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Thermoregulatory responses of lactating dairy cows to an acute heat stress after a pharmacologically-induced LH surge

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To the Graduate Council:

I am submitting herewith a thesis written by Chelsea Ruth Abbot entitled "Thermoregulatory responses of lactating dairy cows to an acute heat stress after a pharmacologically-induced LH surge." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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(Original signatures are on file with official student records.)

Thermoregulatory responses of lactating dairy cows to an acute heat stress after a pharmacologically-induced LH surge

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ABSTRACT

The objective of this study was to develop an in vivo model to assess thermoregulatory response of lactating dairy cows to heat stress. Hyperthermia occurring for 10 to 12 hours after LH surge reduces quality of maturing oocyte, thereby reducing fertility. Between the months of February through May, cows were transported to a climate-controlled facility and maintained at a temperature-humidity index (THI) of 65.9 ± [plus or minus] 0.2 (thermoneutral) or exposed to increases in THI of 0.8 ± 0.1 units per hour (heat stress) for 12 hours before rapidly cooling to thermoneutral conditions. Mixed model regressions with repeated measures were used to test respiration rates (RR) and rectal temperature (RT). Within 40 and 110 min of increasing THI, RR increased in a quadratic fashion (P < [less than] 0.001); RT increased by 0.04 ± 0.1° [degree] C (P < 0.001) per unit THI. Changes in RR lagged THI and preceded rises in RT by 30 min. Average THI 3-days prior to treatment influenced changes in RR (P = [equal] 0.050) and RT (P < 0.001). Increased RR was more noticeable in heat-stressed cows when prior THI was in the 40s than low 60s. Rectal temperature of heat-stressed cows was 0.8 ± 0.02°C lower when prior THI was in the 40s versus low 60s. Progesterone and LH levels before treatment were predictive of thermoregulatory response in heat-stressed cows. Rapid cooling decreased RR by 0.6 ± 0.1 bpm (P < 0.001) and RT by 0.02 ± 0.002°C per min (P < 0.002). Speed and magnitude of thermoregulatory changes to an acute heat stress and after sudden cooling emphasizes the importance of strategic cooling before ovulation. Efforts to do so when prior THI approaches levels expected to induce mild stress are especially important. Respiration

rate is a useful indicator of the degree of hyperthermia a lactating cow is experiencing during an acute heat stress event.

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CHAPTER ONE

INTRODUCTION AND REVIEW OF PERTINENT LITERATURE

Increases in ambient temperatures up to 4°C above long standing averages are projected to occur over the next 50 to 100 years (IPCC, 2014). Yearly average temperature increases have been attributed to longer summers (Jeong et al., 2011, Stine et al., 2009) with more days of record breaking highs (Coumou et al., 2013, IPCC, 2014). This, along with a noted shift in increased night-time temperatures (Alexander et al., 2006) may exacerbate the multitude of negative consequences on agriculturally-important animals during chronic periods of heat stress. The potential for heat waves, defined as 3 or more consecutive days of temperatures exceeding average maximum temperatures (Lau and Nath, 2012), poses additional challenges especially when occurring at unexpected time periods of the year (i.e., winter, early spring/late fall).

Approximately 70% of the world's cattle population reside in subtropical and tropical environments (Robinson et al., 2014) and experience some degree of heat stress. Heat-induced reductions in milk yield at a temperature-humidity index (THI) as low as 65 (Collier et al., 2009) with impacts on fertility at a THI as low as 72 (McGowan et al., 1996, Morton et al., 2007) attest to the global scale of heat stress-related problems for the dairy industry. Although the basis for heat-induced reductions in fertility is multifactorial, hyperthermia is a major contributing factor. Ulberg and Burfening (1967) reported a 20% reduction in pregnancy rate with a 1°C rise in rectal temperature.

Heat-induced hyperthermia is especially problematic during the follicular phase of the cow's reproductive cycle (e.g., peri-estrus; Gwazdauskas et al., 1973; Putney et al., 1989). This is a critical time period when the steroid hormone progesterone is low. Increasing estradiol levels are important for sexual receptivity and trigger the GnRH-induced LH surge which sets forth a series of events important for ovulation some 30 hours thereafter (Giordano et al., 2013, Pursley et al., 1995). The LH surge also induces the oocyte to resume meiosis (begin maturing). Much of the consequences of moderate to severe hyperthermia when occurring for 10 to 12 hours after the LH surge are due to direct effects of elevated temperature to reduce the quality of the maturing oocyte, thereby reducing fertility (Edwards and Hansen, 1996, Putney et al., 1989).

