBAL – Negative-energy balance

NEFA – Non-esterified fatty acids

SOC – Season of calving - variable

STAT-5 – Signal transducer and activator of transcription 5

THI- Temperature-humidity index

TNC – Thermoneutrual conceived

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CHAPTER I: Literature Review

Heat Stress

An animal's core body temperature is maintained through metabolic heat production. This heat production is a result of energy consumption during processes necessary for maintenance and production, e.g. growth, lactation, gestation. Under thermoneutral conditions mammals maintain a core body temperature greater than ambient temperature (Silanikove, 2000; Kadzere et al., 2002; Collier et al., 2006a; Bernabucci et al., 2010; Renaudeau et al., 2012). Heat is able to flow from the animal's warmer core to the cooler environment. This heat exchange occursthrough conduction, convection, and radiation (Silanikove, 2000; Kadzere et al., 2002; Collier et al., 2006a; Bernabucci et al., 2010; Renaudeau et al., 2012). With conduction, heat is exchanged with the environment through physical contact, for example a cow lying on a concrete pad will transfer heat from the body, to the pad. Convection is the exchange of heat through liquids or gasses flowing by each other; in mammals this is heat exchange via air near the surface of the skin, as well as internal heat movement via blood flow. With radiation, heat is emitted or absorbed through electromagnetic energy. Solar radiation is absorbed by the surface of the animal by both direct sunlight and reflection off of surfaces around the animal. The absorption of radiant energy is dependent on an object's surface (i.e. dull or shiny) and color; for example a primarily black cow will absorb more heat than a primarily white cow (Spiers, 2012).

A fourth method of heat loss occurs through a vapor/pressure gradient: evaporation (Silanikove, 2000; Kadzere et al., 2002; Collier et al., 2006a; Renaudeau et al., 2012).Heat is lost as thermal energy is used to transfer liquid into a gaseous phase (Spiers, 2012).

These four heat exchange mechanisms are not mutually exclusive, and as such the ability of a homeothermic animal to maintain body temperature can be affected by numerous environmental variables. These variables include solar radiation, wind speed, humidity, and temperature (Collier et al., 1982; Collier et al., 2006a; Dikmen and Hansen, 2009; Bernabucci et al., 2010; Berman, 2011). Evaporation can be the most effective means of heat transfer. For every gram of water vaporized, approximately 2.4 kJ of heat is lost (Renaudeau et al., 2012). However, evaporation efficacy is highly dependent on the humidity level of the surrounding air.If the air is saturated with water, evaporation no longer serves as an effective means of dissipating heat.

In cattle, the primary modes of heat dissipation include non-evaporative skin-to-air transfer (Berman, 2011; Renaudeau et al., 2012), or the evaporative dissipation of heat through panting and sweating (Bohmanova et al., 2007; Renaudeau et al., 2012). When facing heat stress, a cow’s early response is to pant. Panting increases the rate gas enters and leaves the lungs (i.e. respiratory ventilation rate) and the volume of gas that is inhaled or exhaled from the lungs per minute (i.e. respiratory minute volume), thereby improving evaporative heat loss from the respiratory system (Renaudeau et al., 2012).

As temperature, humidity, and solar radiation increase, mammals reach a point when core body temperature exceeds the individual’s thermoneutral range; that is, a point where the animal experiences discomfort. When the body temperature rises and the animal can no longer dissipate heat, the animal is considered to be heat stressed or hyperthermic (Collier et al., 2006a; Bohmanova et al., 2007; Dikmen and Hansen, 2009; Bernabucci et al., 2010; Hammami et al., 2013). Hyperthermia is important because it is associated with a magnitude of adverse effects that ultimately affect animal well-being andproductivity.

Innate thermoregulatory mechanisms require shifts in energy expenditure as environmental conditions cause ambient temperature to reach or surpass the animal's basal body temperature. Increasing the amount of energy spent on thermoregulation decreases the amount of energy available for other metabolic processes such as growth, lactation, and reproduction. Furthermore physiological changes employed to optimize heat dissipation can result in metabolic disruption. Panting can result in respiratory alkalosis(Collier et al., 1982; Bernabucci et al., 2010; Renaudeau et al., 2012).Water and mineral loss from panting and sweating can lead to moderate-to-severe dehydration and mineral imbalances; for example a large amount of potassium can be lost via sweat (Renaudeau et al., 2012). Shifts in water concentrations and blood flow can disrupt the animal's osmotic balance and blood pressure (Bernabucci et al., 2010). Heat stress may also lead to body temperature rising in an uncontrolled manner in a condition known as heat stroke. Ultimately this can lead to organ dysfunction and death.

Temperature-Humidity Index

It is not practical to constantly monitor a cow's response to the environment (i.e. panting, sweating, alterations in body temperature) to determine if the animal is being affected by heat. For this reason producers and researchers utilize a model to predict when an animal will experience heat stress. A temperature-humidity index (THI) accounts for independent variations in temperature and humidity to determine a range within which an animal is expected to be thermally stressed.

There are a number of temperature-humidity indices; each index takes into account different environmental factors, or weighs environmental factors differently. The environmental variables may include ambient temperature (i.e. dry bulb temperature), the amount of water vapor in air- expressed as a percentage needed for total saturation at the same temperature (i.e.relative humidity), the water-to-air saturation temperature (i.e. dew point temperature), the temperature at 100% humidity (i.e. wet bulb temperature), and the combined effects of solar radiation, ambient air temperature and wind speed (i.e. black globe temperature) (Collier et al., 2006a; Bohmanova et al., 2007; Dikmen and Hansen, 2009; Hammami et al., 2013). Different indices have been developed for various livestock species and climates (Bohmanova et al., 2007; Gaughan et al., 2012); despite this, one index value is typically used to denote heat stress for all the indices. This index value, 72, is considered the threshold of mild heat stress, with heat stress severity increasing with the index value.

DEG		RELATIVE HUMIDITY																				
C	F	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
24	75														72	72	73	73	74	74	75	75
27	80							72	72	73	73	74	74	75	76	76	77	78	78	79	**79**	**80**
29	85			72	72	73	74	75	75	76	77	78	78	79	**80**	**81**	**81**	**82**	**83**	**84**	**84**	**85**
32	90	72	73	74	75	76	77	78	79	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**86**	**87**	**88**	**89**	***90***
35	95	75	76	77	78	79	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	**89**	***90***	***91***	***92***	***93***	***94***	***95***
38	100	77	78	79	**80**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	***90***	***91***	***92***	***93***	***94***	***95***	***97***	***98***	***99***	
41	105	79	**80**	**82**	**83**	**84**	**86**	**87**	**88**	***89***	***91***	***92***	***93***	***95***	***96***	***97***						
43	110	**81**	**83**	**84**	**86**	**87**	**89**	***90***	***91***	***93***	***94***	***96***	***97***									
46	115	**84**	**85**	**87**	**88**	***90***	***91***	***93***	***95***	***96***	***97***											
49	120	**88**	**88**	***89***	***91***	***93***	***94***	***96***	***98***													

Mild Stress
Medium Stress
Severe Stress

Figure 1.1. Temperature-humidity Index (THI)

Temperature-humidity index chart indicating the level of stress expected to be experienced by dairy cattle, using the equation: THI = $T_{dry\ bulb}$ + ((0.36 * $T_{dew\ point}$) + 41.2). Modified from Dr. Frank Weirsma (1990), Department of Agricultureal Engineering, University of Arizona, Tuscon and retrieved from the Dairy Calf & Heifer Association (Weiersma, 1990).

There has been some contention over the validity of THI in modern agriculture. The original THI was developed in 1959 (Johnson, 1976; Zimbelman et al., 2009; Gaughan et al., 2012) and many changes in the environment and genetics of dairy cattle have occurred since then. Zimbleman and colleagues (2009) contended that the typical index value of 72 underestimates the onset of heat stress in modern multiparous lactating Holstein cows. They found that for high producing cows, milk yield first significantly declined when a minimum daily THI reached a threshold of 65, or an average THI over seventeen hours reached a threshold of 68. Similarly, Freitas and colleagues (2006) found that primiparous Holsteins in the Southeastern United States experienced heat stress at a THI of 70. These findings suggest that threshold values of THI vary with parity and location, and as such using a blanket threshold of 72 may not be appropriate for all circumstances.

Heat Stress on Reproduction

Heat stress on endocrine function, cyclicity, and conception:

During periods of heat stress, feed intake is depressed; this decline in intake contributes to negative energy balance and loss of body condition (Collier et al., 1982; Wilson et al., 1998b; Ravagnolo et al., 2000; Bernabucci et al., 2010; Wheelock et al., 2010). Due to reduced intake and increased energy demands for maintenance, heat stressed dairy cows are more likely to experience metabolic disorders such as subclinical or clinical ketosis and liver lipidosis than non-heat stressed contemporaries (Bernabucci, 2012). In addition to the effect of intake on energy balance, a decrease in feed consumption leads toreduced feed in the rumen, thereby causing cows to ruminate less; this decreases stimulation for saliva production and results in decreased buffering agents entering the rumen. With reduced buffering agents, rumen pH declines leading to rumen acidosis, which can enhance the risk of developing other deleterious health conditions (e.g. laminitis; (Bernabucci, 2012). Furthermore, since blood flow has shifted away from the digestive system, products of digestion are inefficiently absorbed (Collier et al., 1982) which can contribute to negative energy balance (NEBAL) and increased strain on the animal.

Despite the decrease in intake, levels of non-esterified fatty acids (NEFA) do not increase as would be expected in a typical NEBAL situation; instead, insulin concentrations gradually rise (Baumgard and Rhoads, 2012; Wheelock et al., 2010). Increase in basal insulin can be attributed to insulin's role in activating and up-regulating the production of heat shock proteins, such as the 70 kilodalton heat shock protein (HSP70; Li et al., 2006). Furthermore, increases in basal and glucose-stimulated insulin can be attributed to the absence of typical NEBAL excess NEFA, which cause pancreas β-cell apoptosis (Baumgard and Rhoads, 2012). The increase in insulin synthesis seen in hyperthemic animals may be important in overcoming heat stress events; however it marks an alteration in typical NEBAL metabolism. Homeothermic lactating cows in a NEBAL status utilize NEFA oxidation and lipid metabolism as a significant source of energy, allowing glucose to be spared for milk production. Heat stressed lactating cows do not employ this typical metabolic shift, but rather continue to use glucose as a primary energy source. The rise in circulating insulin, which has potent antilipolytic properties, is likely responsible for the failure of glucose sparing mechanisms (Baumgard and Rhoads, 2012).

Heat stress also alters the somatotropin axis (Baumgard and Rhoads, 2012), essential for growth and development. Cows experiencing chronic heat stress have reduced somatotropin and a reduction of insulin-like growth factor (IGF-I) (Baumgard and Rhoads, 2012). Hyperthermic lactating cows also have reduced circulating concentrations of thyroxine and triiodothyronine (Gwazdauskas, 1985). Since the reproductive system is sensitive to whole-body health and well-being, any shifts in endocrine function, whole body metabolism, or animal health can lead to alterations in reproductive efficiency. However, that is not to say that hyperthermia does not result in physiological changes that directly reduce reproductive efficiency.

During heat stress, blood is shifted to the periphery to facilitate cooling; this diversion leads to elevated temperatures within the reproductive organs (Gwazdauskas, 1985). Increased temperatures within the reproductive organs may compromise gamete quality or reproductive tract function, thereby reducing fertility. Furthermore, in periods of acute heat stress cows experience changes in circulating concentrations of progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) (Bernabucci et al., 2010) and estradiol (Gwazdauskas, 1985; Wilson et al., 1998b;(Wolfenson and Thatcher, 2012)),which affect estrous cycle length, estrus expression, and conception rates. These changes will be described in detail in the following paragraphs.

Alterations in ovarian function occur in both heifers and cows, with a more severe impediment occurring in the latter, most likely as a result of an increase in metabolic heat production. Heifers experiencing heat stress recruit fewer follicles during each follicular wave. These heifers also have a smaller dominant follicle and slower regression of the dominant follicle in the first two follicular waves compared to contemporaries experiencing thermoneutral conditions. Furthermore, the corpus luteum is maintained for a longer period of time in heifers heat stressed over the course of one estrous cycle, making the cycle in heat stressed heifers longer than those in thermoneutral conditions (Wilson et al., 1998a). An increase in estrous cycle length decreases opportunities for rebreeding by reducing the number of estrous cycles that can occur in the same time frame as normal length cycles and may also decrease developmental competence as a result of oocyte aging.

In cows, the first follicular wave occurs similarly between heat stressed and non-heat stressed individuals. During the second follicular wave the dominant follicle of heat stressed cows initially is larger than the dominant follicle of thermoneutral cows. Thereafter, the dominant follicle of the heat stressed cow grows more slowly resulting in a dominant follicle that is similar in size, or smaller, than what is seen in a thermoneutral cow. During the estrous cycle studied by Wilson and colleagues (1998b), the majority (91%) of thermoneutral cows underwent a cycle with two-follicular waves before ovulation occurred; however, of the cows that experienced heat stress, 82% had three or more follicular waves.

In the same study, Wilson and colleagues (1998b) determined structural and functional luteolysis was delayed in heat stressed cows. In thermoneutral cows, structural regression (as determined by ultrasonography) occurred around d 17 of the estrus cycle; the heat stress cows maintained an observable corpus luteum through d 21. Functional luteolysis, defined as serum progesterone <1 ng/ml, was delayed8.7 ± 2.4 d in the heat stressed cows compared to the thermoneutral cows. By d 21 only 18% of the heat stress cow had undergone functional luteolysis. The persistence of the corpus luteum, and elevated progesterone demonstrated by Wilson and colleagues has been supported by other studies which subjected cows to similar durations of heat stress, i.e. one estrus cycle (Gwazdauskas et al., 1981; Roman-Ponce et al., 1981; Trout et al., 1998). However, studies that evaluated chronic seasonal heat stress (i.e. heat stress for the duration of the summer) have shown progesterone levels to be lower than thermoneutral cows (Wise et al., 1988; Wolfenson et al., 2002). It is likely that the discrepancies in progesterone concentrations between shorter and more prolonged periods of heat stress are related to disrupted follicular development. Heat stress damages theca and granulosa cells (Wolfenson and Thatcher, 2012). Following ovulation, these damaged cells are converted into luteal cells. Subsequently, the previously damaged luteal cells secrete less progesterone (Wolfenson and Thatcher, 2012). Theca and granulosa cells that have been damaged through repeated exposure to heat stress during chronic hyperthermia are more likely to have reduced progesterone synthesis.

Alterations in ovarian dynamics are also exemplified through alterations in estradiol profiles. In cows experiencing chronic heat stress, circulating estradiol is significantly reduced. Furthermore, cows experiencing heat stress have significantly reduced estradiol in follicular fluid. The reduction in estradiol is related to a similar reduction in androstenedione, an androgen precursor (Wolfenson and Thatcher, 2012). Reduced estradiol may contribute to reduced negative feedback on FSH, accounting for the increased frequency of follicular waves observed in the Wilson et al. study (Wilson et al., 1998b). Furthermore, a failure to surpass the appropriate estradiol threshold could contribute to the reduction in surge LH concentrations. Sub-optimal LH surge and estradiol concentrations can result in an ovulation with reduced or no apparent signs of estrus (Bilby et al., 2008). Silent ovulations during heat stress are particularly troublesome because they result in decreased breeding opportunities in both artificial insemination and natural service breeding systems. Furthermore, noted reductions in basal LH (Gwazdauskas, 1985) combined with lower surge LH concentrations may attribute to poor corpus luteum formation, and contribute to the reduced progesterone production described in the previous paragraph.

Altered hormonal patterns and ovarian function is one facet of reduced conception rates under periods of heat stress. Additionally, periods of heat stress may result in damage to the ova within follicles. This damage may decrease ova quality for months creating sub-par conception during that time period (Al-Katanani et al., 2002). Despite the potential for heat stress to create long lasting negative impacts on fertility, hyperthermia is most negatively correlated with reproductive efficacy within the week before and following estrus(Gwazdauskas, 1985; Ealy et al., 1993).

Effects of heat stress on fertility go beyond those related to female physiology. Within the United States, the southern region has the highest percentage of cows bred via natural service compared to the rest of the country (Jordan, 2003). Furthermore, in the southeast, fluid milk pricing drives sire selection (i.e., sires used in the southeastern United States are selected because of total milk production abilities). Bohamanova and colleagues (2008) determined that the predicted transmitting ability of heat-tolerant Holstein sires for milk yield is below average, while sires with a high predicted transmitting ability for milk yield have below average heat-tolerance. Furthermore, heat stress decreases sperm motility, speed, tail beat frequency and tail beat velocity, regardless of genotype (i.e., beef vs dairy vs Brahman influence) (Chandolia et al., 1999). Heat stressed sires and decreased semen quality can be avoided by using cyropreserved semen that was collected from high-fertility bulls housed in thermoneutrual conditions. Unfortunately, this strategy only addresses male subfertility and does not overcome the detrimental effects of heat stress on the female reproductive tract. Hyperthermia alters conditions within the maternal reproductive organs that hinder normal fertilization. Sartori and colleagues (2002) used high-quality semen to show that fertilization was reduced by approximately 45% in heat stressed cows, and this reduction was primarily a result of polyspermy. A reduction in fertilization rates was not seen in heat stressed heifers; however, a reduced number of embryos collected on d 7 has been demonstrated (Bilby et al., 2008).

