Behavioral and physiological differences of dry dairy cattle under heat stress based on median core body temperature

by

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B.S., Kansas State University, 2019

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2021

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Abstract

Heat stress has been demonstrated to have implications on the profitability of the dairy industry. The objectives of this observational study were to investigate behavioral differences of dairy cows having above or below the median core body temperature (CBT) assessed during 1 wk during late gestation as well as measure differences in pregnancy-associated glycoprotein (PAG) concentration. Temperature data were collected every 5 min for 7 d using a temperature logger attached to a intravaginal insert between d 220 and 241 of gestation. Within each of 5 replicates, cows having above median CBT were classified as high temperature (HT) and those below median CBT were classified as low temperature (LT). Behavioral data from 50 cows (10 cows per replicate) were collected using automated activity monitors equipped with accelerometers in addition to visual observations. Accelerometer data were evaluated from d -21 to 21 relative to actual calving date. Cows were observed for 8 h in the far-off pen and 8 h in the close-up pen. Each 8-h observation block consisted of two, 2-h morning observations (0600 to 0800 h) and two, 2-h afternoon observations (1600 to 1800 h). At enrollment, cows were between 220 and 241 days of gestation. Blood samples were collected weekly for 3 wk starting at enrollment. No differences were detected among visually observed behaviors during morning or afternoon observations in the far-off or close-up period. An interaction of temperature and parity ($P = 0.02$) revealed that HT primiparous cows spent the most time eating during afternoon visual observations (30.9%) followed by LT multiparous cows (25.4%), LT primiparous cows (22.5%), and finally HT multiparous cows spent the least time eating (19%). Accelerometer data demonstrated that HT cows had more ($P < 0.01$) high activity time than LT cows. Compared with primiparous cows, multiparous cows spent more time active during both prepartum ($P = 0.02$) and postpartum ($P < 0.01$) periods. High temperature cows were more ($P < 0.01$) inactive

than LT cows before calving. Cows classified as HT had greater (P = 0.05) blood concentrations of PAG during the first 3 wk of the far-off dry period than cows classified as LT. In conclusion, HT and LT cows display different behavior and have different PAG concentrations; however, more research is needed to identify specific thresholds to classify cows as HT and LT.

Table of Contents

List of Figures

List of Tables

Acknowledgments

I thank:

Austin, for his endless encouragement, love, and for his patience as he has listened to me talk ceaselessly about cows during the last 2 years.

My parents, who have taught me to be relentless in pursuing my dreams, and for supporting me through my relentlessness.

Dr. Luis Mendonça, for being an excellent advisor and mentor even during the uncertain times of a pandemic coupled with a career change. Your guidance is invaluable.

Dr. Jeffrey Stevenson, for serving on my committee and providing insight when I needed it.

Dr. Grieger, for serving on my committee and providing me with a chance to learn outside of the classroom. Also, for your perfectly timed comedic relief.

My fellow graduate students, for all the baked goods, comradery, late nights, and early mornings together. I've made some of my closest friends in my time here.

Kansas State University, for facilitating my completion of two degrees and allowing me opportunities to learn I would not have anywhere else.

Thank you all!

Dedication

This thesis is dedicated to my parents. I am the person I am today because of your unending love and support. You have moved mountains for me to chase my dreams and for that I can only hope to make you proud.

Chapter 1 - Heat Stress and Dairy Cow Behavior: Monitoring and Mitigation

Introduction

Heat stress has gained significant attention from the dairy industry in recent years. Many parts of the United States are experiencing higher average temperatures and higher maximum daily temperatures (Wuebbles et al., 2017). Increasing thermal stress because of climate change can have negative effects on key factors that impact profitability of a dairy, such as feed efficiency, livestock health, reproduction, and milk production (Key et al., 2014). Ravagnolo and Misztal (2000) demonstrated that selection for heat tolerance in dairy cattle is possible, but little is known about the genes for which to select until more recently. Basiricò et al. (2011) demonstrated that increased thermotolerance is associated with polymorphisms in the bovine heat shock protein 70.1 and suggested that these polymorphisms can be used as genetic markers for heat tolerance selection. Managers can also help cows mitigate heat stress on their own by implementing a crossbreeding program. By crossing a less heat-tolerant breed such as a Holstein with a more heat-tolerant breed such as a Jersey, the effects of thermal stress on production can be decreased (Nguyen et al., 2018).

It has become clear that increased heat load has a significant effect on the time budget of a dairy cow, and that this change of time budget has implications on a cow's ability to produce milk (Allen et al., 2015). In addition to understanding the implications of hyperthermia on profitability, producers also need to understand how to manage cows in order to reduce the impact of heat stress on milk production. Facility improvements such as feed-line soakers, fans, and shade can be used to mitigate some thermal stress experienced by cows (Correa-Calderon et al., 2004). This literature review will highlight several factors that managers can consider in order to help their cows better cope with hyperthermia.

An Overview of Cow Behavior

Recent attention on cow comfort has unearthed the realization that cows have specific time requirements for performing each of their major behaviors such as eating, drinking, lying, socializing, and ruminating. It has been demonstrated that lactating cows in commercial herds spend an average of approximately 12 h resting in stalls, 2.5 h standing in the alley, 4.3 h eating, and 2.7 h per day moving to and from the milking parlor when housed in a commercial freestall barn (Gomez and Cook, 2010). Cows typically ruminate between 7 and 10 h per day, and rumination is positively associated with milk yield (Mongeon, 2019; Kaufmann et al., 2018). Deviations from this schedule, particularly in lying time, can have detrimental effects on milk production (Munksgaard et al., 2005; Rulquin and Caudal, 1992; Berger et al., 2016). Cows spend up to 90% of the time ruminating while lying down (Mongeon, 2019). Piñeiro et al. (2019) demonstrated that increased lying time during the first 14 d in milk (DIM) has a positive correlation with ketosis diagnosis. With this information, it can be concluded that cows have different lying time requirements at different stages of lactation as demonstrated by Bewley et al. (2009).

Many factors may influence behavior of a modern, freestall-housed dairy cow. These factors range from management tactics such as overstocking, and prolonged time in headlocks or at the milking parlor to factors a manager may have less control over such as increased thermal stress (Lee, 2011). Hyperthermic cows will alter their behavior by standing more (Allen et al., 2015). This reduction in lying time can have impacts on both milk production (Kadzere et al., 2002) and reproduction (Jordan, 2003).

Heat Stress

Heat stress is defined as the inability of an animal to dissipate adequate amounts of heat in order to maintain thermal homeostasis (Bernabucci et al., 2014). Impacts of heat stress on both milk production and reproduction are well-documented (Kadzere et al., 2002; Key et al., 2014; Jordan, 2003; Bernabucci et al., 2014). Heat stress has an estimated economic impact in the dairy industry of approximately $900 million per year (St-Pierre et al., 2003). Milk production losses, increased mortality, and decreased reproductive performance account for most of the economic losses resulting from heat stress (St-Pierre et al., 2003). The evidence is in favor of the economic return of cooling lactating dairy cows; however, little attention has been given to the importance of also cooling dry cows.

Many producers in the United States fail to realize the impact heat stress has on cows during the dry period. Failing to cool cows during the dry period results in an estimated loss of $810 million per year because of decreased future milk production (Ferreira et al., 2016). Although little research has focused on the impact of heat stress during the dry period, there are significant implications associated with ignoring cow comfort during the dry period. Some such implications include suppressed immunity during the transition period, which that can allow for opportunistic infections to present themselves, adverse effects on prepartum mammary gland development, and potential compromise of fetal growth during late gestation (Tao and Dahl, 2013). Scanavez et al. (2017) demonstrated that some cows are more susceptible to heat stress than others. They demonstrated that these more susceptible cows, which they categorized as high-temperature (HT) cows, are more likely to experience postpartum disorders and have lower milk production in their subsequent lactation (Scanavez et al., 2017), resulting in lost revenues. Ferreira et al. (2016) demonstrated that cooling dry cows is profitable for 89% of producers in the U.S. when building a new facility was required. If a new facility was not required, the payback period was less than 1 yr after investing in cooling for dry cows (Ferreira et al., 2016).

Detrimental Effects of Heat Stress on Milk Production

The heat stress threshold for lactating dairy cows when losses in milk production begin is typically defined as starting at a temperature-humidity index (THI) of 72 (Armstrong, 1994). This practical measure of thermal load uses relative humidity and ambient temperature to indicate the thermal load a cow is experiencing. Lying behavior is negatively impacted at a THI as low as 68 (Allen et al., 2015). Because it is known that a relationship exists between milk yield and lying time, it can be concluded that the impact of heat stress on production also starts at a THI of 68. This heat-stress associated reduction in lying time can decrease milk production in mid- to late-lactation cows (Munksgaard et al., 2005; Rulquin and Caudal, 1992; Berger et al., 2016).

As previously mentioned, heat stress results in approximately $900 million in economic loss in the dairy industry in the U.S. Losses in milk production range from 68 to 2,072 kg per cow-year in Wyoming and Louisiana, respectively (St-Pierre et al., 2003). One of the many factors that can be attributed to this production loss is the reduction of dry matter intake (West, 2003). Dry matter intake decreased by 0.82 kg for each degree (°C) increase in average air temperature (West, 2003). Every kilogram of dry matter intake equates to approximately 1.4 to 1.8 kg of milk production (Hutjens, 2005). These factors can have enormous economic implications for dairy producers in temperate climates. Thermal stress can also change the composition of the milk (Kadzere et al., 2002). Several authors have demonstrated that cows under thermal stress produce less fat and protein content in their milk than cows under thermal neutral conditions

(Bandaranayaka et al., 1976; Rhoads et al., 2009). For dairy producers that are paid based on milk components, this is one additional way increased thermal load can affect profitability.