Despite the widely accepted magnitude of hyperthermia-related problems, little progress has been made to identify altered components after the LH surge that could explain heat-induced reductions in fertility. Efforts to do so have been challenging because of the difficulty with study design to restrict heat-induced increases in hyperthermia to the time period after the LH surge. Putney et al. (1989) was successful in doing so by exposing super-ovulated heifers to a constant temperature of 42°C for 10 hours beginning at estrus. Because milk yield is known to impact ability of lactating cows to maintain homeothermy (reviewed by Kadzere et al., 2002), we hypothesized that conditions necessary to develop an in vivo model to assess the impact of hyperthermia in lactating dairy cows will differ. To test this hypothesis, the thermoregulatory response of lactating Holsteins during an acute heat stress event occurring after a pharmacologically-induced LH surge was examined. Effort to steadily

increase THI to induce an acute heat stress event was prioritized to better emulate farm conditions that may occur at the onset of a heat wave. Examining thermoregulatory responses during late winter and spring confined acute heat stress exposure to the time period after the LH surge and eliminated concerns with carryover effects known to be problematic during chronic heat stress. Moreover, efforts to determine where homeothermy is no longer possible in face of an acute heat stress along with effort to determine the rapidness in which hyperthermic cows may be cooled are foundational steps towards devising or revising strategies aimed at mitigating the negative impact of heat stress when occurring at or near the time of estrus.

CHAPTER TWO

MATERIALS AND METHODS

2.1 ANIMALS

Twenty-nine lactating, primiparous (n = 13) and multiparous (n = 16; range = 2 to 4 lactations) Holstein dairy cows were used in this study. Cows were 124 to 390 (mean = 216) days in milk, producing an average of 22.4 to 50.0 (mean = 35.5) kg of milk per day in the 2 days before treatments were initiated. Cows were milked twice daily. A total mixed ration meeting nutrient requirements for lactating dairy cows and fresh water were provided ad libitum. Institutional approval for animal care and use was obtained before the onset of this study.

2.2 PHARMACOLOGICAL-BASED PROTOCOL TO OBTAIN A DOMINANT FOLLICLE AND INDUCE AN ENDOGENOUS LH SURGE

Obtaining a dominant follicle equivalent to that expected at or near the onset of estrus along with effort to induce an LH surge were achieved as previously described by Giordano et al. (2013) with some modifications. Cows with a corpus luteum (confirmed by ultrasound) were administered $PGF_{2\alpha}$ (i.m. dinoprost tromethamine; Lutalyse; Zoetis, Parsippany, New Jersey, USA). Approximately 11 days after $PGF_{2\alpha}$, 100 μg of a GnRH analogue (i.m. gonadorelin hydrochloride; Factrel, Zoetis) was administered and a controlled intravaginal drug release (CIDR) device containing 1.38 g progesterone (Eazi-breed CIDR, Zoetis) was inserted (Figure 1). Seven days later, CIDR was removed and $PGF_{2\alpha}$ was administered. Cows that were confirmed to have undergone luteal regression after initial $PGF_{2\alpha}$ and follicle turnover thereafter and having a