In the United States, the southeastern region experiences a decline in conception rate beginning in May, reaching the lowest point in August, and finally recovering to non-heat stressed levels in December (Huang et al., 2009).This reduction in conception rate is reflected in a high seasonality of calving (Oseni et al., 2003, 2004). While many producers desire, and thus employ management practices to obtain seasonal calving, those that breed throughout the year observe a higher number of days open (i.e. a longer period until conception) in the summer than during other seasons (Oseni et al., 2003, 2004). It is likely that reduced frequency of ovulation and expression of estrus, decreased fertilization, and increased early embryonic death during periods of heat stress are at least partially responsible for this occurrence; however, increased days open may not entirely reflect reproductive failure. In many cases increasing days open can be a sound management decision (Oseni et al., 2003, 2004). It has been demonstrated that in non-heat stressed cows, conception rate increases with days in milk (DIM); however in heat stressed cows, under humid conditions, this trend is not present up to 175 DIM (Huang et al., 2008). Therefore, voluntarily increasing days open during periods of heat stress can increase the likelihood of obtaining a pregnancy. In known periods of severe heat stress, when conception rates are expected to be low, continuing to attempt to breed cows can result in a waste of labor and semen.

Heat stress in early gestation:

Maternal hyperthermia directly reflects the level of heat stress faced by the embryo (Sakatani et al., 2012). Embryos are highly susceptible to heat stress early in development. A significant number of embryos undergo degeneration when heat stress occurs during the first week of gestation. This effect is even more pronounced when heat stress occurs on the third day following conception (Ealy et al., 1993). Compared to thermoneutrual contemporaries, cows and heifers that experience heat stress around the time of estrus, but undergo insemination during thermoneutral conditions, are both more likely to produce degenerate, abnormal, or retarded embryos by day 7 post-insemination(Putney et al., 1989); however, this outcome is more pronounced in cows (Sartori et al., 2002), most-likely as a result of the increased metabolic heat production of mature animals compared to heifers.

A reduced number of oocytes are recovered from cows that have been under chronic (i.e. seasonal) heat stress at the time of collection. This has been documented in oocytes recovered from *in situ* follicle aspirations, and from slashing of slaughterhouse ovaries (Al-Katanani et al., 2002; Ferreira et al., 2011). Furthermore, oocytes that are collected from heat stressed cows have reduced embryological development when used for thermoneutral *in vitro* production procedures (Al-Katanani et al., 2002; Ferreira et al., 2011). When oocytes are collected under thermoneutral conditions, but then exposed to hyperthermia (41°C), *in vitro*-embryos have reduced developmental competence compared to embryos cultured at 38.5 °C. Developmental delays occur regardless of the length of hyperthermia, but degeneration becomes more severe as the length of hyperthermia increases (Edwards and Hansen, 1997; Al-Katanani et al., 2002; Edwards et al., 2009). It has also been shown that small (2 h) reprieves in temperature during periods of heat stress do not lessen the severity of embryo degeneration (Al-Katanani and Hansen, 2002). Santolaria and colleagues (2010) found that a period of heat stress during the first 20 days of gestation increases pregnancy loss and that any subsequent acute (1 d) episodes of heat stress during the first two months of gestation have an additive effect on the risk for early embryonic death/ fetal loss.

Heat stress in late gestation:

Heat stress that occurs during the last trimester of gestation can alter prepartum concentrations of progesterone, estrogen and estrone sulfate. These changes may result in reduced uterine blood flow and compromise placental function and nutrient delivery to the fetus (Collier et al., 1982; Gwazdauskas, 1985). Compromised placental function decreases fetal growth. Severely compromised placental function can result in abortion and still birth. Calves that survive to term often have reduced birth weights, which contributes to compromised health and growth (Collier et al., 1982; Gwazdauskas, 1985;(Yates et al., 2012)).

Cellular and molecular response to heat stress:

Cells exposed to heat stress undergo a dual response. First, the cells initiate a heat stress response which allows for survival of immediate danger; this response is characterized by increased expression of heat shock proteins (Elasser et al., 2012) which are activated and up-regulated by insulin (Baumgard and Rhoads, 2012). The heat shock proteins function as molecular chaperones, promoting cell survival. Collier and colleagues (2006b) established that bovine mammary epithelial cells respond to acute heat stress (42°C) by increasing members of the HSP70 family. Inducible HSP70 gene expression is dramatically stimulated in the first two hours of heat exposure and reaches peak expression within four hours. By eight hours of heat stress exposure, HSP70 expression returned to baseline (Collier et al., 2006b).This return to baseline may mark a shift from immediate cell survival mechanisms, to more adaptive alterations. Through the use of a microarray, Collier and colleagues (2006b) determined that 340 genes in bovine mammary epithelial cellswere heat stress responsive; of these, 31 had significant up- or down-regulation. Up-regulated genes were associated with stress response and DNA and protein repair, while down-regulated genes were associated with cell cycle, metabolism, and structural proteins.

The second response to heat stress is adaptive; cells undergo transcriptional changes that enable continued survival in the new environment (Elasser et al., 2012). Alterations of the genome in response to environmental conditions is epigenetics. Epigenetics involves regulation of gene expression (i.e. activation or repression) as a function of histone changes (i.e. histone modified via acetylation, methylation, and the selective compaction or relaxation of chromatin). This remodeling causes differential gene expression that can be passed on from one generation to the next (Elasser et al., 2012).

Heat Stress on Milk Production and Composition

Estradiol and progesterone are important in mammary duct growth and lobule-alveolar growth, particularly during puberty and gestation(Tucker, 1985). As previously discussed, estradiol and progesterone profiles are altered during periods of heat stress; therefore, heat stress during calf-hood and gestation are likely to contribute to reduced mammary growth and development by reducing duct branching, lengthening, and thickening. Tao and colleagues (2011) showed that multiparous cows experiencing heat stress during the dry period have lower total mammary cell proliferation rate twenty days before calving compared to cooled cows. Consistent with results from other studies, cows that experienced heat stress during the dry period have decreased milk production compared to contemporaries (Moore et al., 1992).

Milk production begins to decline after a temperature threshold has been passed, and the rate of decline increases as temperature increases. Ravagnolo and colleagues (2000) determined that milk yield over the course of a day was relatively constant in Georgia Holsteins; however, once an ambient temperature of surpassed 24°C daily milk production began to decline. Furthermore, humidity concomitantly altered the temperature at which milk production began to decline; yields were similarly reduced when the ambient temperature was 32°C with humidity <30% (i.e. THI between 72 and 77) and when ambient temperature was 26°C with humidity >60% (i.e. THI between 74 and 78). At a THI of 72, milk began to decline at a rate of 0.2 kg/unit of THI. Although heat stress can reduce milk production on a day-to-day basis, a heat stress challenge during midlatation (140 ± 13 d) more severely affects milk yield than heat stress events in early or late lactation (Renaudeau et al., 2012); this is a result of increased metabolic heat production in response to increased energy demand. Despite the stage of lactation when hyperthermia is encountered, a permanent drop in productivity of the current lactation is proportional to the length of heat stress incurred (Ravagnolo et al., 2000).

Classically, the decline in milk productivity during periods of heat stress has been attributed to depressed feed intake. West and colleagues (2003) demonstrated that in hot and humid climates, dry matter intake (DMI) was impacted most significantly by the mean air temperature two days before the milk test date and by the THI three days before the test date. Similarly, the greatest impact on Holstein milk yield was a high THI occurring two- to three-days before milk production is measured (West et al., 2003; Renaudeau et al., 2012). Additionally, West and colleagues (2003) found that Georgia Holsteins maintain both feed intake and milk yield until milk temperature reaches approximately 39°C, after which both decrease at an increasing rate.

As mentioned previously, cattle in NEBAL experiencing heat stress do not undergo the same metabolic shifts as thermoneutral cows in NEBAL. Heat stressed cows are not metabolically flexible, and therefore are unable to preferentially spare glucose for milk production (Baumgard and Rhoads, 2012). The extra energy demand of lactation and heat dissipation combined with a failure to utilize lipid metabolism is likely a major contributor to the decline in milk production. Yet not all factors which contribute to lactation are controlled by NEBAL status. Heat stressed cows have reduced hepatic somatotropin receptors and reduced signal transducer and activator of transcription 5 (STAT-5) phosphorylation. The reduced somatotropin signaling through STAT-5 accounts for a reduction in hepatic IGF-I mRNA abundance. Somatotropin and IGF-I are potent lactogenic hormones. The reduction in somatotropin receptors occurs independently of reduced feed intake (Baumgard and Rhoads, 2012), suggesting that not all reductions in milk synthesis are a result of malnutrition induced NEBAL. Furthermore, thyroxine and triiodothyronine are reduced during heat stress (Gwazdauskas, 1985) and are essential for milk production (Tucker, 1985). Declining milk production during periods of heat stress is likely a result of complex feed intake dependent and independent mechanisms.

As milk yield is altered during periods of heat stress, so is milk composition. Important milk constituents are fat and protein. Milk fat primarily consists of triacylglycerols (97-98%) (Jenness, 1985; Larson, 1985). In association with this lipid layer are fat soluble constituents, including vitamin A, sterols, and tocopherols (Larson, 1985). Milk protein is the aggregate of all proteins, including enzymes, in the milk (Jenness, 1985); however, the majority of these proteins are unique to lactation, including all major caseins, β-latoglobulin and α-lactalbumin (Larson, 1985). Furthermore, some proteins have specific intracellular functions; for example, α-lactalbumin is involved in the formation of lactose synthase (Larson, 1985).

Milk fat and protein are generally lowest during hot months and highest during cool months; this is likely a result of varied seasonal diets and hyperthermic-induced metabolic alterations (Jenness, 1985; Larson, 1985). Milk fat and protein are strongly correlated (Jenness, 1985). Heat stress during the last sixty days of gestation will reduce milk fat production (Moore et al., 1992). Ravagnolo and colleagues (2000) evaluated milk quality from heat stressed Holsteins in Georgia. They showed that milk fat declined with temperature over a wide range of temperature and not with one specific climatic factor. Milk fat production increased slowly until a THI $\geq$ 72 was reached, after which it declined at a rate of 0.012 kg/unit of THI. Milk protein was reduced at a THI $\geq$ 72, and to reduce by an average of 0.009 kg/unit of THI. Collier and colleagues (1982) determined that while total milk protein was reduced, the percentage of milk protein was not.

Summary

Dairy cattle are sensitive to environmental stressors, particularly thermal load. As environmental temperatures rise, cows reach a point when they can no longer dissipate metabolic heat; at this point animals undergo hyperthermia (i.e. heat stress). Heat stress is associated with a range of maladies; these include alterations in feed-intake, endocrine function, whole body metabolism, animal health, lactation, and reproductive efficiency.

Under periods of hyperthermia, dairy cattle experience reduced feed-intake and concomitant NEBAL. These heat stressed cattle exhibit reduced metabolic flexibility which manifests as a failure to employ lipid metabolism, thereby further exacerbating the energy deficit and giving rise to metabolic and health disorders. The reduction in feed consumption and subsequent metabolic state are intimately associated with reduced milk yield and altered milk composition- particularly the reduction of fat and protein constituents.

Furthermore, heat stress can directly disrupt the endocrine system. Periods of hyperthermia are associated with compromised mammary growth and development during the pre-pubertal and dry periods, and the down–regulation of important lactation stimulation hormones (e.g. somatotropin). Additionally, the hormones essential to fertility, specifically progesterone, LH, FSH, and estradiol are all altered; these alterations affect estrous cycle length and reduce estrus expression. Compromised ovarian function and damaged ova greatly contribute to reduced conception. Reduction in conception rates can also be attributed to heat stressed sperm which exhibit reduced motility and increased incidence of polyspermy, thereby resulting in the failure of fertilization.

In incidences where fertilization is successful, heat stress reduces embryo development and often results in early embryonic death. In established pregnancies, heat stress reduces placental function, resulting in compromised nutrient exchange. The fetus is also susceptible to hyperthermia. Because post-insemination stress is capable of affecting the genome of the conceptus (i.e. epigenetics), periconceptional heat stress likely results in long term consequences for productivity.

CHAPTER II: Periconceptional heat stress of primiparous Holstein cows affects subsequent milk production

Abstract

The fertility of lactating Holstein cows is reduced during periods of heat stress. Some inseminations conducted during heat stress result in successful pregnancies from which heifer calves are born; many of these heifer calves are retained and raised to enter the milking herd as replacements. The heat stress experienced by these females around the time they were conceived may confer long-lasting effects that alter subsequent milk production. The objective of this study was to examine the relationship between periconceptional heat stress and measurements of milk production in primiparous cows. National Dairy Herd Improvement Association data was obtained from Dairy Records Management Systems. Records (n=205,060) included Holstein cows born between 2000 and 2010 in FL, GA, and TX. Conception dates were calculated by subtracting 276 d from the recorded birth date. Records for 1st lactation cows conceived within the months of June, July, and August were retained as heat stress-conceived (HSC) cows; 1st lactation cows conceived within the months of December, January, and February were retained as thermoneutral conceived (TNC) contemporaries. For each state, adjusted 305-d mature equivalent milk was evaluated with a mixed model ANOVA using SAS. Of the cows that calved in the spring, the HSC cows produced more milk in all three states. Of the fall and winter calvings, the TNC cows had a higher milk yield than the HSC cows in FL, GA, and TX. The TNC cows that calved in the summer in GA produced more milk than the HSC cows; while no significant differences between HSC and TNC cows that calved in the summer were observed in

FL or TX. The relationship between HSC and milk production suggests that HS at the time of conception and during early pregnancy impacts first lactation performance.

Keywords: Milk production, Heat stress, Primiparous, Periconceptional

Introduction

Over the years, dairy cattle have been selected based on traits that contribute to productivity. In general, body mass has increased to accommodate a large mammary system and other internal organs that contribute to milk synthesis(Renaudeau et al., 2012). Unfortunately, this selection strategy has theoretically decreased heat tolerance of dairy cattle because the heat produced to meet maintenance needs is directly proportional to the body weight and surface area of an animal (Renaudeau et al., 2012). Therefore, as a cow increases in size, metabolic heat production increases. In addition to cow size, metabolic heat production increases as the productive capacity of a dairy cow improves. Furthermore, most of the selection for dairy production takes place in temperate climates (Ravagnolo et al., 2000). This selection process focuses primarily on increasing milk production, and does not account for individual performance in various environmental conditions.

Without selection for heat tolerance (body temperature heritability = 0.37 (Seath, 1947)), modern dairy cattle are highly susceptible to the effects of heat stress. These effects include depressed feed intake, increased ailments, altered metabolism and reduced milk production (Collier et al., 1982; Wilson et al., 1998; Ravagnolo et al., 2000; Bernabucci et al., 2010; Wheelock et al., 2010) In the United States alone, approximately $1 billion is lost annually as a result of poor performance during periods of heat stress (Wheelock et al., 2010). Furthermore, heat stress that is experienced by a conceptus may result in epigenetic alterations of the genome. These changes are an adaptive mechanism that allow cells to survive in a stressful environment, and can be retained in the genome (Elasser et al., 2012) resulting in life-long effects. Therefore, a cow that experienced perioconceptional heat stress, while not selected for heat tolerance ability, may have an advantage over contemporaries during subsequent hyperthermic events.

This objective of this study was to evaluate the effects of periconceptional heat stress on first lactation milk yield. The question that it aims to answer is: does periconceptional heat stress confer long-lasting effects on females that alter milk production during the first lactation? First lactation cows are of a particular interest because, for the most part, the first lactation population within a herd has not yet been subjected to selection via culling. This allows evaluation of a representative sample of all cows rather than just the high performing cows that are retained for multiple years.

Materials and Methods

Inclusion Criteria

Dairy Herd Information Association (DHIA) data was received from Dairy Records Management Systems (Raleigh, NC) for three hot and humid states: Georgia, Florida, and Texas. For the purposes of this study, only first lactation records of Holstein cows born between 2000 and 2011 were retained for evaluation. The records for FL (n=79,790) and TX (n=127,099) were too computationally demanding to complete Proc Mixed in a reasonable amount of time, so a random subset of cows were evaluated; because GA was able to be analyzed in a reasonable manner, data was randomly selected to arrive at similar total observations. Eighty-five percent of Florida first lactation cows were retained for analysis, while fifty-five percent of the first lactation cows in Texas were retained for analysis. Random selection was performed by Proc Surveyselect of SAS. For each state, the total number of cows included in the analysis are listed in Table 2.1.

In order to evaluate heat stress at the time of conception (HSCONCP), conception date was calculated assuming that the average gestation length of a Holstein was 276 d. (Dhakal et al., 2013). The following formula was used: Conception date = Birth date – 276 d. Heat stress at the time of conception was determined by the month in which conception took place. Cows that were conceived during December, January, and February were considered thermoneutral conceived (TNC), while cows conceived during June, July, and August were considered heat stress conceived (HSC). Since fall (September, October and November) and spring (March, April, and May) weather conditions are intermediate and highly variable, cows conceived during these months were excluded from analysis.