Detrimental Effects of Heat Stress on Reproduction

Heat stress has negative effects on reproductive performance of dairy herds (Jordan, 2003). Poor reproductive performance can negatively impact the profitability of a herd (Cabrera, 2014). Scanavez et al. (2019a) evaluated the potential effect of heat stress on herd-level insemination risk (IR) and showed that herd-level IR is seasonal, with the greatest IR occurring in autumn. The authors speculated that this could occur for several reasons. First, poor IR commonly occurs during summer as a result of heat stress (Scanavez et al., 2019a; Jordan, 2003). Second, because of the increased IR during autumn, more cows calve during the subsequent summer and re-inseminated at the same time, increasing the number of cows being submitted to AI during autumn (Mendonça et al., 2017). Knowing that IR at the herd-level is related to heat stress, it is important to evaluate cow-level traits that also can have an impact on the reproductive performance.

Conflicting evidence arises when examining the impact of heat stress on estrus expression. Younas et al. (1993) demonstrated a decrease in the expression of estrus in dairy cattle under heat stress, whereas others reported that thermal load had no effect on the expression of estrus (Howell et al., 1994). Impacts of heat stress were identified at the hypothalamic level, and several authors have demonstrated decreased concentrations of gonadotropins in circulation after exposure to heat stress (Wise et al., 1988; Lee, 1993; Day et al., 1986). Day et al. (1986) demonstrated that LH pulses decreased gradually during summer and were lowest at the autumnal equinox. It can then be concluded that ovarian dominant follicles during summer grow in an environment with less LH than those during winter, and this can lead to a decrease in estradiol concentrations in the blood. This decrease in estradiol can account for reduced expression of estrus (De Rensis and Scaramuzzi, 2003). Mendonça et al. (2017) demonstrated that treating cows with GnRH during heat stress increased pregnancy risk by 12.5%, showing that inducing ovulation by triggering LH release during periods of heat stress can improve reproductive efficiency.

Hormone production is not the only facet of the reproductive system that is impacted by heat stress. Gamete quality is exceedingly important when examining the viability of gametes when under thermal stress. Rutledge et al. (1999) demonstrated that gamete formation in females, much like in males, is temperature sensitive. Blood flow to the uterus is decreased when cows are experiencing thermal stress (Roman-Ponce et al., 1978), and this decrease in blood flow and consequent decrease in nutrients to the uterus can contribute to early embryonic loss. Oocytes exposed to heat stress have a delay in the first two cell divisions after fertilization (Gendelman et al., 2010). Oocytes exposed to thermal shock during *in vitro* maturation had a reduced ability to cleave and become a blastocyst (Wolfenson and Roth, 2019). Thermal stress has been demonstrated to be detrimental to bovine reproduction at all stages of pregnancy.

High thermal load is detrimental to late gestation in bovines as well. Increased thermal load during late gestation decreased calf birth weight and passive transfer of immunoglobulin G into colostrum and may affect the hypophyseal-pituitary axis of calves developed *in utero* during a period of heat stress (Tao et al., 2012). Laporta et al. (2017) demonstrated that calves born to heat-stressed dams had different activity patterns and reduced average daily gain than those born under thermal neutral conditions. The authors suggested that this altered activity pattern of heifers that experienced late gestation heat stress could account for the sub-par post-weaning performance of those heifers (Laporta et al., 2017). The effects of thermal stress during gestation have been demonstrated to follow calves into adulthood as well. Heifers born to dams cooled during late gestation produce more milk during their first lactation compared with heifers born to dams given no form of heat abatement (Dahl et al., 2016). Therefore, calves born during periods of heat stress are at a disadvantage compared when calves born during cooler periods of the year.

Managing Hyperthermic Cows

One of the first, and often, the cheapest methods of heat abatement is to provide shade. For cows housed in covered barns, shade is one of the most readily available resources cows used for heat abatement. It is estimated that the thermal load for dairy cows can be reduced from 30 to 50% by providing shade (Bond and Kelly, 1955). Collier et al. (1981) demonstrated that cows with access to shade had lower rectal temperatures than cows with no access to shade and suggested this occurred because cows had a greater ability to dissipate heat by radiation as well as conduction and convection. In pasture-based herds, trees are an effective and economical option to provide shade (Armstrong, 1994).

Cooling cows in the holding pen also has been shown to be of great importance. Flamenbaum et al. (1986) demonstrated that cows cooled five times per day or more maintained a lower rectal temperature than cows not cooled. Cows cooled five or more times per day also produced more milk than cows that were not cooled (Flamenbaum et al., 1986). It is important to note that use of sprinklers without forced ventilation reduces the ability of cows to dissipate heat by evaporative cooling because it creates an atmosphere saturated with humidity (Flamenbaum et al., 1986). Because of the excess humidity, heat abatement methods utilizing evaporative cooling are not recommended for climates in which humidity regularly exceeds 75% (Fournel et al., 2017).

With industry sustainability becoming more important to consumers and producers alike, using water responsibly is important. Recent research has shown that sprinklers have similar effects on cow cooling regardless of flow rate. It was shown that 1.3 L/min was the most efficient use of water compared with 4.5 L/min or greater (Chen et al., 2015). Although the flow rate of 4.5 L/min decreased body temperature more than the 1.3 L/min, the effects on milk production did not differ between the two flow rates, meaning producers can effectively cool their cows while using less water (Chen et al., 2015). Chen et al. (2016) then demonstrated that a flow rate of 1.3 vs. 4.5 L/min was sufficient to cool cows and mitigate the effect of hyperthermia on dry matter intake as well as milk production. If the installation of sprinklers or extra water usage is not an option, efficiently moving air across the cows can also be an effective cooling method. Cows will benefit at air speeds up to 1 m/sec, but do not seem to benefit additionally when air speed moves beyond 2 m/sec (Mondaca, 2019).

Behavior Changes to Mitigate Thermal Stress

In addition to using resources to reduce heat stress, dairy cows also will alter their own behavior to dissipate more heat. Allen et al. (2015) demonstrated that lactating dairy cows under heat stress spend more time standing. This behavior adaptation manifests itself in several ways. Cows spend less overall time lying down with shorter lying bouts, and their standing bouts are longer (Allen et al., 2015). It can be inferred that dairy cows alter their daily time budget in an effort to maintain thermal homeostasis. Nordlund et al. (2019) demonstrated that the core body temperature of a cow will increase when she lies down. Core body temperature increased, on average, 0.50°C per h for each hour the cow spent lying down (Nordlund et al., 2019). It was also demonstrated that core body temperature decreased, on average, 0.25°C per h for each hour the cow was standing (Nordlund et al., 2019). This implies that a cow must spend approximately twice as much time standing as she spends lying down to completely dissipate the heat she accrued while resting. As previously discussed in this literature review, this can have huge economic implications.

Lying time is not the only behavior that will be altered in times of heat stress. Hyperthermia also reduced eating time (Kanjanapruthipong et al., 2015), rumination time (Soriani et al., 2013), but increased drinking (Tapki and Sahin, 2006). Temperature-humidity index and rumination time are negatively correlated, meaning as THI increases, rumination time decreases or vice versa (Moretti et al., 2017). Rumination is often decreased, particularly in high milk-producing dairy cows, to decrease the amount of metabolic heat produced to help the cow better cope with increased environmental thermal load (Kadzere et al., 2002). Reduced dry matter intake can also partially account for reduced milk production during summer (Tapki and Sahin, 2006). Berman et al. (1985) demonstrated that high milk-producing dairy cows become dehydrated faster than low-producing cows because of increased sweating and respiratory water loss, and to make up for this, high-producing cows drink more water. Anderson (1985) also demonstrated that temperature of the drinking water might influence the amount consumed. Cows drank more water and also produced more milk during heat stress when water was at a temperature of 17°C compared with water offered at a temperature of 3, 10, and 24°C (Anderson, 1985).

Use of Technology to Manage Hyperthermic Cows and Predict Disease

Stangaferro et al. (2016a, 2016b, 2016c) validated the use of automated technologies to identify cows with clinical mastitis, severe metritis, and metabolic and digestive disorders during the early postpartum period. The latter authors used neck-mounted sensors in the experiments, whereas other researchers, such as Borchers et al. (2016), validated the use of ear tag sensors to monitor cow behavior.

Behavior automation allows researchers and dairy producers to identify cows that may have subclinical diseases (Liboreiro et al., 2015) or be experiencing thermal stress (Bar et al., 2019). Borchers et al. (2016) validated use of several popular automated activity monitors including the AfiAct Pedometer Plus, CowManager SensOor, HOBO Data Logger, CowAlert IceQube, Smartbow, and Track A Cow systems. The authors found that these commercially available technologies allowed accurate recording of behavior compared with visual observation and were useful to manage dairy cows (Borchers et al., 2016). Bar et al. (2019) demonstrated that automatic behavior systems that record heavy breathing can be used as a practical method to continuously assess thermal load and that this information can be used to allocate resources accordingly.

Because these technologies have been validated, others are now integrating these technologies into their research. Stevenson et al. (2020) used the CowManager SensOor ear-mounted sensors to determine the relationship between postpartum disease and ovulation risk in fresh cows. The authors found that rumination, activity, and inactivity levels differed between cows that displayed postpartum disease and those that did not (Stevenson et al., 2020). Evidence has also been presented that neck-mounted activity monitors can identify cows with a metabolic or digestive disorder as early as 5 days before the cow presents clinical symptoms (Stangaferro et al., 2016b). These same authors noted that these systems can be used to identify cows with metritis and clinical mastitis but should be used in conjunction with traditional health monitoring methods (Stangaferro et al., 2016a; Stangaferro et al., 2016c). This new technology presents opportunity to develop methods of health management and assessment using automated behavior monitoring systems. Part of this thesis will detail use of ear-mounted behavior tags to monitor dry and fresh cow behavior during thermal stress.

Genetic Markers for Heat Tolerance

As genomic testing has gained popularity, it is now becoming possible to determine if genes for heat tolerance exist in cattle. Thermotolerance is controlled at the cellular level by a family of proteins called heat shock proteins (Hsp). More specifically, thermotolerance is controlled mostly by Hsp70.1 and Hsp70.2, which are the most abundant and the most temperature sensitive of the Hsp family (Beckham et al., 2004). Heat shock proteins are a group of chaperone proteins that are closely tied to general protein folding (Craig, 1993). The job of a chaperone is to provide an environment for other proteins to be able to fold properly (Craig, 1993). Thermotolerance is associated with polymorphisms that are linked to upregulation in the gene that encodes for the heat shock protein 70.1 (Basiricò et al., 2011). This upregulation allows for more of those genes to be expressed.