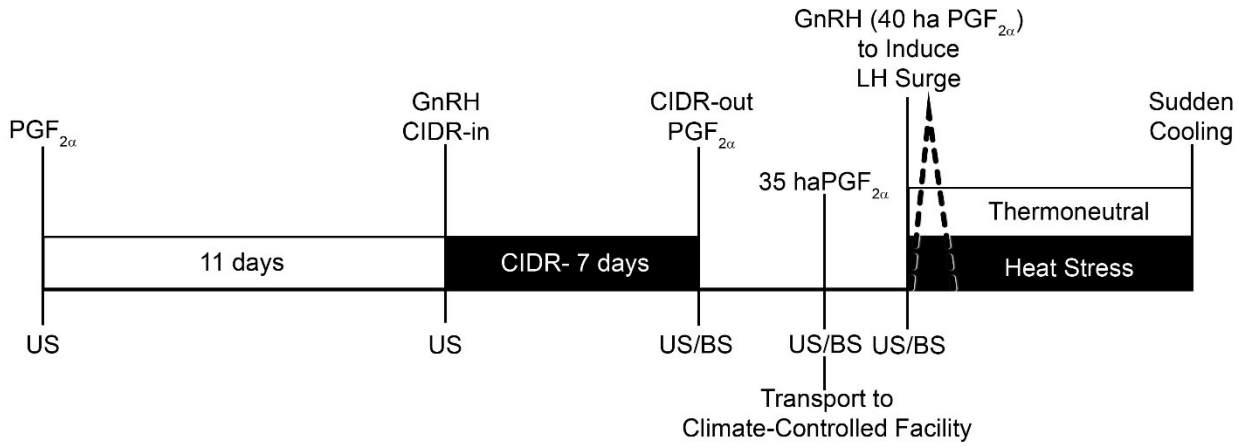

Figure 1. Protocol for synchronizing follicle development and inducing the LH surge in lactating dairy cows that were to be maintained at thermoneutral or heat stress conditions in a climate-controlled room. CIDR = controlled internal drug releasing device, US = ultrasound, BS = blood sample

dominant follicle that had undergone growth after CIDR removal were then transported to a climate-controlled facility 35 hours after final $PGF_{2\alpha}$ administration. Five hours later (40 hours after final $PGF_{2\alpha}$), 100 µg of a GnRH analogue (i.m. gonadorelin diacetate tetrahydrate; Cystorelin, Merial Limited, Iselin, NJ, USA) was administered to induce an endogenous LH surge ~1.9 hours later (Giordano et al., 2013).

2.3 APPLICATION OF TREATMENTS

Cows were randomly assigned for maintenance at thermoneutral conditions or exposed to an acute heat stress in a climate-controlled room equipped with individual pens (Figure 1). Ambient temperature (T_a) and relative humidity (RH) of the room were monitored using HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA). The temperature-humidity index (THI) was calculated using the equation $(0.8 * T_a) + ((RH / 100) * (T_a – 14.4)) + 46.4$ (Dikmen and Hansen, 2009, Mader et al., 2006). A total of 17 thermoneutral cows in 4 different groups and 12 heat-stressed cows in 3

different groups were utilized. Treatments were initiated near the time of final GnRH administration to induce an LH surge. Ambient room conditions remained constant at a THI of 65.8 ± 0.1 for thermoneutral cows (Figure 2). Relevant for heat-stressed cows, THI was steadily increased at a rate of 0.8 ± 0.1 units per hour (83.8 ± 0.1 overall mean; range = 74.1 to 87.3; Figure 2). Treatments were applied between the months of February through May. Days in milk (243.0 ± 18.6 and 198.4 ± 22.1 days; P = 0.135) and milk yield (kg/day; 35.2 ± 1.6 and 35.8 ± 1.8; P = 0.807) were similar between cows that would be exposed to thermoneutral and heat-stress conditions.

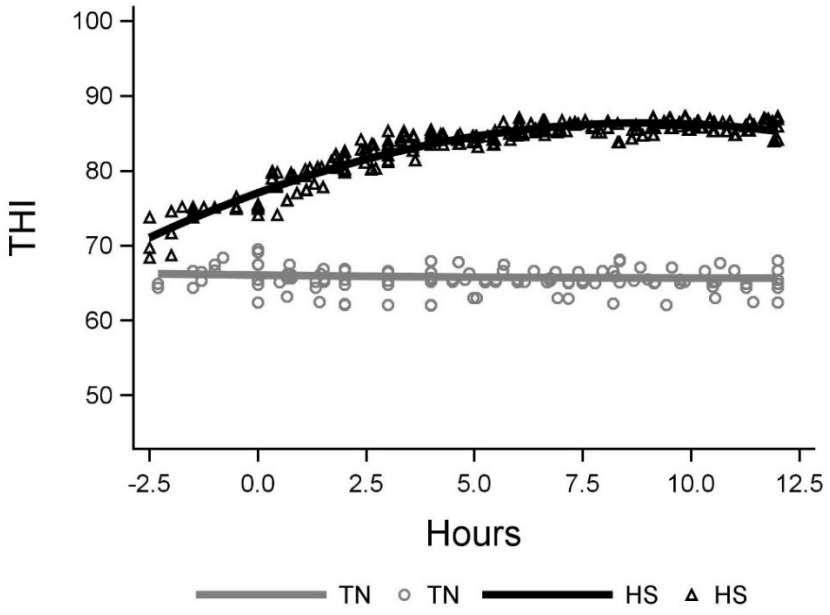

Figure 2. Ambient conditions (temperature-humidity index; THI) that cows were maintained at during thermoneutral (TN; grey line; circles) or heat stress (HS; black line; triangles) treatments.