The season of calving (SOC) for each lactation was also considered because of its inherent effects on milk production. Season of calving was determined using the month of each calving date. The seasons were designated as spring: March, April, May; summer: June, July, August; fall: September, October, November; and winter: December, January and February. Further details regarding data processing can be found in Appendix A.

Data Analysis

Statistical analysis was performed in SAS (SAS Institute Inc., Cary, NC). Due to limited computational ability, and the large size of the data set, each state was analyzed individually. Furthermore, because of the computation power needed to analyze the random effects, covariance parameter estimates were calculated with Proc HPMixed, using Dual Quasi-Newton optimization techniques. The covariance parameter estimates were then used in conjunction with the REML estimation of Proc Mixed. Contrasts were used to make comparisons. Results were considered significant with a p-value ≤ 0.01; results with a p-value of ≤ 0.05 were considered a tendency for significance.

The linear model used to evaluate the data, with the overall intercept μ, for response Y_{ijkl} is given below.

$$Y_{ijkl} = \mu + H_i + \tau_j + C_{k(ij)} + \theta_l + (\tau\theta)_{jl} + e_{ijkl}$$

Herds are denoted by H_i, where i=1, … n_h. Herd was included as a random effect because each herd is assumed to come from an infinite population of similar herds; a herd has a random effect generated from ~ N(0, σ_h^2). Within each herd there were cows that were either conceived under periods of heat stress, or conceived under thermoneutral conditions. The effect of HSC is denoted as τ_1 and the effect of TNC is denoted as τ_2. Each cow has its own effect, denoted by $C_{k(ij)}$where *k(ij)* means the *k*-th cow nested in herd *i* and HSCONCP category *j*. Cow was included as a random effect because each cow is assumed to come from an infinite population of similar cows; a cow has a random effect generated from ~ N(0, σ_c^2). Incorporating both herd and cow as random effects serves to incorporate the fact that observations that come from the same herd/cow are likely correlated. The season of calving is also taken into account, and this seasonal effect is denoted by θ_m, *m*=1,2,3,4 where each number corresponds to a season in the year (spring, summer, fall, winter).

For ANOVA for fixed effects Type III sums of squares was used. Type II sums of squares are used for unbalanced data. To calculate degrees of freedom for error Satterhurthe's Approximation was used. Satterhurthe's Approximation is used when there is not constant variance. The fixed effects include HSCONCP, SOC, and their interaction. The dependent variable is 305-day adjusted mature-equivalent (ME) milk; this variable is discussed further in Appendix A.

Table 2.1. Number of cows from each of the three states that were included in the analysis of first lactation records.

	GA	FL	TX
TNC cows	40,759	43,033	38,872
HSC cows	26,574	24,789	31,033
Total	67,333	67,822	69,905

Results

The results are presented as specific contrasts; contrasts are made where significantly relevant, that is to say the most significant interaction that includes HSCONCP. For Georgia we found HSCONCP and SOC to be significant main effects (P<0.0001; P<0.0001) in addition to the interaction being significant (P<0.0001; Table 2.2; Figure 2.1). Heat stress conceived cows that calved in the spring produced 615 kg more milk than the TNC cows (P=0.0006). The TNC cows that calved all other seasons produced more milk than the HSC cows (summer = 591 kg, P=0.0025; fall = 1,171 kg, P<0.0001; winter = 1,177 kg, P<0.0001).

For Florida we found HSCONCP and SOC to be significant main effects (P<0.0001; P<0.0001) as well as a significant interaction (P<0.0001; Table 2.3; Figure 2.2). Heat stress conceived cows that calved in the spring produced 1,671 kg more milk than the TNC cows (P<0.0001). The TNC cows that calved all other seasons produced more milk than the HSC cows (fall = 2,788 kg, P<0.0001; winter = 2,981 kg, P<0.0001).

For Texas, there was a significant effect of SOC (P<0.0001), while HSCONCP had a tendency for significance (P=0.0242). The interaction was also significant (P<0.0001; Table 2.4; Table 2.3). Heat stress conceived cows that calved in the spring produced 1,105 kg more milk than the TNC cows (P<0.0001). The TNC cows that in calved fall and winter produced more milk than the HSC cows (fall = 1,113 kg, P<0.0001; winter = 1,283 kg, P<0.0001).

Table 2.2. Differences in mature-equivalent milk yield between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Georgia.

Season of Calving	P-Value	Mature-Equivalent Milk Produced[1] (kg)	S.E.	99% Lower Limit	99% Upper Limit
Spring	0.0006	-615	179	-1,076	-153
Summer	0.0025	591	195	88	1,095
Fall	<0.0001	1,171	173	727	1,616
Winter	<0.0001	1,177	213	628	1,725

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

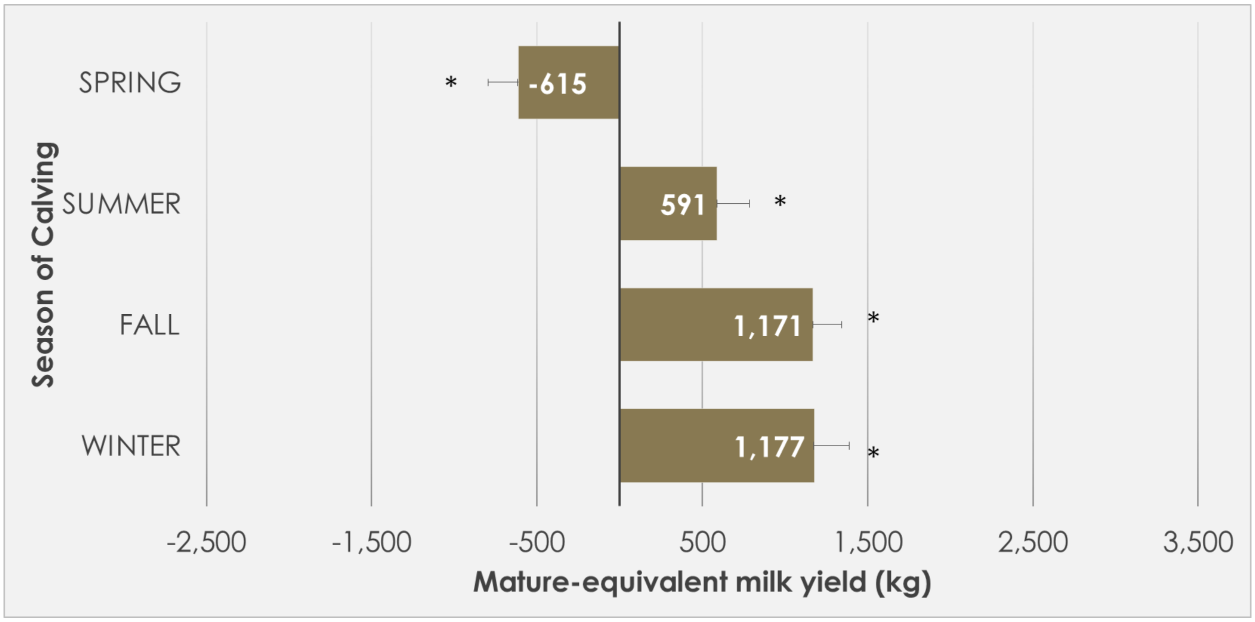

Figure 2.1. Seasonal differences in mature-equivalent milk yield (kg) between thermoneutral conceived (TNC) and heat stress conceived(HSC) cows in Georgia. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows.† denotes P<0.05; * denotes P<0.01.

Table 2.3. Differences in mature-equivalent milk yield between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida.

Season of Calving	P-Value	Mature-Equivalent Milk Produced[1] (kg)	S.E.	99% Lower Limit	99% Upper Limit
Spring	<0.0001	-1,671	201	-2,188	-1,154
Summer	0.5939	-115	216	-669	440
Fall	<0.0001	2,788	188	2,303	3,273
Winter	<0.0001	2,981	227	2,396	3,567

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

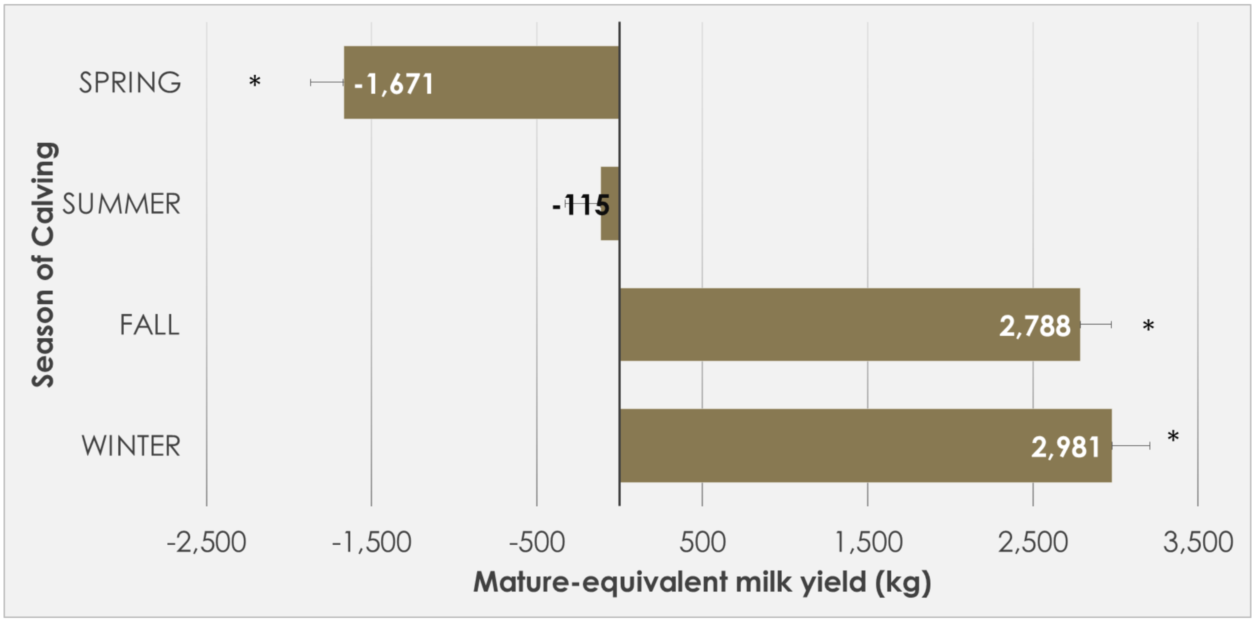

Figure 2.2. Seasonal differences in mature-equivalent milk yield (kg) between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows.† denotes P<0.05; * denotes P<0.01.

Table 2.4. Differences in mature-equivalent milk yield between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas.

Season of Calving	P-Value	Mature-Equivalent Milk Produced[1] (kg)	S.E.	99% Lower Limit	99% Upper Limit
Spring	<0.0001	-1,105	183	-1,575	-634
Summer	0.1214	-352	227	-938	234
Fall	<0.0001	1,113	172	669	1,557
Winter	<0.0001	1,283	231	687	1,879

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

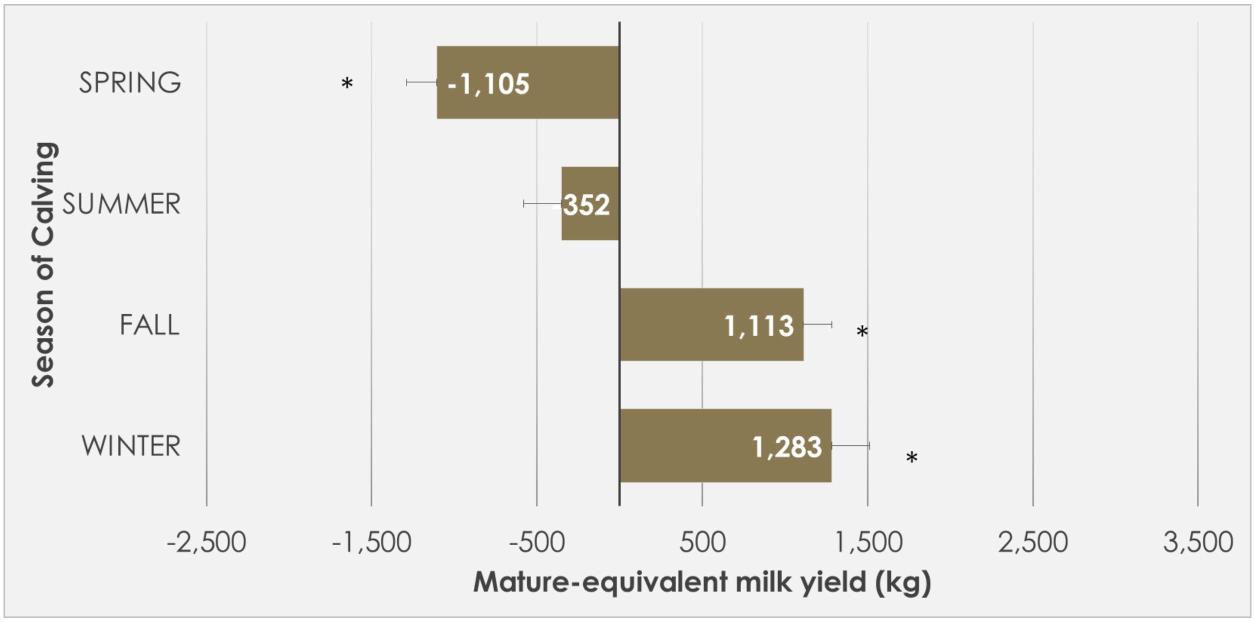

Figure 2.3. Seasonal differences in mature-equivalent milk yield (kg) between thermoneutral conceived (TNC) and heat stress conceived(HSC) cows in Texas. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows.† denotes P<0.05; * denotes P<0.01.

Discussion

The relationship between heat stress at the time of conception and subsequent milk production is complex. There are many confounding factors that cannot be accounted for in such an observational study; these factors include, but are not limited to, nutritional plane and on-farm management practices. The fact that significant differences between HSC cows and TNC cows were observed over a multitude of years, herds, and cows suggests that despite potential confounding factors, heat stress at the time of conception does have long-term effects.

Elucidation of the relationship between HSCONCP and production is made more difficult the confounding of season of conception and season of first calving. Cows are conceived and typically calve thirty-three months later, that is to say, a cow conceived in the winter will calve in the fall; this is discussed further in Appendix A. Additionally, the dependent variable mature-equivalent milk yield, is derived using factors of a cow’s age, breed, season of calving, and state. These factors are taken into account to fit the appropriate lactation curve and to again to appropriately adjust for mature milk production; however by having these components also factored into the model there is difficulty in clarifying the relationship between HSCONCP and production. The mature-equivalent milk variable is discussed further in Appendix A. Furthermore, a year of calving was not taken into account in the model. In doing this, the results are generalized over a ten year period and any the more subtle differences that could arise on a yearly basis, particularly in respect to season, are not available for analysis.

Milk production was significantly affected by HSCONCP in all three states. Of the cows that calved in the spring, the HSC cows produced more milk than the TNC cows in all three states. Of the cows that calved in the summer, the TNC cows produced more milk than the HSC cows in Georgia. There was no significant difference between the HSC and TNC cows that calved in the summer in Florida, or Texas. Of the cows that calved in the fall and winter, the TNC cows produced more milk than the HSC cows over in all three states.

Since the HSC cows that calved during the spring, and thereby produced milk during summer heat stress, outperformed the TNC cows; this suggest that heat stress around the time of conception may offer an advantage to the HSC cows during subsequent periods of heat stress.

Despite potential confounding variables, the data shows that periconceptional heat stress at the time of conception affects subsequent production of primiparous Holstein cows. While this analysis provided new insight into the far-reaching effects of heat stress, it does not provide an indication of the specific mechanisms responsible for the observed effects on production. Further studies are needed to explore the mechanisms responsible for this relationship and resulting effect on dairy production efficiency.

CHAPTER III: Periconceptional heat stress of multiparous Holstein cows affects subsequent milk production and composition

Abstract

Heat stress at the time of conception affects the subsequent milk production of primiparous Holstein cows; however, it is unknown whether these effects are maintained across multiple lactations. Therefore, the objective of the current study was to examine the relationship between periconceptional heat stress and measurements of milk production and composition in cows retained within a herd for multiple lactations (the first three parities). National Dairy Herd Improvement Association data was obtained from Dairy Records Management Systems. Records (n = 80,119) included Holstein cows born between 2000 and 2010 in GA, FL, and TX. Conception dates were calculated by subtracting 276 d from the recorded birth date. Records for cows conceived within the months of June, July, and August were retained as heat stress-conceived (HSC) cows; cows conceived within the months of December, January, and February were retained as thermoneutral conceived (TNC) contemporaries. Adjusted 305-d mature equivalent milk, and protein and fat percent were evaluated with a mixed model ANOVA using SAS. Milk production was significantly affected by perioconeptional heat stress. Across all lactations and seasons of calving, if there was a significant difference between the HSC and TNC cows, the TNC always produced more milk. Alterations in fat and protein percentage were statistically significant, but of little practical importance. The relationship between HSC and milk production variables suggests that heat stress at the time of conception and during early pregnancy impairs cow milk yield throughout her lifetime.