Temperatures ranging from 42 to 45°C induce reversable denaturization of proteins, and Hsp70 can inhibit conformational changes in proteins exposed to heat stress (Stankiewicz et al., 2005). Heat shock protein 70 has also been demonstrated to inhibit stress responses in cells exposed to heat shock (Stankiewicz et al., 2005). Collier et al. (2006) described that increased expression of heat shock proteins provided protection during times of thermal stress. Heat shock protein 70 is the most inducible Hsp, thus providing further evidence for its ease of upregulation after a period of heat shock (Beckham et al., 2004). Upregulation of Hsp70 occurs once an animal’s body temperature exceeds its thermal neutral zone (Basiricò et al., 2011). Above the thermal neutral zone, bound genetic inhibitors dissociate from the genes to which they are inhibiting expression. This allows for the enhancement of Hsp expression (Collier et al., 2008).

More recently, it has been demonstrated that Hsp elevates during periods of general stressors such as temperature, infection, inflammation, exercise, exposure to toxins, dehydration, and water and oxygen deprivation (Wang et al., 2015). Animals differ in their expression of Hsp because of nucleotide differences in the 5'- and 3'- untranslated regions of the genes. These variations affect the inducibility, stability, and degree of expression between animals (Deb et al., 2013). It is important to study and understand the genetic components of heat tolerance because Ravagnolo and Misztal (2000) demonstrated that selection for heat tolerance is possible in dairy cattle. It is also possible to select simultaneously for production traits and heat tolerance (Ravagnolo and Misztal, 2000). Not much research has been done in this area, which presents an opportunity for new and exciting research to be done.

Pregnancy-Associated Glycoproteins and Heat Stress

Though little is known about the function of pregnancy associated glycoproteins (PAGs), their importance is becoming clearer as more research is done. Green and Hennessy (2018) speculated that PAGs have influence on tissue remodeling, immunomodulation of the maternal immune system at parturition, and possible luteotrophic effects on the ovary. PAGs can be used to diagnose an animal pregnant starting around 25 days of gestation (Green and Hennessy, 2018). It is also speculated that PAGs play a role in dampening the maternal immune response as the cow approaches parturition (Hoeben et al., 2000). Because of its suspected luteotrophic effect, it is speculated that PAGs are also associated with pregnancy maintenance since PAGs can elevate PGE_2 concentrations relative to PGF_{2a} (Green and Hennessy, 2018).

Pregnancy loss is a matter of concern at any time of the year for dairy producers. It seems that heat stress exacerbates this issue, be it early embryonic loss (García-Ispierto et al., 2006) or increases in overall spontaneous abortions (Mellado et al., 2016). Pohler et al. (2016a) successfully demonstrated that concentrations of pregnancy-associated glycoproteins (PAG) can be used with 95% accuracy to predict spontaneous abortion between d 28 and 100. These authors noted exceptionally suppressed expression of PAG at d 28 in cows that would later spontaneously abort their fetus between d 28 and 100 (Pohler et al., 2016b). Cows that become pregnant during a warm period also have suppressed expression of PAG (Serrano et al., 2009). Pohler et al. (2016a) later demonstrated that PAG concentrations during early pregnancy < 1.8 ng/mL resulted in a 60% chance of embryonic mortality. Because it has been demonstrated that heat stress results in low PAG concentration during early pregnancy and that this low concentration results in greater embryonic loss, it can be concluded that decreased pregnancy maintenance during the summer is a direct result of decreased PAG expression. Interestingly, Scanavez et al. (2019b) demonstrated that cows classified as having an average high core body temperature before calving had greater PAG concentrations in late gestation than cows classified as having an average low body temperature. This demonstrates that it is possible that core body temperature of dairy cows may potentially impact PAG concentrations.

Summary

Heat stress and its implications on milk production, health, and reproduction have become extremely important in recent years as global temperatures continue to rise. There are several ways to combat the effects of heat stress on dairy cows, including management tactics such as implementing use of sprinklers, fans, and shade. Cows naturally seek ways to reduce body temperature by increasing drinking and time while standing, while reducing rumination and eating times. Automated behavior monitoring is becoming more popular as a method to assess animal health, which opens the door for new research into disease identification and prevention. As genomic testing and genome sequencing also gains traction, it is now a realistic expectation to identify dairy animals with superior heat-tolerant genes to improve thermal tolerance. Concentrations of PAG in cattle tend to be suppressed during times of heat stress. In contrast, the literature has demonstrated that cows with an average high core body temperature have greater concentrations of PAG, implying that core body temperature may be associated with PAG expression.

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Chapter 2 - Use of an Automated Activity Monitoring System to Identify Cows with Increased Susceptibility to Heat Stress and Disease

Abstract

Heat stress has been demonstrated to have implications on the profitability of the dairy industry. The objectives of this observational study were to investigate behavioral differences of dairy cows having above or below the median core body temperature (CBT) assessed during 1 wk during late gestation as well as measure differences in pregnancy-associated glycoprotein (PAG) concentration. Temperature data were collected every 5 min for 7 d using a temperature logger attached to a intravaginal insert between d 220 and 241 of gestation. Within each of 5 replicates, cows having above median CBT were classified as high temperature (HT) and those below median CBT were classified as low temperature (LT). Behavioral data from 50 cows (10 cows per replicate) were collected using automated activity monitors equipped with accelerometers in addition to visual observations. Accelerometer data were evaluated from d -21 to 21 relative to actual calving date. Cows were observed for 8 h in the far-off pen and 8 h in the close-up pen. Each 8-h observation block consisted of two, 2-h morning observations (0600 to 0800 h) and two, 2-h afternoon observations (1600 to 1800 h). At enrollment, cows were between 220 and 241 days of gestation. Blood samples were collected weekly for 3 wk starting at enrollment. No differences were detected among visually observed behaviors during morning or afternoon observations in the far-off or close-up period. An interaction of temperature and parity (P = 0.02) revealed that HT primiparous cows spent the most time eating during afternoon

visual observations (30.9%) followed by LT multiparous cows (25.4%), LT primiparous cows (22.5%), and finally HT multiparous cows spent the least time eating (19%). Accelerometer data demonstrated that HT cows had more ($P < 0.01$) high activity time than LT cows. Compared with primiparous cows, multiparous cows spent more time active during both prepartum ($P = 0.02$) and postpartum ($P < 0.01$) periods. High temperature cows were more ($P < 0.01$) inactive than LT cows before calving. Cows classified as HT had greater ($P = 0.05$) blood concentrations of PAG during the first 3 wk of the far-off dry period than cows classified as LT. In conclusion, HT and LT cows display different behavior and have different PAG concentrations; however, more research is needed to identify specific thresholds to classify cows as HT or LT.

Introduction

With annual economic losses resulting from heat stress in the dairy industry estimated at $0.897 to $1.5 billion (St-Pierre et al., 2003), it is obvious that profitable enterprises focus on cow comfort during times of heat stress. Heat stress in dairy cattle begins at a relatively low temperature-humidity index (THI) of 68 (Bouraoui et al., 2002; De Rensis et al., 2015; Zimbelman et al., 2009). Bouraoui et al. (2002) determined that both milk and reproductive losses occurred at a THI of 69 and greater. Several other researchers have demonstrated that heat stress affects milk production and reproductive efficiency of dairy cows (Cook et al., 2007; Scanavez et al., 2019b; Stevenson et al., 2020; Cook et al., 2008).

At the cow-level, core body temperature (CBT) can be used as a proxy for quantifying to what extent cows are heat stressed. Scanavez et al. (2017) evaluated whether CBT during late gestation was associated with performance after calving. Cows were grouped into high (HT) and low temperature (LT) groups during the dry period based on median body temperature. High temperature cows produced less milk during early lactation than LT cows (Scanavez et al., 2017).

In addition, Scanavez et al. (2019b) demonstrated that HT compared with LT cows had greater concentrations of pregnancy-associated glycoprotein (PAG) concentrations during late gestation (Scanavez et al., 2019b).

Considering physiological differences observed based on CBT, it is possible that HT and LT cows may behave differently. Behavioral differences can be determined by visual observation of cows or by automated activity monitors that capture behavior activities, such as lying, standing, eating, different levels of activity, and rumination. Several researchers have demonstrated the relationship of health status of transition cows and behavior captured by monitoring technologies (Soriani et al., 2012; Liboreiro et al., 2015; Stangaferro et al., 2016a, 2016b, 2016c). Findings from these experiments are encouraging and suggest that rumination time can be used as an indicator or possible predictor of postpartum health disorders.

Heat stress is also associated with behavioral changes, such as lying time (Allen et al., 2015; Nordlund et al., 2019) and rumination (Karimi et al., 2015). Moreover, lying time and rumination are associated with dry matter intake (DMI), which influences heat production and CBT. It is likely that cows classified as HT and LT during late gestation manifest different behavioral patterns that may be identified by automated activity monitors. In addition, it is possible that HT and LT cows are genetically different. Nevertheless, there is limited research investigating the role of specific genes that regulate heat tolerance in dairy cows. Regardless of the culprit for elevated CBT of subpopulations of cows during summer, further research is warranted to investigate associations between CBT and behaviors of heat-stressed dry cows.

The objective of this study was to investigate behavioral differences of HT and LT dry cows during the summer by using both automated activity monitors and visual observations. A secondary objective of this study was to determine PAG concentrations of HT and LT cows. Our hypotheses were that HT cows would have increased PAG concentrations and display different daily proportions of behavioral patterns during the dry period than LT cows.