2.4 MONITORING THERMOREGULATORY RESPONSES

Cows were continuously monitored during treatment. Respiration rates were recorded as breaths per min (bpm) by counting flank movements. For heat-stressed cows, rectal temperatures were recorded every 15 min using a GLA M700 thermometer with a 7.6 cm right angled probe (GLA Agricultural Electronics, San Luis Obispo, CA, USA). After ~12 hours, heat-stressed cows were rapidly cooled by turning off heat source and misters. Doors and windows of the climate-controlled room were opened, and a high speed fan was placed in room. This approach was effective to return room to thermoneutral conditions within ~15 min.

2.5 ASSESSMENT OF OVARIAN ACTIVITY BY ULTRASOUND

Transrectal ultrasound of ovarian structures was performed at the time of final $PGF_{2\alpha}$, 35 hours thereafter, and at GnRH administration to induce an LH surge (Figure 1) using an Aloka SSD-500V ultrasound with a 7.5 MHz linear probe (Aloka Company Ltd, Wallingford, CT, USA). Location and size of ovarian structures were mapped at each ultrasound to assess luteal regression and follicular growth. Follicle size was calculated by averaging perpendicular measurements of the largest diameter.

2.6 BLOOD SAMPLING AND HORMONE ASSAYS

Blood samples were obtained from coccygeal vessels at $PGF_{2\alpha}$ administration, 35 hours thereafter, at final GnRH administration, and 2, 3, and 4 hours after final GnRH. Blood was allowed to clot at 4°C for a minimum of 4 hours. Samples were warmed to room temperature before centrifugation (1000 x g) for 20 min. Serum was stored at -80°C until hormone analysis. Serum progesterone (P4) concentrations were

determined in duplicate using a commercially available radioimmunoassay (RIA) kit (MP Biomedicals, Inc.; Costa Mesa, CA, USA) previously validated for bovine serum (Pohler et al., 2016). Sensitivity of assay was 0.15 ng/ml; intra- and inter-assay coefficients of variation (CV) were 1.6 and 4.1%. Serum estradiol (E2) concentrations were determined as previously described by (Kirby et al., 1997). The sensitivity of the assay was 0.05 pg/ml; intra-assay CV was 3.4%. Concentrations of luteinizing hormone (LH) were quantified using a double antibody RIA previously validated for bovine samples (Moura and Erickson, 1997) with antibodies obtained from the National Institute of Hormone and Peptide program (Harbor-UCLA Medical Center, CA, USA). The sensitivity of the assay was 0.03 ng/ml; intra- and inter-assay CVs were 4.2 and 7.0%.

2.7 STATISTICAL ANALYSES

Statistical analyses were conducted using mixed models in SAS 9.4 (SAS Institute, Cary, NC, USA). Best fit models were determined using backwards manual selection, specifically taking low Akaike information criterion (AIC) and high R^2 values into consideration. R^2 was calculated by dropping random effects since it is not produced by mixed model software. All models included the random variable cow nested within treatment, and repeated measures over time. Statistical differences were noted using a p-value < 0.05. Data are presented as least squares means or slopes ± standard error.

Thermoregulatory responses as related to changes in THI and time were characterized using treatment groups, THI or time regression effects, and all interactions in the model; prior THI (priorTHI, 3-day average before treatments were

initiated) was included as a covariate (section 3.1). Lags up to 4 time points for THI, prior THI and RR were also tested for inclusion in the models. Outcome of efforts to predict thermoregulatory responses based on prior THI is described in section 3.2. Data to determine prior THI were obtained from the meteorological station located at McGhee Tyson Airport, Knoxville, Tennessee, USA (NOAA, 2018), located ~12 miles from dairy.