Keywords: Heat stress, Periconceptional, Dairy cow, Multiparous

Introduction

It is well established that heat stress causes undesirable performance in Holstein cows. Heat stress during the pre-pubertal period and during the dry period decrease mammary cell proliferation (Tao et al., 2011) resulting in reduced secretory capacity during lactation. Furthermore, heat stress reduces feed consumption, contributing to an increased negative energy balance and reducing milk yield and altering milk composition (Collier et al., 1982; Jenness, 1985; Ravagnolo and Misztal, 2002; West et al., 2003; Wheelock et al., 2010; Bernabucci, 2012; Renaudeau et al., 2012). This is particularly detrimental to high producing dairy cows that are already under immense metabolic strain.

In the United States, approximately $1 billion is lost annually as a result of poor performance during periods of heat stress (Wheelock et al., 2010). For cows that are retained within a herd for multiple lactations, any factor that results in a repetitive reduction in milk yield and quality can cause substantial losses to the producer. Embryonic alterations in the genome that affect milk yield and composition throughout adulthood are therefore important to identify. The cellular and molecular changes initiated as a result of exposure to stressful conditions are adaptive mechanisms that allow cells continued survival. These alterations can be retained in the genome (Elasser et al., 2012) resulting in life-long effects. Since heat stress is known to induce epigenetic alterations in *in vitro* bovine mammary epithelial tissues (Collier et al., 2006), it is likely that periconceptional heat stress results in an array of *in vivo* epigenetic alterations in cattle.

The objective of the present study was to evaluate the effects of periconceptional heat stress on subsequent milk production and composition in cows that were retained within one herd for multiple years. It aims to answer the question: does periconceptional heat stress confer long-lasting effects on females that alter milk production and composition for multiple years? Identifying a relationship between periconceptional heat stress and milk production variables will empower producers with information so that they can make sound management decisions about which females will be retained as replacement animals.

A. Milk Production

Materials and Methods

Inclusion Criteria

Dairy Herd Information Association data was received from Dairy Records Management Systems (Raleigh, NC) for three hot and humid states: Georgia, Florida, and Texas. For the purposes of this study cows were required to have at least their first, second, and third lactation within one herd to be included in the analysis; only the first three lactations were evaluated. This selection criteria edits the data and may remove cows that would show an unfavorable result for HSCONCP, since they are removed from the herd before the second or third lactation. For each state, the total number of cows included in the analysis are listed in Table 3.1. Data were processed as previously described in Chapter I.

Data Analysis

Statistical analysis was performed in as previously described in Chapter I. The model, which includes and overall intercept μ, for response Y_{ijklm} is

$$Y_{ijklm} = \mu + H_i + \tau_j + C_{k(ij)} + \beta_l + (\tau\beta)_{jl} + \theta_m + (\tau\theta)_{jm} + (\beta\theta)_{tm} + e_{ijklm}$$

where Hi ~ N(0, σ_h^2) and $C_{k(ij)}$~ N(0, σ_c^2) and $(\tau\beta)_{jl}$ is the interaction effect for heat-stress at conception and parity. Each state analyzed is comprised of n_h herds, denoted by H_i, where *i*=1, …

n_h. Within each herd there were cows that were either conceived under periods of heat stress, or conceived under thermoneutral conditions. This fixed effect, HSCONCP is denoted as $\tau_{j,}$ where the effect of HSC is denoted as τ_1 and the effect of TNC is denoted as τ_2.Multiple measurements are taken for each cow, which are nested within a specific herd and within the HSCONCP designation. Each cow has its own effect, denoted by $C_{k(ij)}$where *k(ij)* means the *k*-th cow nested in herd *i* and HSCONCP category *j*. Each cow has three observed parities, and each parity has effect $\beta_{l,}$ l = 1, 2, 3. The season of calving for each parity is also taken into account, and this seasonal effect is denoted by θ_m, *m*=1,2,3,4 where each number corresponds to a season in the year (spring, summer, fall, winter). Herd (H_i) and cow ($C_{k(ij)}$) are referred to as random effects, which are considered to be a random sample of the population of herds/cows in that state. More precisely, it is assumed that these random effects follow Hi ~ N(0, σ_h^2) and $C_{k(ij)}$~ N(0, σ_c^2); doing this serves to incorporate the fact that observations that come from the same herd/cow are likely correlated.

The response (Y_{ijklm}) is 305-day adjusted ME milk; this variable is discussed further in Appendix A.

Table 3.1. Number of cows from each of the three states that were included in the analysis of the three sequential lactations records.

	GA	**FL**	**TX**
TNC cows	12,466	15,598	21,077
HSC cows	7,261	8,436	15,281
Total	19,727	24,034	36,358

Results

The results are presented as specific contrasts; contrasts are made where significantly relevant, that is to say the most significant interaction that includes HSCONCP. For Georgia we found HSCONCP, LACTNO and SOC to be significant main effects (P<0.0001), but the two-way interactions between LACTNO and SOC, and LACTNO and HSCONCP were also significant (P<0.0001; P=0.0004). The three-way interaction was significant (P=0.0009; Table 3.2; Figure 3.1). Compared to their HSC counterparts, first lactation TNC cows that calved in the fall produced 256 kg more milk (P<0.0001), while those that calved in the winter produced 215 kg more (P=0.0039). There was a slight tendency for the first lactation TNC cows that calved in the summer to produce 139 kg more milk than their HSC counterparts (P=0.0401). For second lactation cows, the TNC cows that calved in summer, fall, and winter produced more milk than the HSC cows that calved in those seasons (326 kg, P<0.0001;165 kg, P=0.0004; 223 kg, P=0.0002; respectively). There was a tendency for second lactation cows that calved in the spring to produce more milk than the HSC cows (168 kg, P=0.0122). In the third lactation, TNC cows that calved in all seasons produced more milk than their HSC contemporaries (Spring: 361 kg; Summer: 260 kg; Fall: 223 kg; Winter: 298 kg; P<0.0001 for all seasons).

For Florida all three main effects: HSCONCP, LACTNO and SOC, were significant (P<0.0001; P<0.0001; P<0.0001). The two-way interaction between LACTNO and SOC was also significant (P<0.0001), and there was a tendency for the two-way interaction between SOC and HSCONCP to be significant (P= 0.0350). Additionally, the three-way interaction was significant (P<0.0001; Table 3.3; Figure 3.2). Thermoneutral conceived cows in their first lactation that calved in the summer and fall produced significantly more milk than the HSC cows

(365 kg, P<0.0001; 158 kg, P=0.0078; respectively). There was a tendency for first lactation TNC cows that calved in the winter to produce 172 kg more milk than their HSC counterparts (P=0.0168). In the second lactation, TNC cows that calved in the spring produced 172 kg (P=0.0059), while the cows that calved in the summer produced 229 kg (P<0.0001) more milk than their contemporary HSC cows. Second lactation TNC cows that calved in the winter had a tendency to produce 140 kg more milk than the HSC cows (P=0.0155). In the third lactation, the TNC cows that calved in the fall and winter produced significantly more milk than their HSC contemporaries (312 kg, P<0.0001; 357 kg, P<0.0001; respectively). Furthermore, there was a tendency for TNC cows to produce 135 kg more milk than the HSC cows (P=0.0422).

For Texas, HSCONCP, LACTNO and SOC were significant main effects (P<0.0001; P<0.0001; P<0.0001). Additionally the two-way interactions were significant (LACTNO and SOC P<0.0001; LACTNO and HSCONCP P=0.0009; SOC and HSCONCP P=0.0064). Furthermore, the three-way interaction was significant (P<0.0001; Table 3.4; Figure 3.3). For first lactation cows, TNC cows that calved in the spring had a tendency to produce more milk than their HSC counterparts (106 kg, P=0.0213), while TNC cows that calved in the summer, fall, and winter produced significantly more milk than the HSC contemporaries (233 kg, P=0.0002; 252 kg, P<0.0001; 447 kg, P<0.0001; respectively). Thermoneutral conceived cows that calved in the spring produced 139 kg milk more than their HSC counterparts (P=0.0041). Of the cows that calved in the summer and winter, the TNC cows produced 197 kg and 182 kg more milk, respectively, than those conceived under heat stress (P<0.0001). Third lactation TNC cows produced significantly more milk than the HSC cows in all seasons (Spring: 140 kg, P=0.0066; Summer: 132 kg, P=0.0016; Fall: 342 kg, P<0.0001; Winter: 181 kg, P<0.0001).

Table 3.2. Differences in mature-equivalent milk yield between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Georgia.

Lactation	Season of Calving	P-Value	Mature-Equivalent Milk Produced[1] (kg)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.1366	-91	61	-249	67
1	Summer	0.0401	139	68	-35	313
1	Fall	<0.0001	256	61	99	413
1	Winter	0.0039	215	75	23	408
2	Spring	0.0122	168	67	-5	340
2	Summer	<0.0001	326	62	167	485
2	Fall	0.0004	165	47	44	286
2	Winter	0.0002	223	60	68	379
3	Spring	<0.0001	361	73	174	548
3	Summer	<0.0001	260	61	102	418
3	Fall	<0.0001	223	45	107	339
3	Winter	<0.0001	298	55	157	439

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

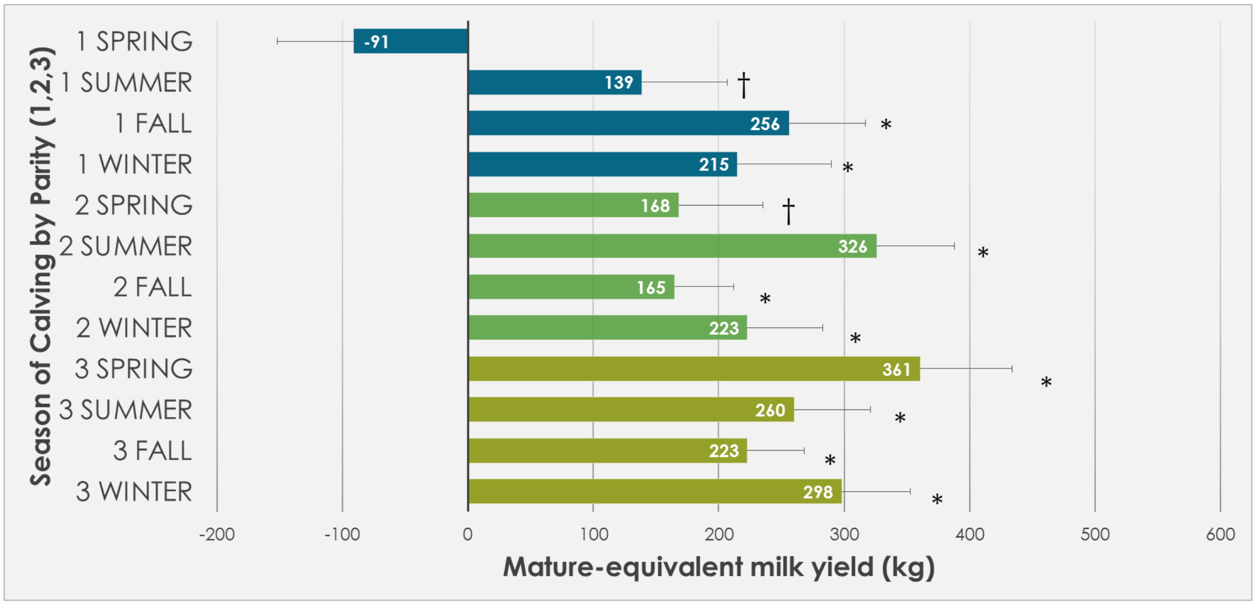

Figure 3.1. Differences in mature-equivalent milk yield (kg; parity x season) between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Georgia.Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. † denotes P<0.05; * denotes P<0.01.

Table 3.3. Differences in mature-equivalent milk yield between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida.

Lactation	Season of Calving	P-Value	Mature-Equivalent Milk Produced[1] (kg)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.1568	-91	65	-258	75
1	Summer	<0.0001	365	66	194	536
1	Fall	0.0078	158	60	5	312
1	Winter	0.0168	172	72	-13	358
2	Spring	0.0059	172	63	11	334
2	Summer	<0.0001	229	56	84	374
2	Fall	0.4907	-31	44	-145	84
2	Winter	0.0155	140	58	-9	289
3	Spring	0.0422	135	66	-36	305
3	Summer	0.1253	-86	56	-230	58
3	Fall	<0.0001	312	42	202	421
3	Winter	<0.0001	357	51	226	489

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

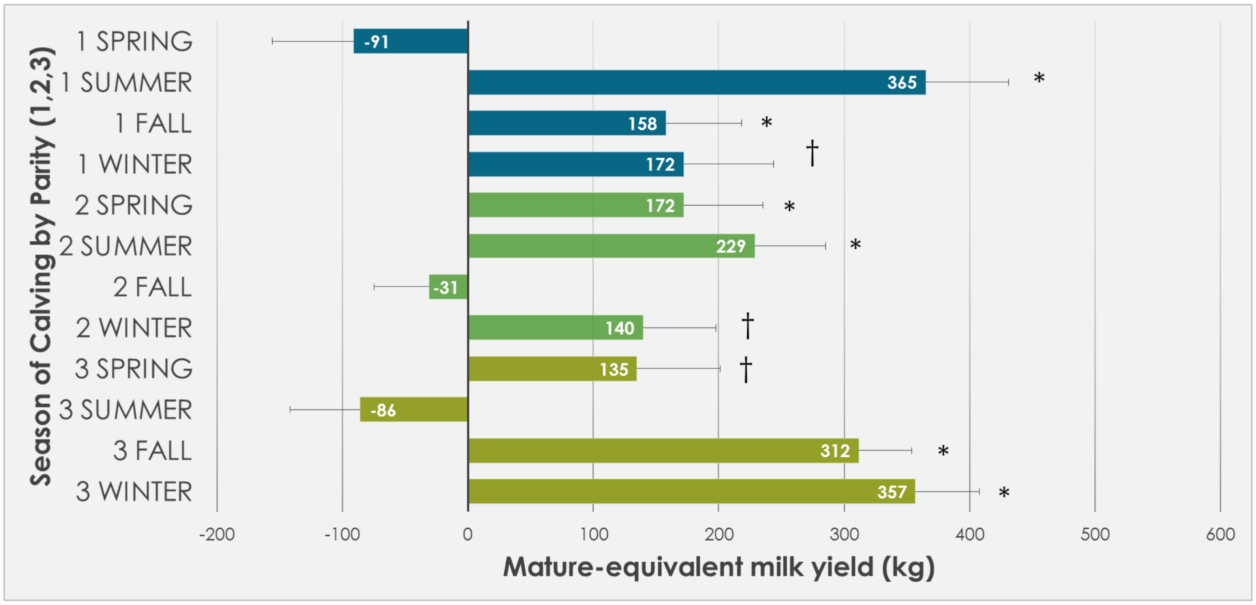

Figure 3.2. Differences in mature-equivalent milk yield (kg; parity x season) between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. † denotes $P<0.05$; * denotes $P<0.01$.

Table 3.4. Differences in mature-equivalent milk yield between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas.

Lactation	Season of Calving	P-Value	Mature-Equivalent Milk Produced[1] (kg)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.0213	106	46	-13	224
1	Summer	0.0002	233	62	72	393
1	Fall	<0.0001	252	45	133	368
1	Winter	<0.0001	447	62	287	607
2	Spring	0.0041	139	48	14	263
2	Summer	<0.0001	197	44	83	312
2	Fall	0.1498	68	36	-25	161
2	Winter	<0.0001	182	47	62	302
3	Spring	0.0066	139	51	7	271
3	Summer	0.0016	132	42	25	240
3	Fall	<0.0001	342	34	253	430
3	Winter	<0.0001	181	41	76	286

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

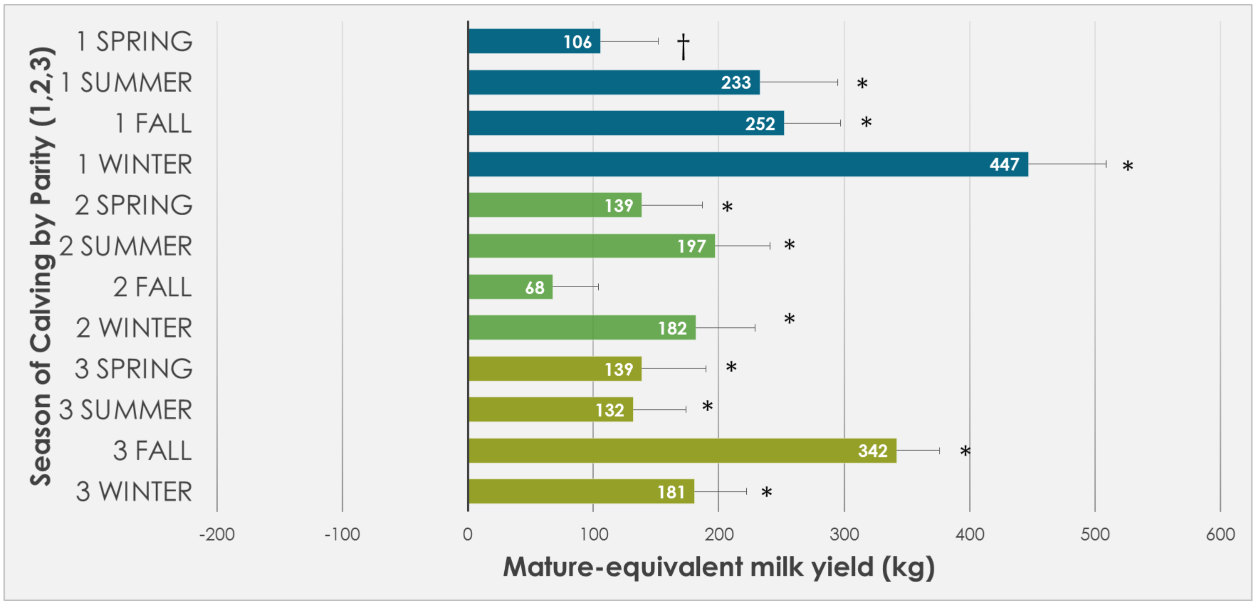

Figure 3.3. Differences in mature-equivalent milk yield (kg; parity x season) between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1 kg more than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. † denotes P<0.05; * denotes P<0.01.