Materials and Methods

Cows and Housing

The procedures described herein were approved by the Kansas State University Institutional Animal Care and Use Committee (# 4256). This experiment was conducted at the Kansas State University Dairy Teaching and Research Center from June through November 2019. Lactating Holstein cows were dried off between 192 and 221 d of gestation and moved to the far-off dry pen. Once dry for at least 6 d, cows at 220 to 241 d of gestation having a locomotion score < 3 (1 = not lame and 3 = noticeably lame; Sprecher et al., 1997) were enrolled in the study. A total of 50 cows were enrolled (19 primiparous and 31 multiparous [19 second lactation cows and 12 third-lactation or greater cows]). Cows were reconfirmed pregnant and enrolled in five replicates as they reached enrollment criteria. Body condition score was evaluated on day of enrollment, 7, and 14 d after enrollment using a 5-point scale with 0.25-point increments (Ferguson et al., 1994). Cows were moved to the close-up pen between 247 and 261 d of gestation. Cows in the far-off pen were housed in a free-stall barn and fed a total mixed ration once daily with free choice access to prairie hay and water. No active cooling was provided in the far-off pen; however, shade was provided over free-stall beds and feed bunks. Cows in the close-up pen were housed in an open-front barn on straw-bedding over concrete, provided a total mixed ration once daily, and had free choice access to water. The back wall of the barn was equipped with cellulose cooling pads and fans. After calving, lactating cows were housed in free-stall barns equipped with sprinklers and fans and fed a TMR once daily (twice daily during summer). Lactating cows are milked thrice daily starting at 0400 and 1600 h.

Assessment of Core Body Temperature and Ambient THI

Core body temperature (CBT) was recorded by attaching a temperature logger (iButton DS1922L, Embedded Data Systems, Lawrenceburg, KY) to a blank intravaginal insert (CIDR, Zoetis, Parsippany, NJ). The iButton was attached to the insert with silicone aquarium sealant (Loctite®, Henkel Corporation, Rocky Hill, CT) and wrapped with Parafilm (Parafilm®, Carolina Biological Supply Company, Burlington, NC) once the sealant was dry. The insert was then placed intravaginally and removed after 7 d. The temperature logger recorded CBT every 5 min. This resulted in approximately 2000 temperature observations per cow. Temperature loggers were calibrated for an accuracy of ± 0.13°C. Temperature loggers were placed in cows between d 225 and 239 of gestation and removed between 232 and 248 days of gestation.

Upon removal, data from the loggers were downloaded to a computer and average CBT was calculated as described by Scanavez et al. (2017). Median values were then calculated for each of the five replicates. Within replicate, 2 CBT groups were created: cows whose average CBT exceeded the median value were classified as HT and cows with an average CBT below the median value were classified as LT.

Ambient temperature and humidity were monitored in both the far-off and close-up pens by fixing a temperature logger (HOBO U23 Pro v2, Onset Computer Corp., Pocasset, MA) in each pen. Loggers were located approximately 3 m above the ground. Temperature and humidity measurements were recorded every 5 min in both pens. Temperature data were downloaded from the loggers and used to calculate THI as described in Scanavez et al. (2019b).

Assessment of Dry Cow Behavior

Visual Observations

To quantify cow behavior, each cow was observed for a total of 16 h during the dry period. Cows were observed for 8 h in the far-off pen and 8 h in the close-up pen. Each 8-h observation block consisted of two, 2-h morning observations (0600 to 0800 h) and two, 2-h afternoon observations (1600 to 1800 h). Behaviors were recorded during continuous visual observation by a total of 7 different observers. Observers were trained in continuous behavior observation before their first observation period and shadowed by another observer during their first observation period to ensure proper observation. Furthermore, observers were blinded to which cows were HT or LT.

Each observer was randomly assigned two cows to monitor for each 2-h block. Observations in the far-off pen occurred during the first 10 d after cows were enrolled and during the first 10 d after cows were moved to the close-up pen. The five behaviors recorded were: lying, standing, eating, drinking, and perching (standing with only front feet in the free stall). Perching was only recorded in the far-off pen because the close-up pen did not have free stalls.

Automated Behavior Monitoring

Data were collected using CowManager SensOor ear tags affixed to the left ear of each cow (Agis Automatisering BV, Harmelen, Netherlands). Data captured by the ear tag equipped with accelerometers included ear surface temperature, high activity, general activity, inactivity, eating, and rumination. The ear tags collect behavior data on a minute-by-minute basis, in which the behavior that occurs during the majority the minute is the behavior that is recorded. Ear and jaw movement aid in the classification of rumination and eating behaviors, and all other behaviors that are not eating, rumination, or resting (inactivity) are classified as active. High activity is used as an indicator of estrus-like activity. Accelerometer data from d -21 to 21 relative to calving (d 0) were analyzed. Ear surface temperature was collected every hour by the ear tags. Ear surface temperature was also analyzed from d -21 to 21 relative to calving (d 0 being calving). For bunk-fed dairy cows equipped with the SensOor ear tags, feeding and ruminating activities are well correlated with visual observations (simple and concordance correlations exceeding 0.82 and 0.59, respectively, Borchers et al., 2016).

Blood Sampling, Processing, and Analysis

Blood samples were collected from the coccygeal vein or artery into an evacuated EDTA tube (BD Vacutainer, Becton, Dickinson and Company, Frankin Lakes, NJ) while cows were restrained in a palpation rail at enrollment, as well as 7, and 14 d after enrollment. Blood samples were kept on ice and transported to the laboratory. Blood plasma was harvested after centrifugation (1,000 × g at 5°C) using disposable plastic pipettes (one pipette per sample). Plasma samples were stored frozen at -18˚C. Plasma samples were later evaluated using PAG ELISA as described by Green et al. (2005) to determine concentration of pregnancy-associated glycoprotein. Intra- and inter-assay coefficient of variation was 4.62% and 13.92%, respectively.

Statistical Analyses

Of the total 50 cows enrolled, two cows were removed from the study because of lameness and spontaneous late-term abortion during the far-off period. Prepartum and postpartum behaviors assessed by the accelerometers were analyzed separately by ANOVA for repeated measures using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Each behavior captured by the accelerometers was analyzed separately. For example, prepartum rumination was analyzed separately from postpartum rumination. For each visual observation assessment, percentage of time attributed to each behavior of interest was calculated.

Percentage for each activity was analyzed separately for cows during the far-off and close-up periods, and morning and afternoon assessments using the GLIMMIX procedure of SAS. For example, standing in the morning in the far-off period was analyzed separately from standing in the afternoon in the far-off period. Log transformation before analysis was conducted when behavior trait residuals were not normally distributed.

Concentrations of PAG were analyzed by ANOVA for repeated measures using PROC MIXED in SAS. All other continuous variables were analyzed by ANOVA using the MIXED procedure of SAS. Models for the repeat-measure analyses of accelerometer data included the following fixed effects: CBT group, parity (multiparous vs. primiparous), day, two-way interactions between effects, and three-way interaction of CBT group, parity, and day. Models for the analyses that evaluated visual behavior observations included CBT group, parity, and the interaction between CBT group and parity. Assessment period (far-off or close-up, and morning or afternoon observation) was included as a random variable in the models that evaluated visual behaviors. The model for the analyses that evaluated PAG concentrations included CBT group, parity, week (1, 2, or 3), two-way interactions between effects, and three-way interaction of CBT group, parity, and week. Replicate block was included as a random variable in all models. Independent variables and interactions were removed from the models using a stepwise backward elimination method when $P > 0.10$. Statistical significance was defined as $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$.

Results

Temperature-Humidity Index and Overall Descriptive Data

In the far-off pen, average daily, average minimum, and average maximum THI were 75.9, 60.8, and 85.7, respectively. In the close-up pen, average daily, average minimum, and average maximum THI were 69.2, 50.3, and 82.6, respectively. Median CBT values for each of the 5 replicates were as follows: 38.78°C, 38.85°C, 38.84°C, 38.92°C, and 38.67°C for groups 1 through 5 respectively. Core body temperature ranged from 38.8°C to 39.0°C for HT cows and from 38.6°C to 38.8°C for LT cows. Although mean lactation number was greater ($P < 0.01$) for cows in the LT compared with the HT group (Table 1), percentage of multiparous cows did not differ ($P \geq 0.16$) between groups. Core body temperature was greater ($P < 0.01$) for HT cows than LT cows (Table 1). Days in milk at dry-off did not differ ($P > 0.93$) between HT and LT cows (Table 1). Days of gestation at enrollment were less ($P = 0.04$) for LT cows compared with HT. Cows classified as HT spent fewer days in the close-up pen ($P = 0.03$) compared with LT cows (Table 1). Gestation length tended ($P = 0.09$) to be shorter in HT than LT cows (Table 1). Two HT cows and one LT cow delivered twins. Projected 305-d mature equivalent milk yield for the subsequent lactation did not differ ($P = 0.90$) between HT and LT cows.

Automated Behavior Monitoring

Before calving, HT cows had displayed greater ($P = 0.01$) daily periods of high activity compared with LT cows (215 ± 6 vs. 199 ± 6 min. respectively; Figure 1). Parity also affected high activity (P=0.02), in which primiparous cows had greater periods of high activity than multiparous cows before calving (214.1 ± 6.1 min vs. 200.0 ± 5.2 min, respectively). The interaction between parity and day tended ($P = 0.08$) to influence high activity time during the prepartum period.

During the postpartum period, no difference ($P = 0.96$) was detected between HT and LT cows (175 ± 8 vs. 1759 ± 8 min. respectively; Figure 1). In contrast, day ($P = 0.01$) and parity ($P = 0.01$) affected high activity time (Figure 1), with a tendency ($P = 0.10$) for an interaction between parity and day. Multiparous cows spent an average of 162.3 ± 7.4 min per day in high activity and primiparous cows spent approximately 187 ± 8.3 minutes per day in high activity.

Daily overall activity time did not differ between CBT groups ($P = 0.95$) before calving (157 ± 11 vs. 156 ± 10 min; Figure 2) for HT and LT cows, respectively. In contrast, parity ($P = 0.01$; 172.6 ± 10.1 min for multiparous vs. 140.4 ± 11.3 min for primiparous) and day ($P = 0.02$) affected daily overall prepartum activity with an interaction ($P = 0.06$) between parity and day as well as a tendency ($P = 0.07$) for an interaction between CBT group and day (Figure 2).