Models to characterize thermoregulatory responses with ambient temperature and relative humidity individually were also tested to address if THI captures the information in both variables (section 3.3). Respiration rate and RT were characterized using treatment groups, prior ambient temperature and prior relative humidity (3-day average before experiment was started), ambient room temperature, and room relative humidity regression effects and all interactions in the model. The best time and THI models identified in section 3.1 were also used to test the addition of individual regression variables of milk yield, parity, hormone (progesterone, estradiol, and LH) levels, dominant follicle size at various time points (section 3.4) and analyzed to determine their association with thermoregulatory responses. A separate set of models characterized RR and RT during the cooling period using treatment groups and time regression effects and interactions (section 3.5).

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 THERMOREGULATORY RESPONSES OF LACTATING COWS AFTER EXPOSURE TO AN ACUTE HEAT STRESS

Respiration rates, after transport to climate controlled facility but before treatments were initiated, were similar in cows that were to be maintained in thermoneutral versus heat stress conditions (60.3 ± 4.7 versus 72.1 ± 6.3 bpm; P = 0.149). Rectal temperature of cows that were to be maintained in thermoneutral or heat stress conditions were also similar before treatments were initiated (38.9 ± 0.1 versus 38.4 ± 1.1°C; P = 0.635). In response to treatment, RR of heat-stressed cows were ~2.5 times higher than thermoneutral cows (111.5 ± 0.7 versus 42.8 ± 1.3 bpm; P < 0.001). Incremental increases in THI elevated RT above those observed in thermoneutral counterparts (39.7 ± 0.01 versus 38.3 ± 0.02°C; P < 0.001). When examining changes as a function of time, RR of heat-stressed cows were different from the RR of thermoneutral cows 40 min after initiating increases in THI (37.2 ± 2.2 versus 95.5 ± 1.5 bpm for thermoneutral and heat stress; P < 0.001). A quadratic relationship (P (linear) < 0.001; P (quadratic) < 0.001) was observed between time and RR of heat-stressed cows ($RR = -1.7x^2 + 27.8x + 19.1$; $R^2 = 0.90$; P < 0.001; Figure 3, Panel A). Heat-induced elevations in RT (P < 0.001) in heat-stressed cows were different from RT of thermoneutral cows 110 min after initiating increases in THI (38.5 ± 0.03 versus 39.4 ± 0.02°C for thermoneutral and heat stress). Heat-induced increases in RT increased at a rate of 0.07 ± 0.01 °C per hour (P < 0.001; Figure 3, Panel B).