B. Milk Composition

Materials and Methods

Inclusion Criteria and Calculations

Cows included in the analysis were required to have at least their first three lactations within one herd. Only the records for the first three lactations were evaluated. Fat percentage was calculated as ME fat divided by ME milk, multiplied by 100; similarly, protein percentage was calculated as ME protein divided by ME milk, multiplied by 100. Records with a zero for fat or protein percentage were removed from the data. Records with a value > 100 for either fat or protein percentage were removed from the data. For each state the total number of cows analyzed, for each lactation, are listed in Table 3.9.

Data Analysis

Statistical analysis occurred as previously described in the Chapter II. The model for response Y_{ijklm} is

$$Y_{ijklm} = \mu + H_i + \tau_j + C_{k(ij)} + \beta_l + (\tau\beta)_{jl} + \theta_m + (\tau\theta)_{jm} + (\beta\theta)_{tm} + e_{ijklm}$$

where the response (Y_{ijklm}) are fat percent and protein percent.

Table 3.5. Number of cows from each of the three states that were included in the analysis of fat and protein percentage.

	LACTNO	GA	FL	TX
TNC cows	1	7,523	7,757	17,857
	2	7,609	8,142	17,817
	3	7,788	8,490	17,428
HSC cows	1	3,532	4,247	12,313
	2	3,542	4,384	12,285
	3	3,639	4,430	12,216

Results

Fat Percentage

For Georgia we found the main effect: LACTNO, to be significant (P<0.0001), and HSCONCP to have a tendency for significance (P=0.0443; Table 3.14). The TNC cows produced 0.018 percentage points more than the HSC cows over all lactations and seasons of calving (P=0.0443).

For Florida we found LACTNO and SOC to be significant main effects (P<0.0001; P=0.0002), and the two-way interaction to be significant (P=0.0002). Additionally the two-way interaction between SOC and HSCONCP was significant (P<0.0001). The three-way interaction between LACTNO, SOC, and HSCONCP was significant (P<0.0001; Table 3.15). Thermoneutral conceived cows in their first lactation that calved in the spring had a tendency to have a higher percentage of fat in their milk than the HSC cows (0.04%, P=0.0397). First lactation HSC cows produced a significantly higher percentage of fat in their milk in fall calvings (0.115 percentage points, P<0.0001), while first lactation TNC cows produced a significantly higher percentage of fat in their milk in winter calvings (0.088 percentage points, P=0.0008). In second lactation cows that calved in the spring, the TNC cows produced a significantly higher percentage of fat in their milk than the HSC cows (0.087 percentage points, P=0.0001).

For Texas, HSCONCP, LACTNO and SOC were a significant main effects (P<0.0001; P<0.0001; P<0.0001). The two-way interaction of SOC and LACTNO was significant (P<0.0001); the interaction between SOC and HSCONCP was also significant (P<0.0001; Table 3.16). For all lactations, the HSC cows that calved in the fall produced 0.038 percentage points more fat in their milk than their TNC contemporaries (P<0.0001), and the HSC cows that calved in the winter produced 0.046 percentage points more fat in their milk than their TNC contemporaries (P<0.0001).

Table 3.6. Differences in fat percent between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Georgia.

Lactation	Season of Calving	P-Value	Percent Fat Produced[1] (%)	S.E.	99% Lower Limit	99% Upper Limit
All	All	0.0443	0.018	0.009	-0.005	0.040

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1% would indicate that TNC cows performed 1 percentage point better than the HSC cows, while an estimate of -1% would indicate HSC cows performed 1 percentage point better than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

Table 3.7. Differences in fat percent between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida.

Lactation	Season of Calving	P-Value	Percent Fat Produced[1] (%)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.0397	0.045	0.023	-0.011	0.102
1	Summer	0.6444	-0.011	0.023	-0.070	0.102
1	Fall	<0.0001	-0.115	0.022	-0.172	-0.057
1	Winter	0.0008	0.088	0.026	0.021	0.155
2	Spring	0.0001	0.087	0.022	0.029	0.144
2	Summer	0.6469	0.009	0.020	-0.041	0.059
2	Fall	0.7251	-0.006	0.017	-0.049	0.037
2	Winter	0.2750	-0.024	0.022	-0.079	0.032
3	Spring	0.1186	0.037	0.024	-0.024	0.097
3	Summer	0.0779	0.034	0.019	-0.016	0.084
3	Fall	0.1404	-0.023	0.016	-0.064	0.017
3	Winter	0.9018	0.002	0.019	-0.046	0.051

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1% would indicate that TNC cows performed 1 percentage point better than the HSC cows, while an estimate of -1% would indicate HSC cows performed 1 percentage point better than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

Table 3.8. Differences in fat percent between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas.

Lactation	Season of Calving	P-Value	Percent Fat Produced[1] (%)	S.E.	99% Lower Limit	99% Upper Limit
All	Spring	0.7633	0.003	0.009	-0.020	0.026
All	Summer	0.9038	-0.001	0.009	-0.025	0.023
All	Fall	<0.0001	-0.038	0.007	-0.057	-0.019
All	Winter	<0.0001	-0.046	0.009	-0.071	-0.021

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1% would indicate that TNC cows performed 1 percentage point better than the HSC cows, while an estimate of -1% would indicate HSC cows performed 1 percentage point better than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

Protein Percentage

For Georgia, LACTNO and SOC were found to be significant main effects (P<0.0001), but we also found their interaction to be significant (P<0.0001). Additionally, the three-way interaction was significant (P<0.0001; Table 3.21).The first lactation TNC cows that calved in the spring produced 0.031 percentage points more protein in their milk than the HSC cows calving in the same period (P<0.0001). Similarly, the first lactation HSC cows that calved in the fall produced 0.030 percentage point more protein in their milk than the TNC cows calving in the same season (P<0.0001).

For Florida, the significant main effects were: LACTNO and SOC (P<0.0001; P=0.0022). The two-way interaction was also significant (P=0.0009). Additionally the two-way interaction between SOC and HSCONCP had a tendency for significance (P=0.0184). The three-way interaction was also significant (P<0.0001; Table 3.22). First lactation TNC cows that calved during in the spring produced 0.034 percentage points more protein in their milk than the HSC cows (P=0.0001); in contrast first lactation HSC cows that calved in the summer and fall produced 0.029 percentage points and 0.026 percentage points more protein in their milk, respectively (P=0.0016; P=0.0034). In the second lactation, HSC cows that calved in the winter had a strong tendency to produce 0.021 percentage points more protein in their milk than the TNC cows calving in the same season (P=0.0125).

For Texas we found HSCONCP, LACTNO, and SOC to be the significant main effects (P<0.0001; P<0.0001; P<0.0001). The two-way interactions between LACTNO and SOC, and SOC and HSCONCP were significant (P<0.001; P=0.0025; respectively). Additionally, the three-way interaction was significant (P<0.0001; Table 3.23). First lactation TNC cows that calved during in the spring produced 0.022 percentage points more protein in their milk than the HSC cows (P=0.0022); in contrast first lactation HSC cows that calved in the fall produced 0.030 percentage points more protein in their milk (P<0.0001). In the second lactation, HSC cows that calved in the spring and winter produced 0.029 percentage points and 0.038 percentage points more protein in their milk than the TNC cows calving in the same season, respectively (P=0.0002; P<0.0001). Second lactation HSC cows that calved in the fall had a tendency to produce 0.014 percentage points more protein in their milk than the TNC contemporaries (P=0.0128). Of the third lactation cows that calved in the summer, fall, and winter, the HSC cows produced more protein in their milk than the TNC cows (Summer: 0.020 percentage points, P=0.0029; Fall: 0.023 percentage points, P<0.0001; Winter: 0.018 percentage points, P=0.0053).

Table 3.9. Differences in protein percent between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Georgia.

Lactation	Season of Calving	P-Value	Percent Protein Produced[1] (%)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	<0.0001	0.031	0.008	0.011	0.052
1	Summer	0.7778	0.002	0.008	-0.019	0.024
1	Fall	<0.0001	-0.030	0.008	-0.049	-0.011
1	Winter	0.1036	-0.015	0.009	-0.039	0.009
2	Spring	0.1448	-0.014	0.010	-0.038	0.011
2	Summer	0.3254	0.008	0.008	-0.013	0.028
2	Fall	0.9123	0.001	0.006	-0.016	0.017
2	Winter	0.1533	-0.011	0.008	-0.032	0.009
3	Spring	0.8067	0.002	0.010	-0.024	0.028
3	Summer	0.7662	-0.002	0.008	-0.023	0.018
3	Fall	0.8283	0.0001	0.006	-0.014	0.017
3	Winter	0.4339	-0.006	0.007	-0.024	0.013

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1% would indicate that TNC cows performed 1 percentage point better than the HSC cows, while an estimate of -1% would indicate HSC cows performed 1 percentage point better than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

Table 3.10. Differences in protein percent between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida.

Lactation	Season of Calving	P-Value	Percent Protein Produced[1] (%)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.0001	0.034	0.009	0.011	0.056
1	Summer	0.0016	-0.029	0.009	-0.053	-0.005
1	Fall	0.0034	-0.026	0.009	-0.049	-0.003
1	Winter	0.2159	-0.013	0.010	-0.039	0.014
2	Spring	0.2045	-0.011	0.009	-0.034	0.012
2	Summer	0.6319	0.004	0.008	-0.016	0.024
2	Fall	0.7525	0.002	0.007	-0.015	0.019
2	Winter	0.0125	-0.021	0.009	-0.044	0.001
3	Spring	0.8292	0.002	0.009	-0.025	0.007
3	Summer	0.9885	0.0001	0.008	-0.020	0.020
3	Fall	0.1656	-0.009	0.006	-0.025	0.007
3	Winter	0.4672	-0.005	0.007	-0.025	0.014

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1% would indicate that TNC cows performed 1 percentage point better than the HSC cows, while an estimate of -1% would indicate HSC cows performed 1 percentage point better than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

Table 3.11. Differences in protein percent between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas.

Lactation	Season of Calving	P-Value	Percent Protein Produced[1] (%)	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.0022	0.022	0.007	0.004	0.041
1	Summer	0.4104	-0.008	0.010	-0.034	0.017
1	Fall	<0.0001	-0.030	0.007	-0.050	-0.011
1	Winter	0.1375	-0.015	0.010	-0.042	0.011
2	Spring	0.0002	-0.029	0.008	-0.049	-0.009
2	Summer	0.4547	0.0053	0.007	-0.013	0.024
2	Fall	0.0128	-0.014	0.006	-0.029	0.001
2	Winter	<0.0001	-0.038	0.008	-0.058	-0.019
3	Spring	0.3725	-0.007	0.008	-0.029	0.014
3	Summer	0.0029	-0.020	0.007	-0.038	-0.003
3	Fall	<0.0001	-0.023	0.005	-0.037	-0.009
3	Winter	0.0053	-0.018	0.007	-0.036	-0.001

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1% would indicate that TNC cows performed 1 percentage point better than the HSC cows, while an estimate of -1% would indicate HSC cows performed 1 percentage point better than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

Discussion

There is a complex interaction between heat stress and milk production and composition; this is particularly true when heat stress occurs around the time of conception. Firstly, cows are conceived and typically calve thirty-three months later, therefore a cows conceived in the summer will calve in the fall; this confounding makes it difficult to definitively attribute results to heat stress around conception. Secondly, elucidating the reasoning behind this relationship is unfeasible because a multitude of confounding factors that cannot be accounted for in such an observational study. These factors include, but are not limited to, nutritional plane and on-farm management practices. Therefore, only the relationship between periconceptional heat stress and subsequent performance parameters can be discussed. Although this relationship is likely affected by an array of factors, the fact that significant differences were observed between the HSC and TNC cows over a multitude years, herds, and cows suggests that despite potential confounding factors, the long-term effects observed in this study are a result of heat stress around the time of conception.

It should also be acknowledged that the dependent variable mature-equivalent milk yield, is derived using factors of a cow's age, breed, season of calving, and state. These factors are taken into account to fit the appropriate lactation curve and to again to appropriately adjust for mature milk production. The model used for evaluation also takes into account these factors. Therefore, any results that show a difference within these fixed effects suggests that the adjustment does not reflect actual production; this is discussed further in Appendix A.

Milk production was significantly affected by HSCONCP, with TNC cows always producing more than the HSC cows. Of the cows that calved in the spring, the TNC cows produced more milk than the HSC cows in all lactations in Texas. In Georgia and Florida, the TNC cows outperformed the HSC cows in the second and third lactations. Of the cows that calved in the summer, the TNC cows produced more milk than the HSC cows in all lactations in Georgia, and Texas. In Florida, the TNC cows produced more milk than the HSC cows in both the first lactation and second lactation. Of the cows that calved in the fall, the TNC cows produced more milk than the HSC cows over all lactations in Georgia. In Florida and Texas, TNC cows produce more milk than the HSC cows in both the first and third lactations. Of the cows that calved in the winter, the TNC cows produced more milk than the HSC cows over all lactations, in all three states.

The summer and winter are more stable seasons, with the average temperature-humidity index values for those seasons more consistent than in the spring or fall (listed in Appendix A); as such, it makes sense that the TNC cows would more frequently outperform the HSC cows in the summer and winter than the spring or fall. The TNC cows producing more milk in the winter and fall than in the summer and spring respectively suggest that the HSC cows may have some advantage during subsequent periods of heat stress.

Third lactation TNC cows outperformed the HSC cows more often than cows in their first and second lactations, suggesting that heat stress at the time of conception can not only result in long term economic losses but that these losses may become more severe the longer a cow remains in a herd.

Milk composition was much more variable. In some circumstances the HSC cows produced a higher percentage of fat and protein in their milk, as would be expected with HSC cows producing a smaller volume of milk overall; in other circumstances the TNC cows produced a higher percentage of fat and protein in their milk. Despite the statistically significant differences in between the HSCONCP groups, there were no biologically meaningful differences

Unlike the trend seen in total milk produced, the frequency in alterations of milk composition decreased as lactation number increased. The fat and protein percentages were found to differ between TNC and HSC cows more frequently in first lactation animals than those in their second or third lactation. Similarly, second lactation cows more frequently had altered milk composition than those in their third lactation.

Changes in milk constituents were not consistent or biologically significant. Although heat stress has been shown to alter fat and protein composition, it is generally attributed to the seasonal alterations in forage availability and depressed nutrient acquisition resulting from reduced feed intake (Jenness, 1985; Larson, 1985). It is likely that the small variations of the proportions of milk fat and protein relative to total milk content observed here are a direct result of alterations in feed intake and quality.

Overall, the improved performance of the TNC cows compared to their HSC contemporaries is worth attention. Despite potential confounding variables, the data shows that periconceptional heat stress can have significant long-term economic impacts, particularly in cows that are retained in a herd for multiple years.

CHAPTER IV: Periconceptional heat stress of multiparous Holstein cows affects subsequent days open

Abstract

Heat stress experienced by females around the time they were conceived could confer long-lasting effects on subsequent reproductive efficiency. This study aimed to examine if there was a relationship between periconceptional heat stress and days open in cows retained within a herd for a minimum of three lactations. National Dairy Herd Improvement Association data was obtained from Dairy Records Management Systems. Records (n=87,202) included Holstein cows born between 2000 and 2010 in GA, FL, and TX. Conception dates were calculated by subtracting 276 d from the recorded birth date. Records for cows conceived within the months of June, July, and August were retained as heat stress-conceived (HSC) cows; cows conceived within the months of December, January, and February were retained as thermoneutral conceived (TNC) contemporaries. Days open was evaluated with a mixed model ANOVA using SAS. One consistent pattern emerged; when the cows were bred in the summer, TNC cows remained open longer than HSC cows. The difference between TNC and HSC cows during other seasons and throughout three lactations was highly variable. However, the relationship between HSC and days open suggests that heat stress at the time of conception and during early pregnancy does alter a cow's subsequent reproductive performance.

Keywords: Heat stress, Periconceptional, Days Open, Holsteins

Introduction

Periconceptional heat stress has the potential to alter the genome during critical developmental processes; these transcriptional changes can carry on throughout animal's life and result in differential behavior during subsequent periods of heat stress (Elasser et al., 2012).This is particularly important un heifers that are retained in the breeding herd.

Heat stress is known to cause a variety of maladies in dairy cattle. These included alterations in health, feed efficiency and intake, milk production and quality, and reproductive performance (Wheelock et al., 2010). These factors all drive economic loss to the dairy producer. In the United States alone, approximately $1 billion is lost annually as a result of poor performance during periods of heat stress (Wheelock et al., 2010).