During the postpartum period, activity between CBT groups did not differ (186 ± 17 vs. 175 ± 17 min; $P = 0.16$); however, parity was significant, with multiparous cows spending more ($P < 0.01$) active time than primiparous cows (Figure 2; 198.3 ± 16.7 min vs. 162.3 ± 17.1 min, respectively). Day affected postpartum activity as it decreased ($P < 0.01$) from a peak at parturition through d 20, but less so in HT than LT cows (interaction between CBT group and day, $P = 0.05$; Figure 2).

High temperature cows spent less ($P < 0.01$) time being inactive than LT cows during the prepartum period (411 ± 10 vs. 439 ± 9 min; Figure 3). Multiparous cows were more ($P = 0.02$; 437.5 ± 8.9 min) inactive compared with primiparous cows (412.5 ± 10.3 min), with some fluctuations across the prepartum period (interaction of parity and day, $P = 0.06$).

From a peak at calving, inactive time slowly decreased ($P < 0.01$) until d 20 (Figure 3). Core body temperature did not affect ($P = 0.11$) postpartum inactivity, with HT cows spending an average of 412 ± 14 min per day being inactive compared with LT cows at 388 ± 13 min per day (Figure 3). Multiparous cows spent more ($P = 0.02$; 418.3 ± 12.1 min) time being inactive than primiparous cows (381.8 ± 14.3 min).

Eating time was not affected by CBT (*P* > 0.91) during the prepartum period (214 ± 12 vs. 213 ± 12 min; Figure 4) for HT and LT cows, respectively. No other factors affected prepartum eating time. In contrast, postpartum eating time was at a nadir on the day of calving and slowly increased (*P* < 0.01) to d 20 (Figure 4). Although postpartum eating time was not affected by CBT (108 ± 11 vs. 111 ± 11 min; *P* = 0.85) in HT and LT cows, respectively, an interaction (*P* = 0.03) of CBT group and day was detected. Primiparous cows tended (*P* = 0.07) to spend more time eating than multiparous cows (120.9 ± 12 min vs. 97.9 ± 10.1 min, respectively), with a tendency (*P* = 0.07) for some differences between parity groups across the postpartum period to d 20.

Core body temperature did not affect (*P* > 0.88) rumination before calving (441 ± 15 vs. 438 ± 14 min; Figure 5) in HT and LT cows, respectively, but rumination decreased (*P* < 0.01) acutely during the last 48 h before calving (Figure 5).

Rumination time increased (*P* < 0.01) rapidly from a nadir at parturition until reaching a peak at the end of the first week. Although HT cows tended (*P* = 0.07) to spend less time ruminating compared with LT cows after calving (547 ± 12 vs. 571 ± 12 min; Figure 5), no difference (*P* > 0.30) in postpartum rumination time was detected between primiparous and multiparous cows.

Core body temperature was not associated (*P* = 0.20) with ear tag temperature prepartum (Figure 6). Days relative to calving had an effect (*P* < 0.01) on ear tag temperature because temperature decreased as cows approached calving. Parity and the interaction of CBT and days relative to calving did not affect ear tag temperature prepartum.

Core body temperature was associated with ear tag temperature postpartum (*P* = 0.02; Figure 6). Days relative to calving (*P* = 0.36), parity (*P* = 0.40), and the interaction between CBT and days relative to calving (*P* = 0.84) did not affect ear tag temperature postpartum.

Visual Behavior Monitoring

Far-off Period

Core body temperature had no effect on standing, lying, or drinking activity in the morning and afternoon periods (Table 2). During the morning observations, multiparous cows spent less (*P* < 0.01) time lying (54.8%) than primiparous cows (67.3%). During the afternoon observations, multiparous cows tended (*P* = 0.10) to spend more time eating than primiparous cows (Table 2). There were no interactions between CBT and parity for morning or afternoon observations during the far-off period (Table 2).

Close-up Period

Core body temperature did not affect standing, lying, or drinking activity in the morning or afternoon periods (Table 3). Multiparous cows spent more time standing in the afternoon (*P* = 0.05) than primiparous cows (58.0 vs. 51.2%; Table 3). High temperature primiparous cows spent the most time eating during afternoon observations (30.9%), followed by LT multiparous cows (25.4%), LT primiparous cows (22.5%), and finally HT multiparous cows spent the lowest percentage of time eating (19.0%; interaction of parity and CBT, *P* = 0.02).

Pregnancy-Associated Glycoprotein

High temperature cows had greater (*P* = 0.05) PAG concentration compared with LT cows at each weekly sampling (Figure 7). The interaction between parity and week was significant (*P* = 0.01). For multiparous cows, weekly concentrations of PAG for cows were 1.75 ± 0.22 ng/mL, 1.99 ± 0.23 ng/mL, and 1.81 ± 0.23 ng/mL, respectively, whereas those for primiparous cows were 1.42 ± 0.28 ng/mL, 1.54 ± 0.30 ng/mL, and 1.81 ± 0.30 ng/mL, respectively. The interaction of CBT group and week number was not significant ($P = 0.14$). Twinning had no significant impact on PAG concentration and therefore was not included in the model.

Discussion

To our knowledge, this is the first study in which variation in CBT of dry cows during summer heat stress was demonstrated to have effects on activity. The authors hypothesized that cows with different CBTCBT would have different behaviors. It was also hypothesized that placental function of cows may differ by HT cows producing greater concentrations of PAGs than LT cows. Our results present evidence that it may be possible to identify cows with, HT and LT cows display different CBT during heat stress using behaviors that can be captured by automated activity monitoring systems (prepartum high activity, pre- and postpartum general activity, and prepartum inactivity), but not specific activities based on visual observations. We also confirmed that HT cows have greater concentrations of PAGs compared with LT cows (Scanavez et al., 2019b).

Effects of heat stress on dairy cow behavior is well-documented in the literature. Allen et al. (2015) demonstrated that standing bout duration increases and lying bout duration decreases as CBT rises (Allen et al., 2015). These same authors demonstrated that the majority of cows begin to stand more once their CBT is greater than 38.89°C (Allen et al., 2015). Standing and lying time did not differ between HT and LT cows in the present study. Anderson et al. (2013) demonstrated that fans and misters reduce CBT of dairy cows, and in turn, increase lying duration and number of lying bouts. It is possible that other behavioral differences were not detected during visual observations in the current study because of the number of observations, which could explain the lack of major differences in behavior between HT and LT cows. In future studies of this nature, longer, more visual observation periods, or both, should be employed. One could employ automated monitoring technology to evaluate actual standing and lying times.

It was hypothesized that HT and LT cows would have different behavior during the dry period. Although this may not have been captured in full by visual observations, differences were detected by the automated activity monitors. In the current study, HT cows tended to ruminate less compared with LT cows in the first 21 d after calving. In a previous experiment (Scanavez et al., 2017), HT cows were more likely to be diagnosed with postpartum diseases. Moreover, rumination is expected to remain suppressed longer after calving in cows that experienced dystocia compared with cows that did not experience dystocia (Kovács et al., 2017). Schirmann et al. (2016) showed that prepartum rumination time is different between healthy and diseased cows. In the current study, no differences in prepartum rumination time were detected between HT and LT cows; however, daily rumination time tended to be less in HT than LT cows during the first 3 wk after calving. Activity also differed between HT and LT cows in several facets. High temperature cows were less inactive, and therefore, registered more high active time during the prepartum period than LT cows. In contrast, no differences in activity were observed after calving.

Rising global temperatures have impacted dairy producers' profitability (Key et al., 2014). St-Pierre et al. (2003) estimated potential annual industry losses of approximately $0.897 to $1.5 billion because of heat stress. More recently, Ferreira et al. (2016) stated that heat stress on dry cows has serious economic implications to U.S. dairy producers. Cooling cows during the entire dry period has been demonstrated to increase DMI during periods of heat stress (Adin et al., 2009). Studies have shown that maximizing DMI during the dry period is important for postpartum health (Grummer et al., 2004; Huzzey et al., 2007). Transition diseases cost the industry just less than $500 million on an annual basis (Bellows et al., 2002). Furthermore, HT primiparous cows and LT multiparous cows spent the most time eating. It is possible that these two groups of cows have fewer postpartum health issues by maximizing prepartum DMI.

Relatively extensive work has been done to validate the use of monitoring technology to detect behavioral differences in sick vs. healthy cows after calving. Liboreiro et al. (2015) demonstrated that cows diagnosed with metritis have decreased postpartum rumination time. Stangaferro et al. (2016a, 2016b, 2016c) showed that activity monitors can be used to detect different postpartum disorders. Stangaferro et al. (2016a) describes the detection of metabolic and digestive disorders during the early postpartum period using activity monitors. The latter authors indicated that activity monitors detected cows with ketosis and displaced abomasum up to 5 d earlier than farm personnel. These same authors demonstrated that activity monitors can be used to identify cows with severe cases of metritis (Stangaferro et al., 2016c). Stevenson et al. (2020) further demonstrated that there is a difference in activity between healthy and diseased cows. Others have evaluated the use of activity monitors to identify postpartum health disorders in dairy cows (Rutherford et al., 2016; Omontese et al., 2020). In contrast, little research has been conducted to investigate behavioral differences before calving between sick and healthy cows. It is possible that the conditions of heat stress made detection of differences more difficult because cows alter their behavior to better cope with hyperthermia. More research in this area is warranted as disease prevention, rather than treatment, becomes the focus of modern dairy production.

One report demonstrated that prepartum lying time is associated with postpartum health outcomes. Piñeiro et al. (2019) reported a quadratic relationship between lying time and postpartum non-esterified fatty acid concentrations, demonstrating that extremes of prepartum lying time (> 8 h or <14 h/d) could be associated with postpartum ketosis. Thorough understanding of lying time is important because it is associated with the welfare of cows (Tucker et al., 2021). Sahar et al. (2020) demonstrated that prepartum feeding behaviors can be used to successfully distinguish between cows that will develop postpartum disease and those that will remain healthy. They determined that cows had increased risk of postpartum disease if the time they spent eating increased while prepartum feed intake was high (Sahar et al., 2020). These studies show promise for developing new approaches to identify cows predisposed to disease.