Reproductive efficiency is essential to dairy cow productivity. Reproductive performance is compromised when cows experience hyperthermia. Heifers and cows undergo ovarian deregulation as circulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) profiles are altered (Bernabucci et al., 2010). Furthermore, ova are retained within follicles through prolonged follicular waves (Wilson et al., 1998a; Wilson et al., 1998b) which reduces developmental competence through the detrimental effects of heat and aging (Al-Katanani et al., 2002). These ova may also be released in silent ovulations (Bilby et al., 2008) which result in decreased breeding opportunities. A decline in breeding opportunities, in combination with reduced ova competence and early embryonic death loss result in increased days open.

This study examines the effects of periconceptional heat stress on days open of cows retained within one herd for multiple years. It seeks to answer the question: does periconceptional heat stress experienced by these females confer long-lasting effects that alter the number of days open during the first three lactations? Days open is an economically important reproductive measurement as it represents a lengthened calving interval and potentially semen lost due to failed breeding attempts.

Materials and Methods

Inclusion Criteria

Dairy Herd Information Association data was received from Dairy Records Management Systems (Raleigh, NC) for three hot and humid states: Georgia, Florida, and Texas. For the purposes of this study cows were required to have at least their first, second, and third lactation within one herd to be included in the analysis; only the first three lactations were evaluated.

Records for FL (n=26,950) were too computationally demanding to complete Proc Mixed, so a random subset of the records were evaluated. Ninety percent of Florida cows were retained for analysis; random selection was performed by Proc Surveyselect of SAS. For each state, the total number of cows included in the analysis are listed in Table 4.1. Data was processed as previously mentioned in Chapter I.

Data Analysis

Statistical analysis was performed as previously mentioned in Chapter II. Herd, denoted by $H_{i,}$ where i=1, … $n_{h,}$ is considered a random effect because each herd is assumed to come from an infinite population of similar herds; a herd has a random effect generated from a Normal (0, σ_h^2). Within each herd there were cows that were either conceived under periods of heat stress, or conceived under thermoneutral conditions. The effect of HSC is denoted as τ_1 and the effect of TNC is denoted as τ_2.Multiple measurements are taken for each cow, which are nested within a specific herd and within the HSCONCP designation. Cow is considered random effect because each cow is assumed to come from an infinite population of similar cows; a cow has a random effect generated from a Normal (0, σ_c^2). Each cow has its own effect, denoted by

$C_{k(ij)}$where *k(ij)* means the *k*-th cow nested in herd *i* and HSCONCP category *j*. Each cow has three observed parities, and each parity has effect β_l, l = 1, 2, 3. The season of calving for each parity is also taken into account, and this seasonal effect is denoted by θ_m, *m*=1,2,3,4 where each number corresponds to a season in the year (spring, summer, fall, winter).

For ANOVA for fixed effects Type III sums of squares was used. Type II sums of squares are used for unbalanced data. To calculate degrees of freedom for error Satterhurthe's Approximation was used. Satterhurthe's Approximation is used when there is not constant variance. The fixed effects include HSCONCP, SOC, and all of their interactions. The model, which includes and overall intercept μ, for response Y_{ijklm} is

$$Y_{ijklm} = \mu + H_i + \tau_j + C_{k(ij)} + \beta_l + (\tau\beta)_{jl} + \theta_m + (\tau\theta)_{jm} + (\beta\theta)_{tm} + e_{ijklm}$$

where Hi ~ N(0, σ_h^2) and $C_{k(ij)}$~ N(0, σ_c^2) and $(\tau\beta)_{jl}$ is the interaction effect for heat-stress at conception and parity. The response (Y_{ijklm}) is days open (i.e., days not pregnant). Days open is calculated by DRMS for DHIA; according to DHIA days open is the days from calving until successful breeding date if pregnant.

Table 4.1. Number of cows from each of the three states that were included in the analysis of three sequential parity records.

	GA	FL	TX
TNC cows	13,745	15,609	23,712
HSC cows	8,098	8,686	17,352
Total	21,843	24,295	41,064

Results

The results are presented as specific contrasts; contrasts are made where significantly relevant, that is to say the most significant interaction that includes HSCONCP. For Georgia we found HSCONCP, LACTNO and SOC to be significant main effects (P=0.0019; P<0.0001; P<0.0001). The interaction between LACTNO and SOC was also significant (P<0.0001), as well as the interaction between HSCONCP and SOC (P=0.0004; Table 4.2; Figure 4.1). Thermoneutral conceived cows that calved in the spring had 11 more days open than the HSC cows across all lactations (P<0.0001).

For Florida we found LACTNO and SOC to be significant main effects (P<0.0001; P<0.0001), and HSCONCP to have a tendency for significance (P=0.0189). The two-way interactions between LACTNO and SOC, and SOC and HSCONCP were significant (P<0.0001; P<0.0001). The three-way interaction also had a tendency for significance (P=0.0114; Table 4.3; Figure 4.2). First lactation TNC cows that calve in the spring have a slight tendency to have 7 more days open than the HSC cows (P=0.0494).Of the first lactation cows that calved in the summer, the HSC cows had a statistical tendency to have 8 more days open than the TNC cows (P=0.271). In the second lactation, TNC cows that calved in the spring had 12 more days open than the HSC cows (P=0.0003), while the HSC cows that calved in the winter had a tendency to have 7 more days open than the TNC cows (P=0.0248). In the third lactation, TNC cows that calved in the spring had 14 more days open than the HSC cows (P<0.0001), while the TNC cows that calved in the winter had a tendency to have 6 more days open than the HSC cows (P=0.0351).

For Texas the main effects HSCONCP, LACTNO and SOC to be significant ($P<0.0001$; $P<0.0001$; $P<0.0001$), and the interaction between HSCONCP and SOC was also significant ($P<0.0001$). Additionally the three-way interaction was significant ($P<0.0001$; Table 4.4; Figure 4.3). Of the first lactation cows that calve in the summer, fall, and winter, the TNC cows have longer days open than their HSC counterparts ($P=0.0023$; $P=0.0023$; $P<0.0001$; respectively). Of the second lactation cows, only the cows that calved in the spring had a statistical difference in days open; the TNC had 13 more days open than the HSC cows ($P<0.0001$). Of the third lactation cows, TNC cows that calved in the spring and winter had more days open than the HSC cows (spring: 13 days open, $P<0.0001$; winter: 7 days open, $P=0.0025$). Of the third lactation cows that calved in the fall, the HSC cows had 6 more days open than the TNC cows ($P=0.0012$).

Table 4.2. Differences in days open between thermoneutral conceived (TNC) and heat stress conceived(HSC) cows in Georgia.

Lactation	Season of Calving	P-Value	Days Open[1]	S.E.	99% Lower Limit	99% Upper Limit
All	Spring	<0.0001	11	2	5	16
All	Summer	0.6761	-1	2	-6	4
All	Fall	0.3275	2	2	-3	6
All	Winter	0.5328	1	2	-4	7

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 day would indicate that TNC cows were open 1 day longer than the HSC cows, while an estimate of -1 day would indicate HSC cows were open 1 day longer than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

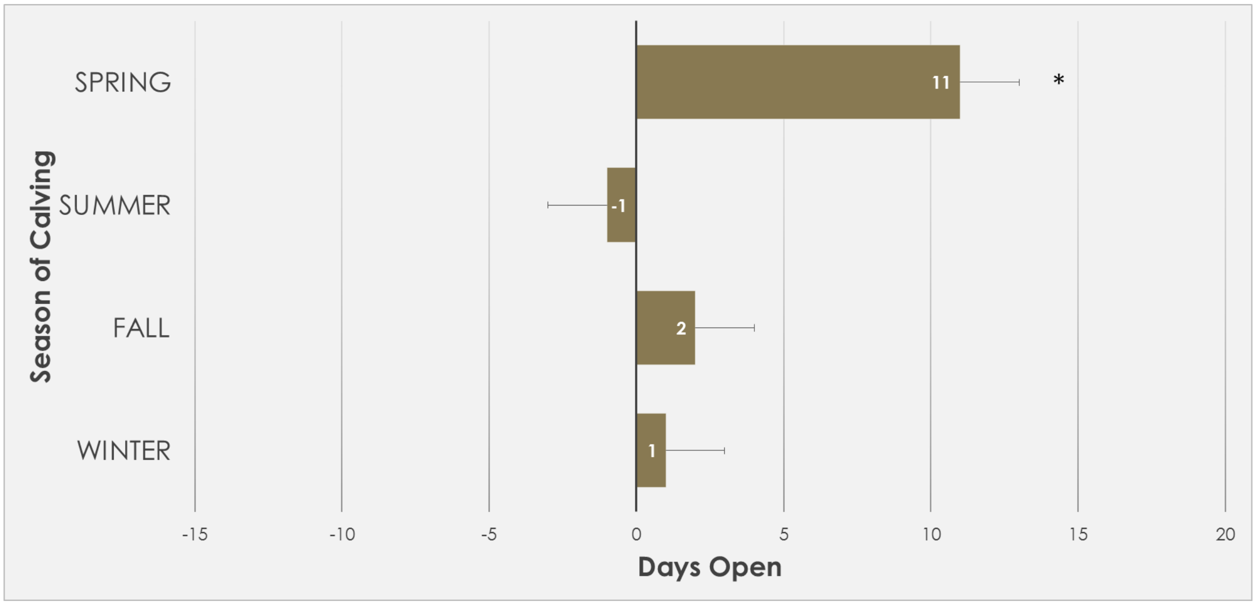

Figure 4.1. Seasonal differences in days open between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Georgia.Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 day would indicate that TNC cows were open 1 day longer than the HSC cows, while an estimate of -1 day would indicate HSC cows were open 1 day longer than the TNC cows.† denotes P<0.05; * denotes P<0.01.

Table 4.3. Differences in days open between thermoneutral conceived (TNC) and heat stress conceived(HSC) cows in Florida.

Lactation	Season of Calving	P-Value	Days Open[1]	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.0494	7	5	-2	16
1	Summer	0.0271	-8	4	-18	1
1	Fall	0.2226	4	3	-4	12
1	Winter	0.9444	0	4	-10	10
2	Spring	0.0003	12	3	4	21
2	Summer	0.4467	2	3	-6	10
2	Fall	0.3627	-2	2	-8	4
2	Winter	0.0248	-7	3	-15	4
3	Spring	<0.0001	14	4	5	24
3	Summer	0.8034	1	3	-7	9
3	Fall	0.4808	-2	2	-7	4
3	Winter	0.0351	6	3	-1	13

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 day would indicate that TNC cows were open 1 day longer than the HSC cows, while an estimate of -1 day would indicate HSC cows were open 1 day longer than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

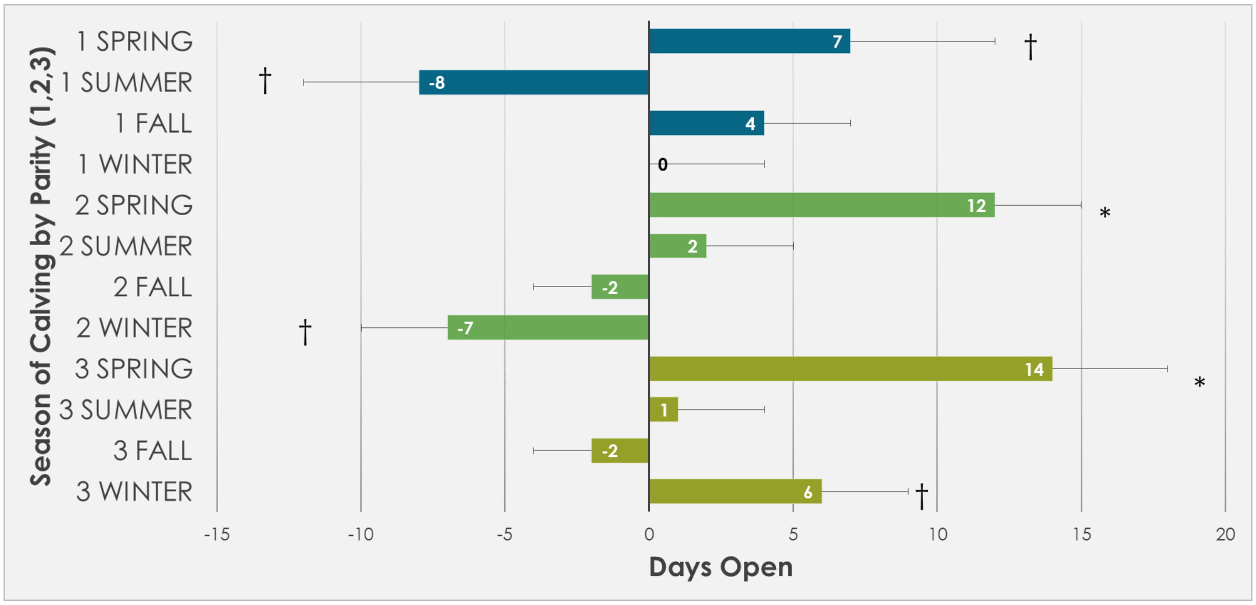

Figure 4.2. Seasonal differences in days open between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Florida. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 day would indicate that TNC cows were open 1 day longer than the HSC cows, while an estimate of -1 day would indicate HSC cows were open 1 day longer than the TNC cows.† denotes $P<0.05$; * denotes $P<0.01$.

Table 4.4. Differences in days open between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas.

Lactation	Season of Calving	P-Value	Days Open[1]	S.E.	99% Lower Limit	99% Upper Limit
1	Spring	0.2785	-3	3	-9	4
1	Summer	0.0023	11	3	2	19
1	Fall	0.0023	7	2	1	14
1	Winter	<0.0001	15	3	7	24
2	Spring	<0.0001	13	3	6	20
2	Summer	0.0666	4	2	-2	11
2	Fall	0.1640	-3	2	-8	2
2	Winter	0.0531	5	3	-2	11
3	Spring	<0.0001	13	3	6	20
3	Summer	0.5947	1	2	-5	20
3	Fall	0.0012	-6	2	-11	-1
3	Winter	0.0025	7	2	1	12

[1]Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 day would indicate that TNC cows were open 1 day longer than the HSC cows, while an estimate of -1 day would indicate HSC cows were open 1 day longer than the TNC cows. Negative and positive values that span the confidence interval indicate that the estimate is not a reliable indicator of how HSCONCP effects cow performance.

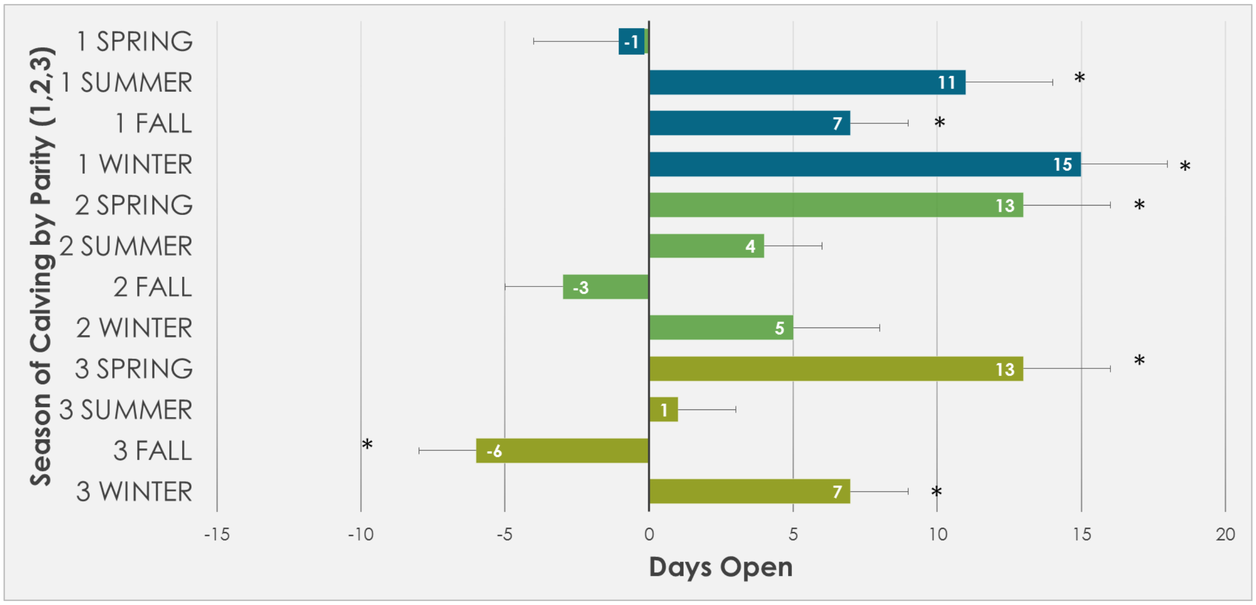

Figure 4.3. Seasonal differences in days open between thermoneutral conceived (TNC) and heat stress conceived (HSC) cows in Texas. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 day would indicate that TNC cows were open 1 day longer than the HSC cows, while an estimate of -1 day would indicate HSC cows were open 1 day longer than the TNC cows.† denotes P<0.05; * denotes P<0.01.