Relatively little work has been done to evaluate the use of ear surface temperature readings in cow health diagnosis. Stevenson et al. (2020) demonstrated that ear surface temperatures captured by the CowManager ear tags is highly correlated with environmental conditions (r=0.96). The authors also speculated that because ear surface temperature and rectal temperature were in the same correlation ranges as rectal and environmental temperatures, that ear skin temperature was more closely related to environmental temperature than rectal temperature (Stevenson et al., 2020). They also demonstrated that ear surface temperature was not different between healthy and diseased cows (Stevenson et al., 2020). Understanding the literature surrounding disease and core body temperature (i.e., fever), one would be hesitant to use ear surface temperature as an indicator for anything other than a measurement of the cow's environment. More research should be done in this area to evaluate ear surface temperature and if it can be used for an indicator of any health traits, if at all.

This experiment also corroborates the findings of Scanavez et al. (2017; 2019b), in that HT cows spent less time in the close-up pen than LT cows because HT cows calved earlier with shorter gestations. In the present experiment, LT cows spent approximately 3 d more in the close-up pen than HT cows. This finding is similar to Scanavez et al. (2017), where LT cows spent approximately 19.4 d in the close-up pen and HT cows spent 14.3 d. In another report (Scanavez et al., 2019b), LT cows spent 29.3 d in close-up and HT cows spent 25.2 d in close-up. Under normal circumstances, it is believed that the space limitations of the uterus induce fetal stress during late gestation, causing the fetus to release adrenal corticotropin, or ACTH (Senger, 2003). Tao and Dahl (2013) described the relationship between fetal and maternal body temperature, detailing that a fetus will experience heat stress along with its dam. It is possible that the increased CBT of the HT cows cause fetal stress, inducing the fetus to release ACTH sooner than it would under thermal-neutral conditions and thus earlier parturition. Further research is needed to validate this supposition.

The time spent in the close-up pen has its own set of implications for cows. Prepartum diets with a negative dietary cation-anion difference (DCAD) have positive benefits for postpartum health, therefore, ensuring cows receive the appropriate diet during the last 21 d of gestation. A meta-analysis by Charbonneau et al. (2006) demonstrated that lowering DCAD from +300 to 0 mEq/kg reduced risk of clinical milk fever from 16.4 to 3.2%. Evidence from Lopera et al. (2018) indicates that feeding anionic salts to acidify the prepartum diet for more than 21 d might be detrimental to postpartum performance of dairy cows. In contrast, Degaris et al. (2008) demonstrated that optimal exposure to a prepartum DCAD diet is approximately 25 d. It is generally accepted that exposure to a negative DCAD diet for less than 21 d is less effective in preventing postpartum milk fever. Therefore, dairy producers should ensure that cows spend at least 21 d in the close-up pen when feeding a DCAD diet. Better understanding the physiological differences between HT and LT cows can help producers better manage their cows and prevent disease.

Scanavez et al. (2017) demonstrated an association between increased vaginal temperature and susceptibility to postpartum health disorders. In addition, cows classified as HT produced less milk than LT cows during the early stages of lactation. In a subsequent experiment, Scanavez et al. (2019b) showed that HT cows had distinctly greater concentrations of PAG than LT cows. Similar findings were observed in the current experiment, in which HT cows had greater PAG concentration in late gestation than LT cows. Scanavez et al. (2019b) speculated that PAG clearance may be altered in HT cows because of decreased portal blood flow and increased peripheral blood perfusion caused by heat stress. Concentration of PAG differ in cows bearing single vs. twin fetuses (Serrano et al., 2009). Scanavez et al. (2019b) demonstrated that HT cows have a greater incidence of twinning than LT cows, which may partially explain the greater average PAG expression. Even though the present study supports previous findings that HT cows experience a greater plasma concentration of PAG than LT cows, further research is warranted to clarify the relationship between CBT before calving and PAG concentration.

Parity differed significantly between HT and LT cows in which cows in the HT group had greater average number of previous lactations than LT cows in the present study. Scanavez et al. (2019a) speculated that parity is among some of the animal-level factors that influence CBT. Although their study demonstrated that parity did not differ between their CBT groups for dry cows, Suthar et al., (2011) demonstrated that primiparous cows had a greater CBT than multiparous cows during the first 10 d of lactation. Scanavez et al. (2019a) speculated that midlactation multiparous cows would have a greater CBT than primiparous cows because of their increased feed intake and milk yield. Scanvez et al. (2019a) extensively explores the relationship between parity and CBT. Further research is needed to explore the mechanism of this relationship and to validate that a relationship truly exists.

In conclusion, the present study demonstrated that behavioral differences can be detected according to CBT in late gestation of heat-stressed dairy cows. In addition to behavioral differences, HT cows have greater plasma concentrations of PAG than LT cows. Visual observation did not detect major behavioral difference between HT and LT cows, but information from automated activity monitors captured important differences. High temperature compared with LT cows tended to spend less time ruminating after calving. Furthermore, LT compared with HT cows spent more time being inactive during the prepartum period. More research must be conducted in this area to further understand the relationship between CBT and animal welfare. Future experiments should explore the ability to establish thresholds or cut-points for PAG concentrations that may serve as a proxy for detecting cows with greater than average prepartum CBT. In addition, further study of behavior indicators for rumination and active time may serve as predictors of cows susceptible to postpartum health disorders.

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Table 1. Prepartum descriptive data (mean ± SEM) of cows classified as having low or high core body temperature before calving[1]

	Core body temperature (CBT) group[1]		
Item	Low	High	*P*-value
Number of cows	25	25	
Percentage of multiparous cows	41.9	58.1	0.16
Lactation number at enrollment	1.7 ± 0.1	2.2 ± 0.1	< 0.01
Average core body temperature, °C	38.70 ± 0.05	38.95 ± 0.01	< 0.01
Days in milk at dry off	317 ± 12.6	316 ± 12.6	0.93
Days of gestation at enrollment	226 ± 1.1	229 ± 1.1	0.04
Days spent in close-up pen	26.7 ± 1.1	23.5 ± 1.1	0.03
Gestation length, d	280.1 ± 0.9	278.0 ± 0.9	0.09

[1] Core body temperature group: low = cows with vaginal temperature below the median value; high = cows with vaginal temperature above the median value. Median values were calculated separately for each of 6 replicates based on core body temperature data collected during 7 d between d 225 and 239 of gestation.

Table 2. Least squares means ± SEM of percentage of daily time spent in each activity during the 2 h visual observations in the far-off period[1]

Item[3]	Core body temperature (CBT) group[2] Low Temperature (LT)	High Temperature (HT)	*P*-value CBT	Parity	CBT x parity
AM					
Drinking, %[4]	2.2 ± 1.5	3.0 ± 1.5	0.43	0.11	0.90
Eating, %[4]	15.0 ± 1.4	12.8 ± 01.4	0.84	0.24	0.29
Lying, %	63.3 ± 11.9	58.5 ± 11.9	0.35	0.01	0.84
Standing, %[4]	9.6 ± 1.7	13.2 ± 1.7	0.36	0.95	0.12.
Perching, %[4]	7.2 ± 1.2	6.1 ± 1.2	0.37	0.94	0.21
PM					
Drinking, %[4]	2.7 ± 1.4	1.8 ± 1.4	0.12	0.35	0.53
Eating, %	22.7 ± 7.2	24.6 ± 7.2	0.25	0.10	0.24
Lying, %[4]	26.2 ± 1.5	26.1 ± 1.5	0.82	0.32	0.70
Standing, %[4]	25.0 ± 1.4	24.2 ± 1.4	0.75	0.22	0.71
Perching, %[4]	5.3 ± 1.2	9.8 ± 1.2	0.16	0.45	0.59

[1] Cows were observed for a total of two AM sessions and two PM sessions in the far-off pen. Cows in the far-off pen between d 220 and 261 of gestation).

[2] Core body temperature group: low = cows with vaginal temperature below the median value; high = cows with vaginal temperature above the median value. Median values were calculated separately for each replicate based on core body temperature data collected during 7 d between d 225 and 239 of gestation.

[3] AM observations occurred between 0600 and 0800 h and PM observations occurred between 1600 and 1800 h.

[4] Data were log-transformed for analysis and back-transformed for reporting.

Table 3. Least squares means ± SEM of percentage of daily time spent in each activity during the 2 h visual observations in the close-up period[1]

Item[3]	Core body temperature (CBT) group[2] Low temperature (LT)	Core body temperature (CBT) group[2] High temperature (HT)	P-value CBT	P-value Parity	P-value CBT x parity
AM					
Drinking, %[4]	2.1 ± 1.4	2.6 ± 1.2	0.84	0.93	0.24
Eating, %[4]	13.3 ± 1.2	12.4 ± 1.4	0.87	0.84	0.96
Lying, %	79.8 ± 2.8	85.2 ± 2.9	0.36	0.72	0.39
Standing, %[4]	8.6 ± 1.2	8.8 ± 1.2	0.92	0.75	0.81
PM					
Drinking, %[4]	3.1 ± 1.2	3.2 ± 1.2	0.83	0.44	0.21
Eating, %[4]	23.9 ± 1.1	24.2 ± 1.1	0.91	0.14	0.02
Lying, %[4]	19.9 ± 1.4	18.3 ± 1.4	0.88	0.48	0.89
Standing, %	54.3 ± 5.6	54.8 ± 5.7	0.76	0.05	0.20

[1] Cows were observed for a total of two AM sessions and two PM sessions in the close-up pen. Cows were in the close-up pen from d 247 to 285 of gestation.

[2] Core body temperature group: low = cows with vaginal temperature below the median value; high = cows with vaginal temperature above the median value. Median values were calculated separately for each replicate based on core body temperature data collected during 7 d between d 225 and 239 of gestation.

[3] AM observations occurred between 0600 and 0800 h and PM observations occurred between 1600 and 1800 h.

[4] Data were log-transformed for analysis and back-transformed for reporting.

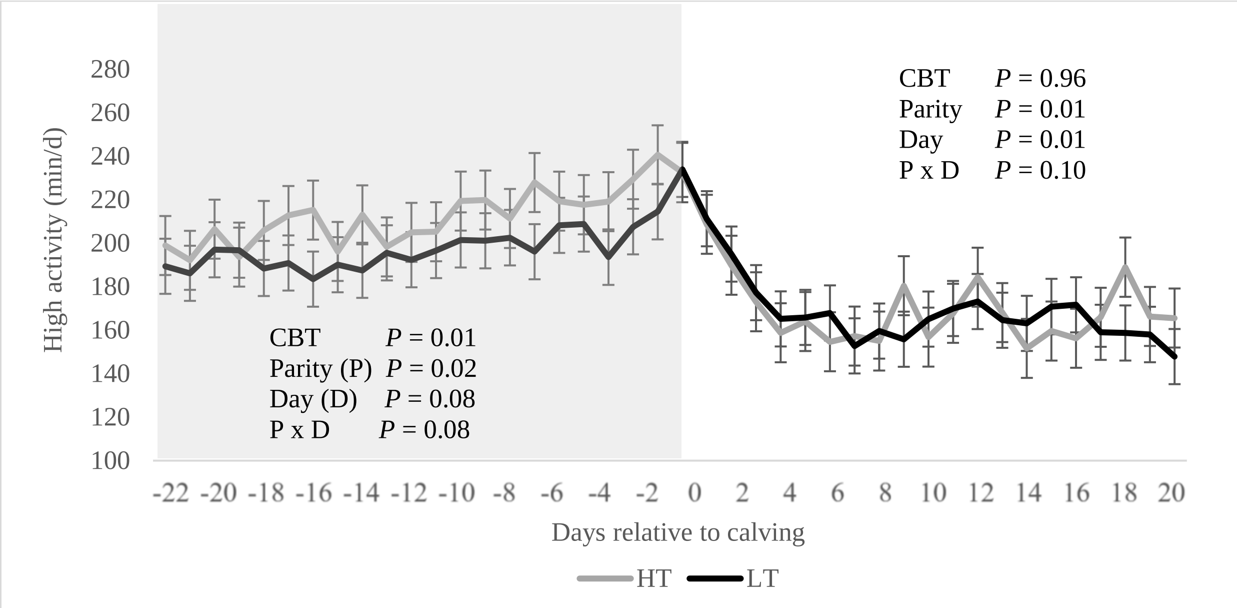

Figure 1. Total minutes per day (least squares means ± SEM) quantified as high activity by the activity monitoring ear tag from d -21 through +21 (d 0 = calving) for cows with either high median (HT; gray line) or low median (LT; black line) core body temperature (CBT). Data collected during the prepartum and postpartum periods were analyzed separately (CBT = HT or LT cows). *P*-values listed include all fixed effects used in the final model for each analysis.

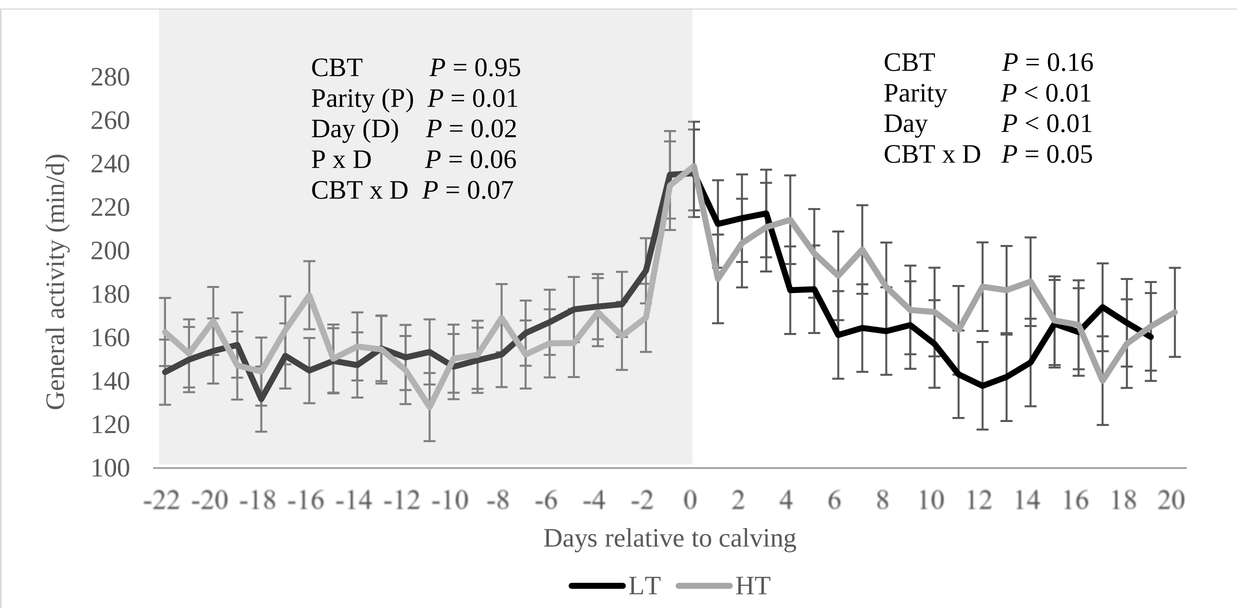

Figure 2. Total minutes per day (least squares means ± SEM) quantified as general activity by the activity monitoring ear tag from d -21 through +21 (d 0 = calving) for cows with either high median (HT; gray line) or low median (LT; black line) core body temperature (CBT). Data collected during the prepartum and postpartum periods were analyzed separately (CBT = HT or LT cows). *P*-values listed include all fixed effects used in the final model for each analysis.

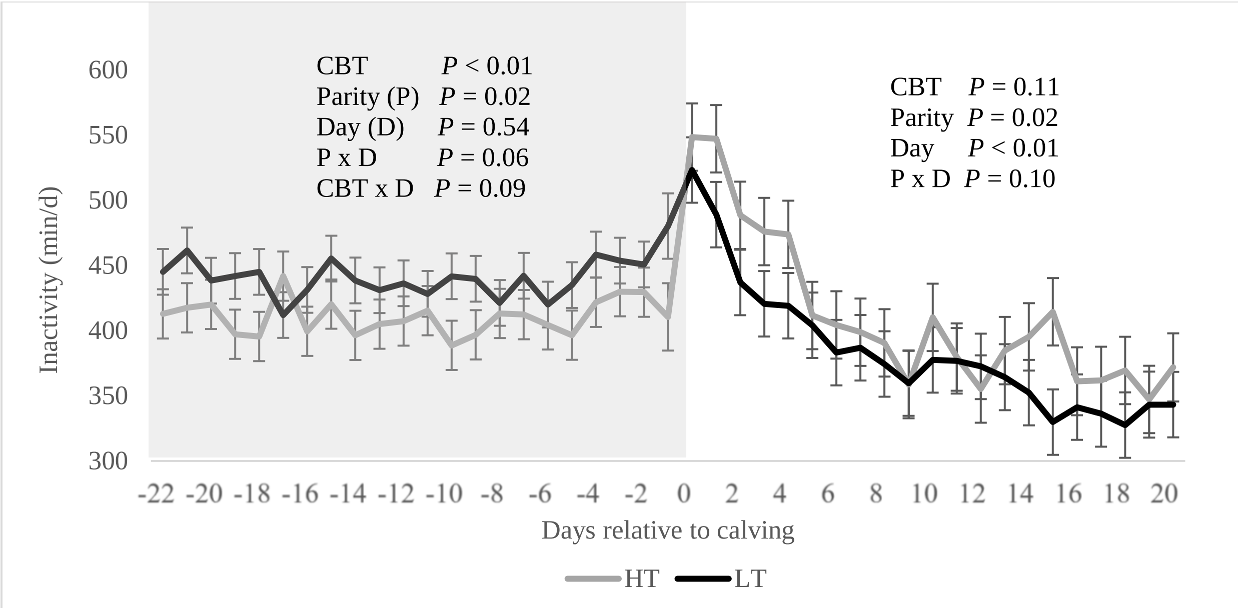

Figure 3. Total minutes per day (least squares means ± SEM) quantified as inactive time by the activity monitoring ear tag from d -21 through +21 (d 0 = calving) for cows with either high median (HT; gray line) or low median (LT; black line) core body temperature (CBT). Data collected during the prepartum and postpartum periods were analyzed separately (CBT = HT or LT cows). *P*-values listed include all fixed effects used in the final model for each analysis.