Discussion

The relationship between heat stress at the time of conception and days open is affected by a multitude of factors including nutritional plane and on-farm management practices. These are confounding factors that cannot be accounted for in such an observational study. The potential for differences in on-farm management practices are particularly important to keep in mind because variations in voluntary-waiting periods and breeding protocols (i.e. artificial insemination vs. natural service vs. a combination of artificial insemination and a clean-up bull) will make substantial differences in reproductive measurements- especially days open.

Increasing voluntary-waiting periods is often a sound management decision (Oseni et al., 2003, 2004); however, with the data available for analysis it was impossible to account for the length of the voluntary-waiting period. Therefore, it was important to make comparisons within SOC, as seasonal variations in voluntary-waiting periods are to be expected (Oseni et al., 2003, 2004). Furthermore, male fertility is sensitive to heat stress, with sperm motility and fertilization ability becoming compromised (Chandolia et al., 1999; Sartori et al., 2002); the status of sperm quality and breeding practices for the data used in this analysis were unknown. However, the fact that significant differences between HSC cows and TNC cows were observed over the a multitude of years, herds, and cows suggests that despite potential confounding factors, heat stress at the time of conception does have long-term effects.

Days open was significantly affected by HSCONCP in all three states. Of the cows that calved in the spring, and therefore were bred in the summer, the TNC cows had more days open than the HSC cows in all lactations in Georgia and Texas. The TNC in Texas had more days open in both the second and third lactations. Of the cows that calved in the summer, and therefore were bred in the fall, only first lactation cows in Florida and Texas had a significant difference in days open between TNC and HSC. In Florida the HSC cows had more days open than the TNC, while the TNC cows had more days open in Texas. In Texas, the TNC cows had more days open than the HSC cows only in their first lactation, but in the third lactation, the HSC cows had more days open. Of the cows that calved in the winter, and therefore were bred in the spring, only Florida and Texas had significant differences in TNC and HSC cows. Second and third lactation HSC cows in Florida had more days open than their TNC counterparts. In Texas, the TNC cows had more days open than the HSC in both the first and third lactation.

The results are highly variable, with only one consistent pattern emerging; when the cows were bred in the summer, TNC cows remained open longer than HSC cows. This suggests that periconceptional heat stress may offer some reproductive advantage during subsequent heat stress exposure. Studies performed by Oseni and colleagues (2003, 2004) have demonstrated that cows in the southeastern region of the United States have the highest number of days open in the summer compared to any other season. While the mean number of days open for each season was not determined for this study, the results from Oseni and colleagues (2003, 2004) and the significant differences between TNC and HSC cows seen here, further supports the assumption that HSC cows have some genetic advantage during periods of heat stress. Furthermore, the difference in days open between cows that experienced periconceptional heat stress and those that did not, demonstrated that heat stress can have significant long-term economic impacts, particularly in cows that are retained in a herd for multiple years. Further studies are needed to explore the mechanisms responsible for this relationship and the resulting effect on reproductive efficiency.

CHAPTER V: Conclusions

Milk yield and days open throughout the first three productive years of Holstein cows are related to the periconceptional heat stress status of that cow. Overall, cows that endured periconceptional heat stress performed poorly compared to the TNC contemporaries. Although the HSC cows appeared to possess some advantage that enabled during periods of heat stress (i.e., summer), this did not make up for inferior production under predominantly thermoneutral conditions (i.e., fall and winter). This is particularly apparent when all first lactation cows are compared to those that are retained within one herd for multiple years. Of the entire first lactation population, the HSC cows were able to outperform the TNC cows following spring calving; however, of the cows that were retained for at least three parities, the TNC cows always performed similarly or produced a greater quantity of milk than those that were HSC. This is possibly a result of the low producing first lactation cows being culled from the herd.

Because observational studies are limited to identifying relationships rather than causative effects, further research should be performed to determine the mechanisms underlying periconceptional heat stress, and the scope of effects encountered through adulthood. The results determined thorough these studies suggest that HSC cows may experiences alterations in mammary development, uterine environment and endocrine profiles. Therefore, experiments that focus on embryological development and epigenetic changes that occur in mammary and reproductive tissues as a result of periconceptional heat stress would shed the most light on the suspected mechanisms. Furthermore, experiments evaluating circulating progesterone, FSH, LH and estradiol during periods of heat stress in addition to the ovarian structures present over the course of an estrous cycle will provide insight into the mechanism driving a smaller number of days open observed in the HSC cows during summer months.

Other studies would provide further insight into the effects of periconceptial heat stress. It would be particularly interesting to evaluate whether TNC and HSC cows experience hyperthermia at the same THI values, or if HSC cows are able to withstand a higher THI than the TNC cows. Additionally it would be interesting to evaluate the heritability of traits altered by perconceptional heat stress; specifically to answer the question: if a dam is conceived during a period of heat stress, will her TNC calf subsequently produce less milk than the TNC calf of a TNC dam? However, most of the future periconceptional heat stress experiments proposed are incredibly limited as a result of the time necessary for a cow to reach maturity following periconceptional treatment. This creates a substantial financial burden on the research; furthermore, controlling confounding factors during such a prolonged time period would be extremely difficult.

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Appendix A: Data Retrieval and Processing

Dairy Herd Information Association Data

Dairy Herd Information Association data was received from Dairy Records Management Systems (Raleigh, NC) for seven hot and humid states: South Carolina, Alabama, Mississippi, Louisiana, Georgia, Florida, and Texas. Total data received included 14,189,891 records spanning from 1977 through 2010.

To remove variability that could be attributed to differences in breed, only cows that were listed as Holsteins were retained. To help ensure the accuracy of the breed data, cows that were listed as Holstein but whose parents and grandparents were not identified, were removed. To evaluate modern Holstein production, data was pared down to only include cows that were born within the time frame of 2000 through 2010. Any records with >600 DIM were removed.

In order to evaluate heat stress at the time of conception (HSCONCP), conception date was calculated assuming that the average gestation length of a Holstein was 276 d. (Dhakal et al., 2013). The following formula was used: Conception date = Birth date – 276 d. Heat stress at the time of conception was determined by the month in which conception took place. Cows that were conceived during December, January, and February were considered thermoneutral conceived (TNC), while cows conceived during June, July, and August were considered heat stress conceived (HSC). Since fall (September, October and November) and spring (March, April, and May) weather conditions are intermediate and highly variable, cows conceived during these months were excluded from analysis.

The season of calving (SOC) for each lactation was also considered because of its inherent effects on milk production. Season of calving was determined using the month of each calving date. The seasons were designated as spring: March, April, May; summer: June, July, August; fall: September, October, November; and winter: December, January and February.

The dependent variable used for milk yield evaluations was mature-equivalent milk yield. This variable is computed by DRMS from DHIA test day milk weights and corresponding days in milk that have been fitted to a 305 day lactation curve. The cow's age, breed, season of calving, and state are taken into account to fit the appropriate lactation curve. Finally, the 305 day milk yield value is adjusted to the amount of milk each cow would produce as a mature animal. The mature-equivalent factors vary based on cow's age, breed, season of calving, and state. The adjustment factors were developed by Bob Everett at Cornell in 1974, and therefore likely do not appropriately reflect the performance of modern cows.

To show determine if 305 day milk yield and mature-equivalent milk yield were similar, analyses were run for first-lactation records for Georgia and Florida using the model:

$$Y_{ijkl} = \mu + H_i + \tau_j + C_{k(ij)} + \theta_l + (\tau\theta)_{jl} + e_{ijkl},$$

where $H_i \sim N(0, \sigma^2_h)$, $C_{k(ij)} \sim N(0, \sigma^2_c)$, and $(\tau\theta)_{jl}$ is the interaction between season of calving and HSCONCP. The response variable Y_{ijkl} was 305 d milk yield, which was compared to the results discussed in Chapter II. Only Georgia and Florida first lactation records were compared because of computational demand (each analysis takes >48 hrs to run); however, it is believed the results will be similar for the other states and parities. The analyses contained the same number of cows for both dependent variables (Georgia n=67,333; FL n=67,822).

For both Georgia and Florida, the interaction between season of calving and HSCONCP was significant (P=0.0005; P<0.0001). In Georgia there was no significant difference in 305 d milk production of the cows that calved in spring (P=0.6018) or summer (P=0.0816); however numerically, of the cows that calved in the spring the HSC cows produced more milk, and of the cows that calved in the summer, the TNC cows produced more milk. Of the cows that calved in the fall and winter, the TNC cows produced significantly more milk than their HSC counterparts (P<0.0001; P=0.0049). In Florida, of the cows that calved in the spring and summer, the HSC cows produced more milk (P<0.0001; P<0.0064). Conversely, of the cows that calved in the fall and winter, the TNC cow produced more milk (P<0.0001; P<0.0001). The results were similar to those seen when the dependent variable was mature-equivalent milk production. A comparison between the results for both dependent variables can be seen for Georgia in Figure A.1, and for Florida in Figure A.2. The similar trend in results suggest that both dependent variables tell us similar information, and therefore it is not inaccurate to use mature-equivalent milk yield as the dependent variable.

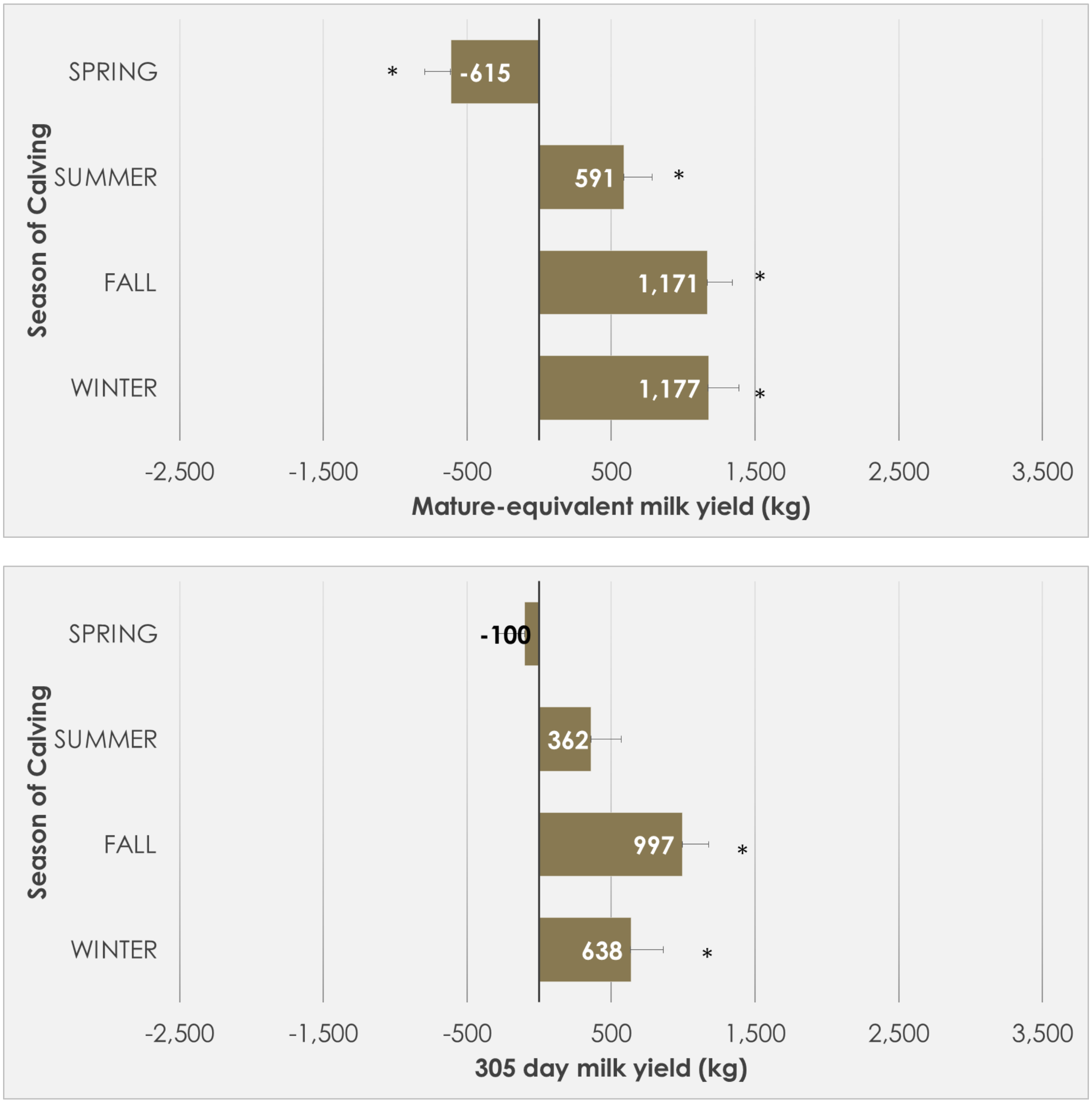

Figure A.1. Comparison between 305 day milk yield and mature-equivalent milk yield results for Georgia. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1kg more milk than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows.†denotes P<0.05; * denotes P<0.01.

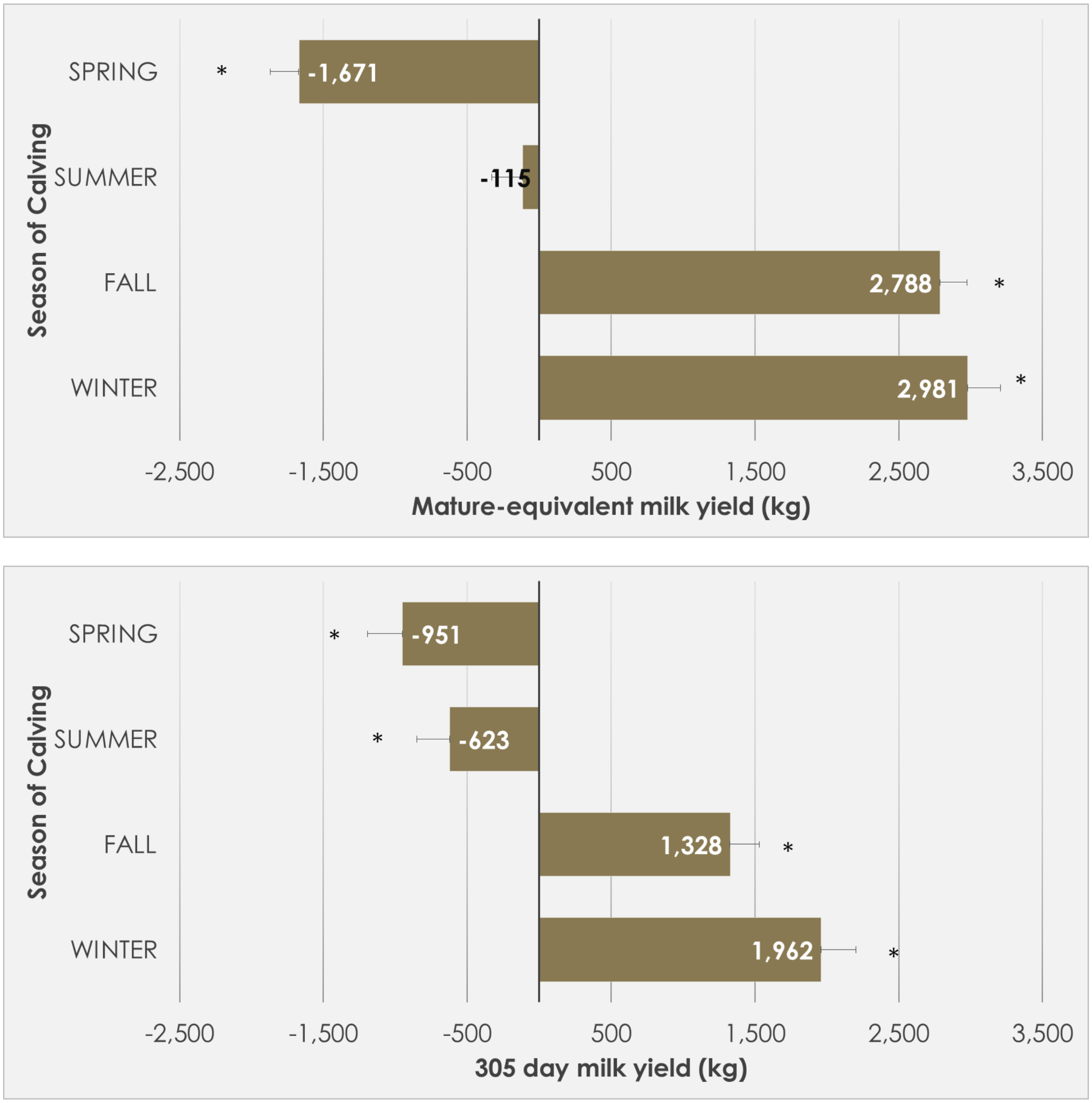

Figure A.2. Comparison between 305 day milk yield and mature-equivalent milk yield results for Florida. Positive values indicate the superior performance of TNC cows, while negative values indicate the superior performance of HSC cows; e.g. an estimate of 1 kg would indicate that TNC cows produced 1kg more milk than the HSC cows, while an estimate of -1 kg would indicate HSC cows produced 1 kg more than the TNC cows. † denotes P<0.05; * denotes P<0.01.

There, is a confounding of season of conception and season of first lactation. Cows are conceived and typically calve thirty three months later, at twenty four months of age. Therefore a cow's first calving is typically the season before the one in which she was herself conceived (i.e. a cow conceived in the winter will calve in the fall); this makes it difficult to definitively attribute results to heat stress around conception. The confounding between season of conception and season of first calving can fall apart when producers choose to breed for certain calving seasons; however, for the majority of cows this is not the case. The distribution of primiparous cows used in the analysis of mature-equivalent milk yield based on season of calving is shown Table A.1. Following the first lactation, if a cow is bred within the first three services following the voluntary waiting period of 60 days, she will continue to calve in the same season on an annual basis. The distribution of multiparous cows used in the analysis of mature-equivalent milk yield based on season of calving is shown Table A.2.

Table A.1. Distribution of primiparous cows included in analysis of mature-equivalent milk production, based on season of calving.

State	HSCONCP	Spring	Summer	Fall	Winter
Georgia	TNC	4,792 (12%)	3,789 (9%)	16,125 (40%)	16,053 (39%)
Florida	TNC	3,489 (8%)	2,972 (7%)	20,197 (47%)	16,465 (38%)
Texas	TNC	4,134 (11%)	2,329 (6%)	17,046 (44%)	15,363 (40%)
Georgia	HSC	9,912 (37%)	9,718 (37%)	4,388 (17%)	2,556 (10%)
Florida	HSC	10,132 (41%)	8,866 (36%)	3,517 (14%)	2,272 (9%)
Texas	HSC	12,410 (40%)	12,075 (39%)	4,438 (14%)	2,110 (7%)

Table A.2. Distribution of multiparous cows included in analysis of mature-equivalent milk production, based on season of calving.

State	Parity	HSCONCP	Spring	Summer	Fall	Winter
Georgia	1	TNC	1,394 (11%)	1,029 (8%)	5,058 (41%)	4,985 (40%)
Florida	1	TNC	1,068 (7%)	1,067 (7%)	7,543 (48%)	5,920 (38%)
Texas	1	TNC	2,118 (10%)	983 (5%)	9,301 (44%)	8,675 (41%)
Georgia	2	TNC	1,460 (12%)	1,608 (13%)	4,846 (39%)	4,552 (37%)
Florida	2	TNC	1,620 (10%)	1,923 (12%)	6,583 (42%)	5,472 (35%)
Texas	2	TNC	2,376 (11%)	2,534 (12%)	8,497 (40%)	7,670 (36%)
Georgia	3	TNC	1,516 (12%)	1,912 (15%)	4,802 (39%)	4,336 (34%)
Florida	3	TNC	1,901 (12%)	2,320 (15%)	6.262 (40%)	5,115 (33%)
Texas	3	TNC	2,556 (12%)	3,352 (16%)	8,272 (39%)	6,897 (33%)
Georgia	1	HSC	2,689 (37%)	2,757 (38%)	1,128 (16%)	687 (9%)
Florida	1	HSC	3,616 (43%)	2,939 (35%)	1,142 (14%)	739 (9%)
Texas	1	HSC	6,658 (44%)	5,615 (37%)	2,072 (14%)	936 (6%)
Georgia	2	HSC	1,589 (22%)	2,041 (28%)	2,441 (34%)	1,190 (16%)
Florida	2	HSC	1,940 (23%)	2,580 (31%)	2,622 (31%)	1,294 (15%)
Texas	2	HSC	3,766 (25%)	5,462 (36%)	4,083 (27%)	1,970 (13%)
Georgia	3	HSC	1,094 (15%)	1,726 (24%)	2,840 (39%)	1,601 (22%)
Florida	3	HSC	1,321 (16%)	2,134 (25%)	3,110 (37%)	1,871 (22%)
Texas	3	HSC	2,539 (17%)	4,779 (31%)	4,994 (33%)	2,969 (19%)

Climatic Data

Climatic data was received from the National Climatic Data Center. Data included hourly temperature, humidity, and dew-point observations from airports during 1999 through 2011. The locations (listed in Table A.3) were chosen to represent the average climate of each state. Data with error reports was removed, as well as any data outside of reasonable ranges for temperature (-50 to 50 °C), dew point (-50 to 50 °C) and relative humidity (0 to 100%).

Table A.3. Airports used for climatic data.

State	Airport
South Carolina	Greenvile/Greenvil
	Beaufort Mcas
Alabama	Huntsville/Madison
	Montgomery/Dannelly
Mississippi	McComb Pike Co Joh
	Tupelp/C.D. Lemons
Louisiana	Shreveport Regional
	Lafayette Rgnl
Georgia	Atlanta Hartsfield Intl Ap
	Macon / Lewis B. Wilso
Florida	Tallahassee Municip
	West Palm Beach/In
Texas	Fort Worth Nas JRB
	San Antonio Intl
	Lubbock/Lubbock Int
	Midland/Midland Reg.

Temperature-humidity index values were calculated using an equation derived for use with dairy cattle (Gaughan et al., 2012).

$$THI = T_{dry\ bulb} + ((0.36 * T_{dew\ point})+41.2)$$

A THI value was calculated for each hourly observation, at each airport; those THI values were then averaged to create one hourly THI for each state. The hourly THI values were averaged to create daily THI value, and the daily values were used to create average weekly values. From the weekly average THI value, corresponding average monthly THI value was calculated. Monthly THI were used to determine the treatment periods for HSCONCP, and to calculate seasonal THI values. The average THI for each season is listed in Table A.4.

Table A.4. Average temperature-humidity (THI) index per season for each state.

	SC		AL		MS		LA		GA		FL		TX	
	THI	**S.E.**	**THI**	**S.E.**	**THI**	**S.E.**	**THI**	**S.E.**	**THI**	**S.E.**	**THI**	**S.E.**	**THI**	**S.E.**
Spring	62.25	3.421	62.47	3.498	63.28	3.538	65.82	3.004	62.00	3.376	68.11	2.345	62.47	3.498
Summer	**74.71**	**0.623**	**74.77**	**0.537**	**75.81**	**0.699**	**76.01**	**0.473**	**74.38**	**0.606**	**76.60**	**0.411**	**74.77**	**0.537**
Fall	63.69	4.123	63.13	4.359	63.84	4.273	66.22	3.883	62.99	4.220	70.12	3.099	63.13	4.359
Winter	**50.10**	**0.570**	**49.65**	**0.693**	**50.03**	**0.770**	**54.42**	**0.713**	**49.69**	**0.632**	**60.09**	**0.628**	**49.65**	**0.693**

Appendix B: Selected SAS Code

```
/****************************************************/
* Analysis for dependent variable:
      MilkME
for first lactation cows. Analysis run separately for each state.

Example is for South Carolina.
/***************************/

/* Doing comparisons within Herd and within Cow will increase sensitivity to
differences */
/* We make them random effects because they are a random sample of all
possible herds and cows within herds */

/*Order of Effects*/
/*
      HSconcp:  Normal, HS
      SOC:  Fall, Spring, Summer, Winter
*/

/* Use this just to get covariance parameter estimates, which we will input
into proc mixed*/
prochpmixeddata=DHIA.SC_1;
      class herdcode cowindex soc hsconcp;
      model MilkME1k=hsconcp|soc;
      random intercept/subject=herdcode;
      random intercept/subject=cowindex(herdcode*hsconcp);
      odsoutput COVParms=HerdCowRE;
run;

/*Using the covariance parameters, we run proc mixed*/
procmixeddata=DHIA.SC_1 covtest;
      class herdcode cowindex  soc hsconcp;
      model MilkME1k=hsconcp|soc/ddfm=satterth;
      random intercept/subject=herdcode;
      random intercept/subject=cowindex(herdcode*hsconcp);
      parms / pdata=HerdCowRE noiter;
      estimate 'Norm-HS Across Lactno and SOC' hsconcp 1 -1/cl alpha=0.01;
      estimate 'Norm-HS @ Fall Across Lactno'
            hsconcp 3 -3
            soc 0000
            soc*hsconcp 3 -3000000/cl alpha=0.01;
      estimate 'Norm-HS @ Spring Across Lactno'
            hsconcp 3 -3
            soc 0000
```

```
            soc*hsconcp 003 -30000/cl alpha=0.01;
      estimate 'Norm-HS @ Summer Across Lactno'
            hsconcp 3 -3
            soc 0000
            soc*hsconcp 00003 -300/cl alpha=0.01;
      estimate 'Norm-HS @ Winter Across Lactno'
            hsconcp 3 -3
            soc 0000
            soc*hsconcp 0000003 -3/cl alpha=0.01;

run;


/****************************************************/
* Analysis used for dependent variables:
      Days Open
      MilkME
      Protein Percent
      Fat Percent
for multiparous cows. Analysis run separately for each state and each
dependent variable.

Example is for Florida days open.
/***************************/

/* Doing comparisons within Herd and within Cow will increase sensitivity to
differences */
/* We make them random effects because they are a random sample of all
possible herds and cows within herds */
/*Order of Effects*/
/*
      HSconcp:  Normal, HS
      LactNo: 1, 2, 3
      SOC:  Fall, Spring, Summer, Winter
*/
/* Use this just to get covariance parameter estimates, which we will input
into proc mixed*/
prochpmixeddata=DHIA.FL_r32;
      class herdcode cowindex lactno soc hsconcp;
      model daysop=hsconcp|lactno|soc;
      random intercept/subject=herdcode;
      random intercept/subject=cowindex(herdcode*hsconcp);
      odsoutput COVParms=HerdCowRE;
run;

/*Using the covariance parameters, we run proc mixed*/
procmixeddata=DHIA.FL_r32 covtest;
      class herdcode cowindex lactno soc hsconcp;
      model daysop=hsconcp|lactno|soc/ddfm=satterth;
      random intercept/subject=herdcode;
```

```
random intercept/subject=cowindex(herdcode*hsconcp);
parms / pdata=HerdCowRE noiter;
estimate 'Norm-HS Across Lactno and SOC' hsconcp 1 -1/cl alpha=0.01;
estimate 'L3-L1 Across HSConcp and SOC' lactno -101/cl alpha=0.01;
estimate '(L1+L3)/2-L2 Across HSConcp and SOC' lactno 1 -21
            /divisor=2 cl alpha=0.01;
estimate 'Norm-HS @ Fall Across Lactno'
      hsconcp 3 -3
      lactno 000
      lactno*hsconcp 1 -11 -11 -1
      soc 0000
      soc*hsconcp 3 -3000000
      lactno*soc 000000000000
      lactno*soc*hsconcp  1   -1    0     0     0     0     0     0
                        1     -1    0     0     0     0     0     0
                        1     -1    0     0     0     0     00
                        /divisor=3 cl alpha=0.01;
estimate 'Norm-HS @ Spring Across Lactno'
      hsconcp 3 -3
      lactno 000
      lactno*hsconcp 1 -11 -11 -1
      soc 0000
      soc*hsconcp 003 -30000
      lactno*soc 000000000000
      lactno*soc*hsconcp  001 -1    0     0     0     0
                        001   -1    0     0     0     0
                        001   -1    0     0     0     0
                        /divisor=3 cl alpha=0.01;
estimate 'Norm-HS @ Summer Across Lactno'
      hsconcp 3 -3
      lactno 000
      lactno*hsconcp 1 -11 -11 -1
      soc 0000
      soc*hsconcp 00003 -300
      lactno*soc 000000000000
      lactno*soc*hsconcp  00001     -1    0     0
                        00001 -1    0     0
                        00001 -1    0     0
                        /divisor=3 cl alpha=0.01;
estimate 'Norm-HS @ Winter Across Lactno'
      hsconcp 3 -3
      lactno 000
      lactno*hsconcp 1 -11 -11 -1
      soc 0000
      soc*hsconcp 0000003 -3
      lactno*soc 000000000000
      lactno*soc*hsconcp  0000001   -1
                        0000001     -1
                        0000001     -1/divisor=3 cl alpha=0.01;
```

```
estimate 'Norm-HS @ L1 Across SOC'
      hsconcp 4 -4
      lactno 000
      lactno*hsconcp 4 -40000
      soc         0000
      soc*hsconcp       1     -1    1     -1    1     -1    1     -1
      lactno*soc  000000000000
      lactno*soc*hsconcp 1    -1    1     -1    1     -1    1     -1
                        0     0     0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        /divisor=4 cl alpha=0.01;
estimate 'Norm-HS @ L2 Across SOC'
      hsconcp 4 -4
      lactno 000
      lactno*hsconcp 004 -400
      soc         0000
      soc*hsconcp       1     -1    1     -1    1     -1    1     -1
      lactno*soc  000000000000
      lactno*soc*hsconcp 0000 00    00
                        1-1   1-1   1-1   1-1
                        00    00    00    00/divisor=4 cl alpha=0.01;

estimate 'Norm-HS @ L3 Across SOC'
      hsconcp 4 -4
      lactno 000
      lactno*hsconcp 00004 -4
      soc         0000
      soc*hsconcp       1     -1    1     -1    1     -1    1     -1
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        1     -1    1     -1    1     -1    1     -1
                        /divisor=4 cl alpha=0.01;
estimate 'Norm-HS @ L1/Fall'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 1 -10000
      soc         0000
      soc*hsconcp       1     -1    0     0     0     0     0     0
      lactno*soc  000000000000
      lactno*soc*hsconcp 1    -1    0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L1/Spring'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 1 -10000
      soc         0000
```

```
      soc*hsconcp       0     0     1     -1    0     0     0     0
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     1     -1    0     0     0     0
                        0     0     0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L1/Summer'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 1 -10000
      soc         0000
      soc*hsconcp       0     0     0     0     1     -10   0
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     1     -10   0
                        0     0     0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        /divisor=1 cl alpha=0.01;

estimate 'Norm-HS @ L1/Winter'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 1 -10000
      soc         0000
      soc*hsconcp       0     0     0     0     0     01    -1
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     01    -1
                        0     0     0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L2/Fall'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 001 -100
      soc         0000
      soc*hsconcp       1     -1    0     0     0     0     0     0
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                        1     -1    0     0     0     0     0     0
                        0     0     0     0     0     0     0     0
                        /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L2/Spring'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 001 -100
      soc         0000
      soc*hsconcp       0     0     1     -1    0     0     0     0
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                        0     0     1     -1    0     0     0     0
```

```
                       0     0     0     0     0     0     0     0
                       /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L2/Summer'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 001 -100
      soc         0000
      soc*hsconcp      0     0     0     0     1     -10   0
      lactno*soc  000000000000
      lactno*soc*hsconcp  0   0     0     0     0     0     0     0
                       0     0     0     0     1     -10   0
                       0     0     0     0     0     0     0     0
                       /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L2/Winter'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 001 -100
      soc         0000
      soc*hsconcp      0     0     0     0     0     01    -1
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                       0     0     0     0     0     01    -1
                       0     0     0     0     0     0     0     0
                       /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L3/Fall'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 00001 -1
      soc         0000
      soc*hsconcp      1     -1    0     0     0     0     0     0
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                       0     0     0     0     0     0     0     0
                       1     -1    0     0     0     0     0     0
                       /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L3/Spring'
      hsconcp 1 -1
      lactno 000
      lactno*hsconcp 00001 -1
      soc         0000
      soc*hsconcp      0     0     1     -1    0     0     0     0
      lactno*soc  000000000000
      lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                       0     0     0     0     0     0     0     0
                       0     0     1     -1    0     0     0     0
                       /divisor=1 cl alpha=0.01;
estimate 'Norm-HS @ L3/Summer'
      hsconcp 1 -1
      lactno 000
```

```
        lactno*hsconcp 00001 -1
        soc         0000
        soc*hsconcp       0     0     0     0     1     -10   0
        lactno*soc  000000000000
        lactno*soc*hsconcp  0   0     0     0     0     0     0     0
                          0     0     0     0     0     0     0     0
                          0     0     0     0     1     -1    0     0
                                /divisor=1 cl alpha=0.01;
    estimate 'Norm-HS @ L3/Winter'
        hsconcp 1 -1
        lactno 000
        lactno*hsconcp 00001 -1
        soc         0000
        soc*hsconcp       0     0     0     0     0     01    -1
        lactno*soc  000000000000
        lactno*soc*hsconcp 0    0     0     0     0     0     0     0
                          0     0     0     0     0     0     0     0
                          0     0     0     0     0     01    -1
                          /divisor=1 cl alpha=0.01;
    estimate 'Norm L1-(L2+L3)/2 Across SOC'
        hsconcp 00
        lactno 8 -4 -4
        lactno*hsconcp 80 -40 -40
        soc         0000
        soc*hsconcp       0     0     0     0     0     00    0
        lactno*soc  2222 -1 -1 -1 -1 -1 -1 -1 -1
        lactno*soc*hsconcp 2    0     2     0     2     0     2     0
                          -1    0     -1    0     -1    0     -1    0
                          -1    0     -1    0     -1    0     -10
                          /divisor=8 cl alpha=0.01;
    estimate 'HS L1-(L2+L3)/2 Across SOC'
        hsconcp 00
        lactno 8 -4 -4
        lactno*hsconcp 080 -40 -4
        soc         0000
        soc*hsconcp       0     0     0     0     0     00    0
        lactno*soc  2222 -1 -1 -1 -1 -1 -1 -1 -1
        lactno*soc*hsconcp 02   0     2     0     2     0     2
                          0 -1  0     -1    0     -1    0     -1
                          0 -1  0     -1    0     -1    0     -1
                          /divisor=8 cl alpha=0.01;
run;
```