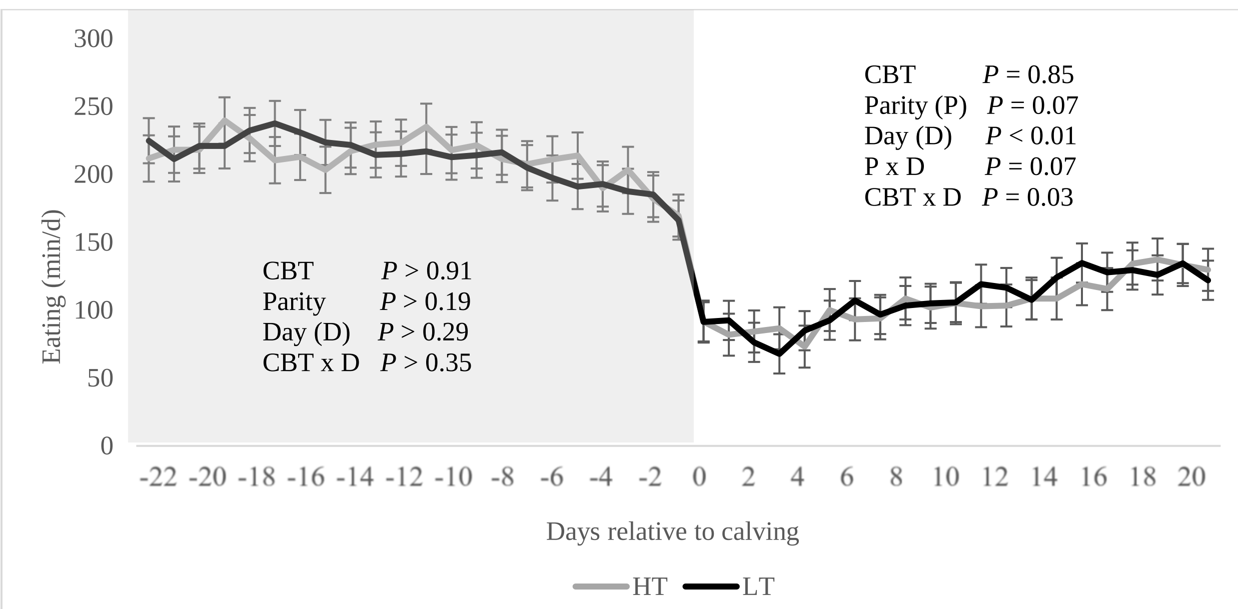

Figure 4. Total minutes per day (least squares means ± SEM) quantified as eating by the activity monitoring ear tag from d -21 through +21 (d 0 = calving) for cows with either high median (HT; gray line) or low median (LT; black line) core body temperature (CBT). Data collected during the prepartum and postpartum periods were analyzed separately (CBT = HT or LT cows). *P*-values listed include all fixed effects used in the final model for each analysis.

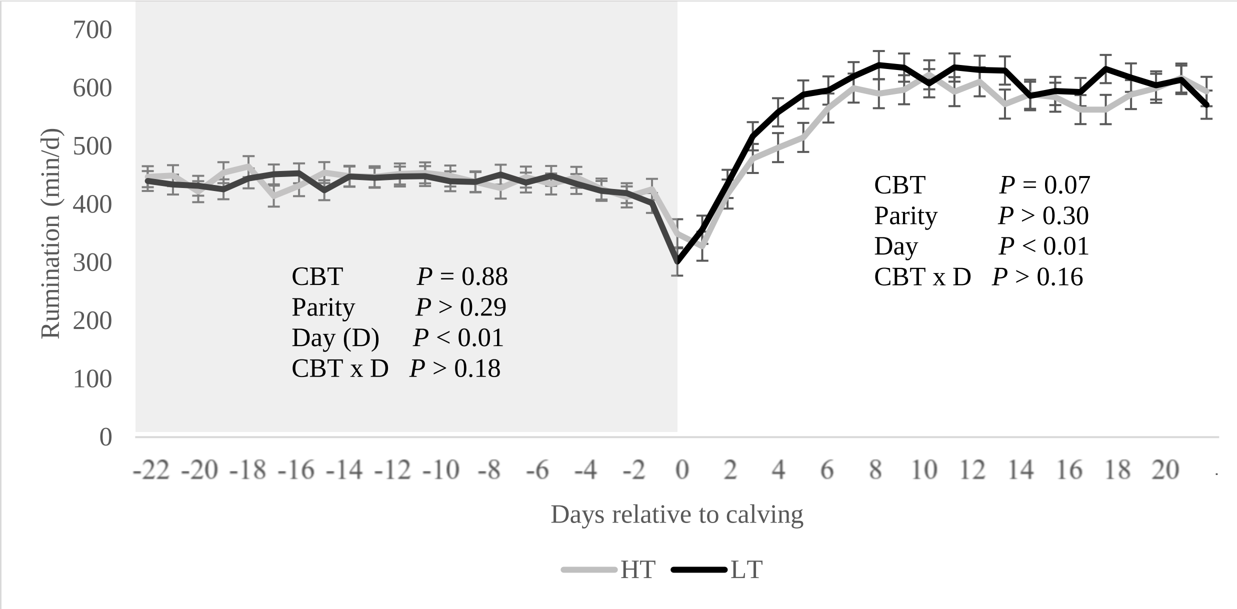

Figure 5. Total minutes per day (least squares means ± SEM) quantified as rumination by the activity monitoring ear tag from d -21 through +21 (d 0 = calving) for cows with either high median (HT; gray line) or low median (LT; black line) core body temperature (CBT). Data collected during the prepartum and postpartum periods were analyzed separately (CBT = HT or LT cows). *P*-values listed include all fixed effects used in the final model for each analysis.

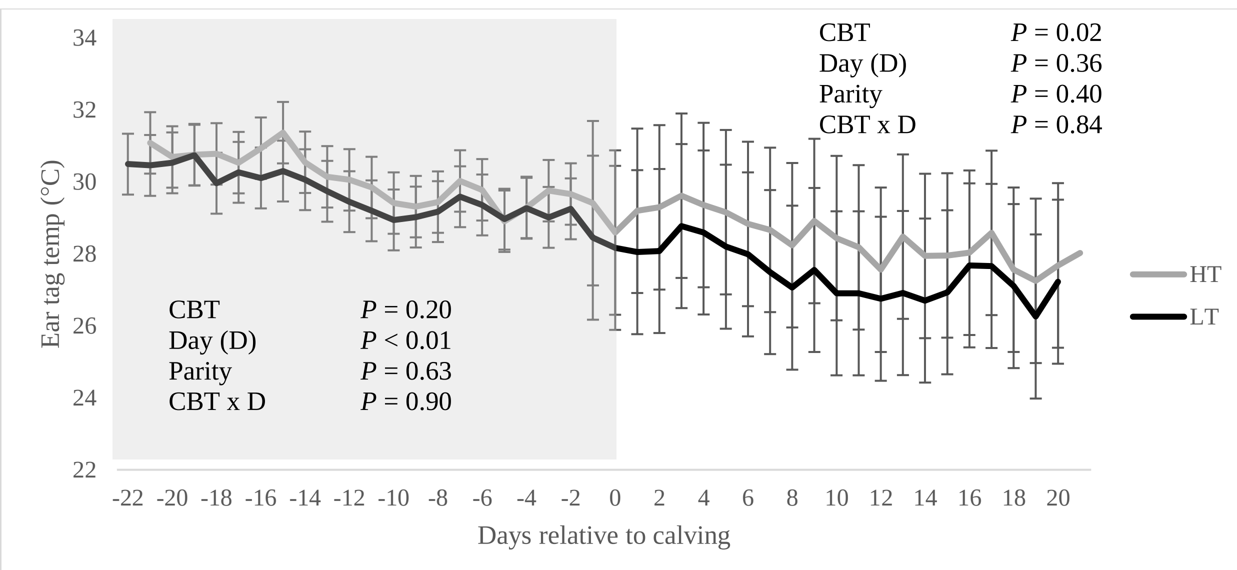

Figure 6 Average ear tag temperature per day (least squares means ± SEM) quantified by the activity monitoring ear tag from d -21 through +21 (d 0 = calving) for cows with either high median (HT; gray line) or low median (LT; black line) core body temperature (CBT). Data collected during the pre- and postpartum periods were analyzed separately. (CBT = HT or LT cows). *P*-values listed include all fixed effects used in the final model for each analysis.

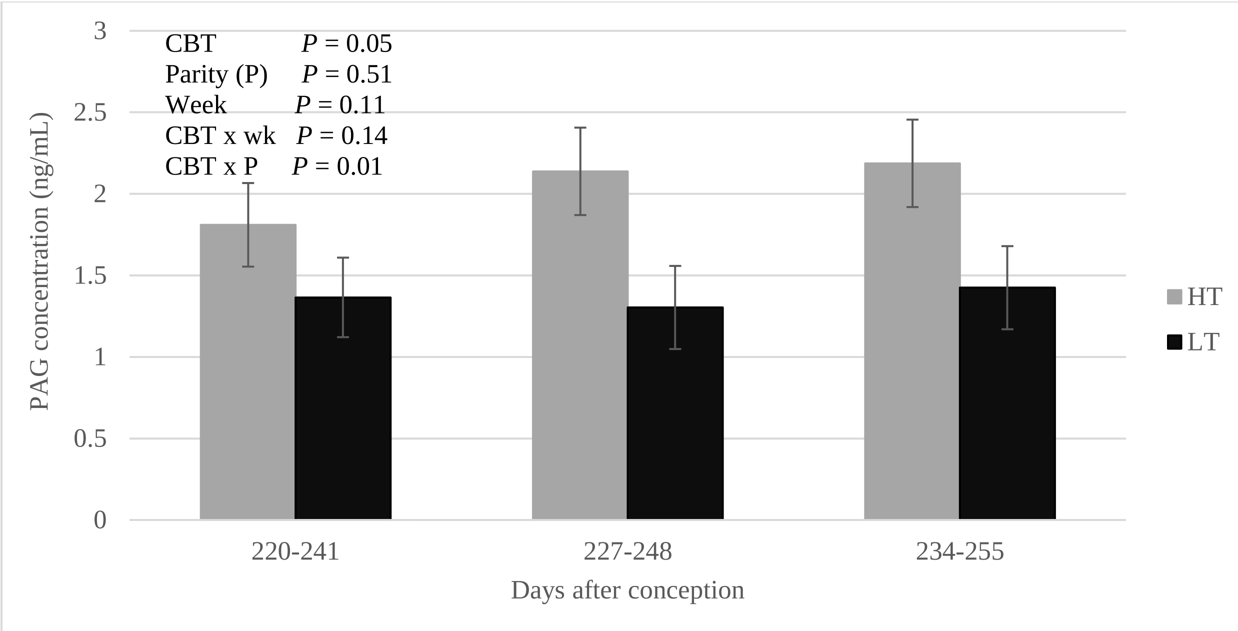

Figure 7 . Pregnancy associated glycoprotein (PAG) concentrations from 3 samples collected between d 220 and 255 of gestation, each 7 days apart, for high temperature (HT; grey bar) and low temperature (LT; black bar) cows [core body temperature (CBT) = HT or LT cows; BS = blood sample number]. All *P*-values listed include all fixed effects used in the final model for this analysis.