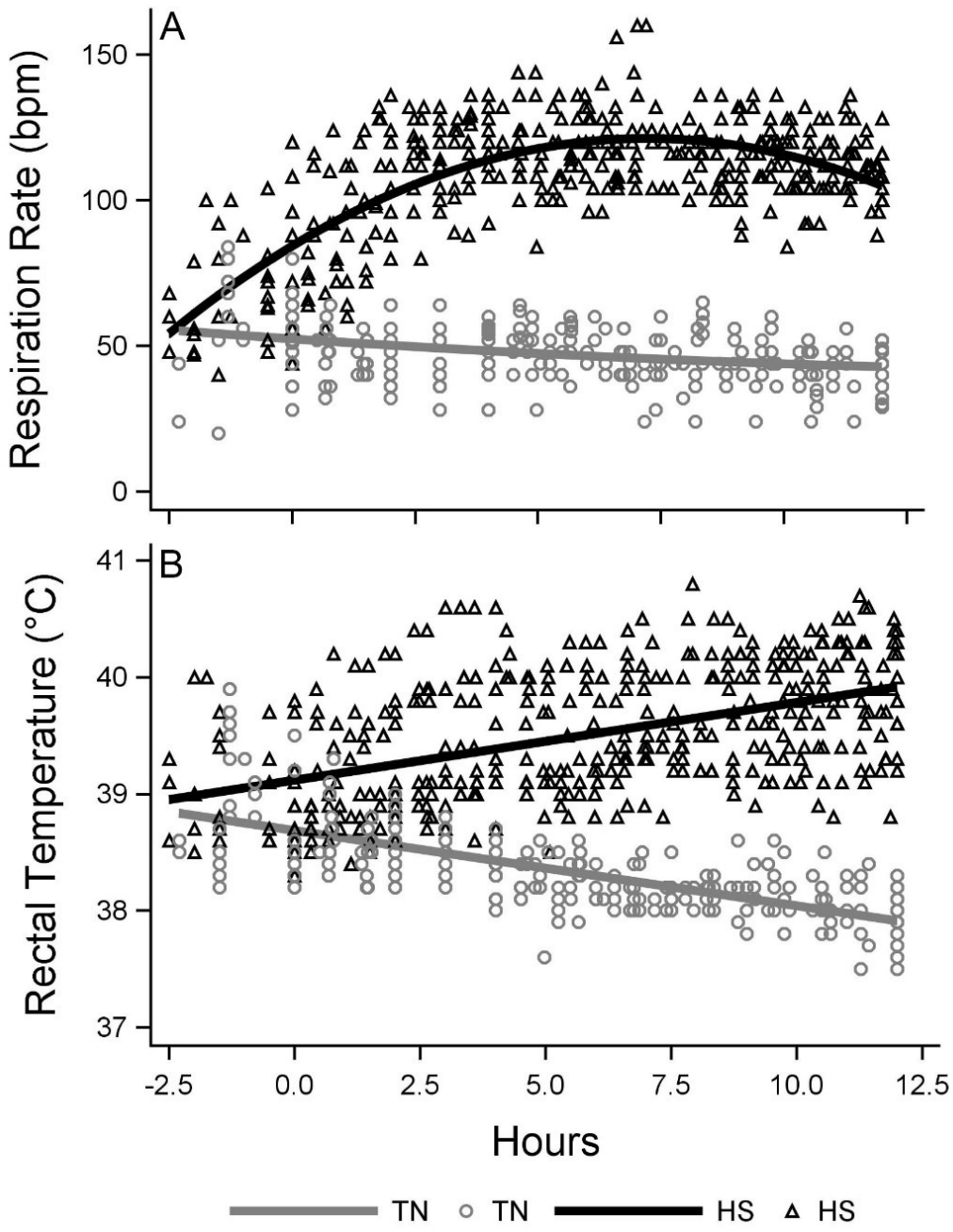

Figure 3. Changes in respiration rate (RR; Panel A) and rectal temperature (RT; Panel B) of thermoneutral (TN; grey line; circles) and heat-stressed (HS; black line; triangles) cows as a function of time. For thermoneutral and heat-stressed cows, a linear (RR = -0.3x + 18.4) and quadratic (RR = $-1.7x^2 + 27.8x + 19.1$) relationship, respectively, was noted between RR and THI. A linear relationship with RT and THI was noted for thermoneutral (RT = -0.05x + 38.5) and heat-stressed (RT = 0.07x + 37.1) cows. bpm = breaths per minute

When examining changes as a function of THI, a quadratic relationship (P (linear) = 0.003); P (quadratic) = 0.007) was observed between THI and RR in heat-stressed cows ($RR = -0.8x^2 + 141.7x – 6216.4$; $R^2 = 0.90$; $P < 0.001$; Figure 4, Panel A). Heat-induced changes in RR lagged changes in THI by 30 min (slope = 0.7 ± 0.3 bpm per hour; P = 0.016). THI-induced elevations in RT occurred at a rate of 0.04 ± 0.01°C for each unit THI (P < 0.001; Figure 4, Panel B). Heat-induced changes in RT lagged THI changes by 1 hour (slope = 0.05 ± 0.01°C per hour; P < 0.001). Heat-induced increases in RT lagged changes in RR by 30 min (slope = 0.01 ± 0.001°C per hour; P < 0.001).

Cattle are homeotherms capable of dissipating heat to maintain body temperature in face of changing ambient conditions. Heat loss is possible through a number of different mechanisms including convection, conduction, radiant exchange, and evaporative cooling (reviewed by Aggarwal & Upadhyay, 2013 and Spiers, 2012). Evaporative cooling is especially effective with ~15% of heat loss occurring from the respiratory tract (McLean, 1963). A difference in temperature and vapor pressure between the animal and environment is required for evaporative cooling (Hoff, 2013). Although most effective when humidity is low, heat-induced elevations in RR is a useful and important initial mechanism to maintain homeothermy when ambient conditions begin to exceed thermoneutral zone (Kadzere et al., 2002). In our study, changes in RR occurred within 30 to 40 min after steady increases in THI were initiated. Changes in RT were not noticeable until ~30 min after heat-induced changes in RR.

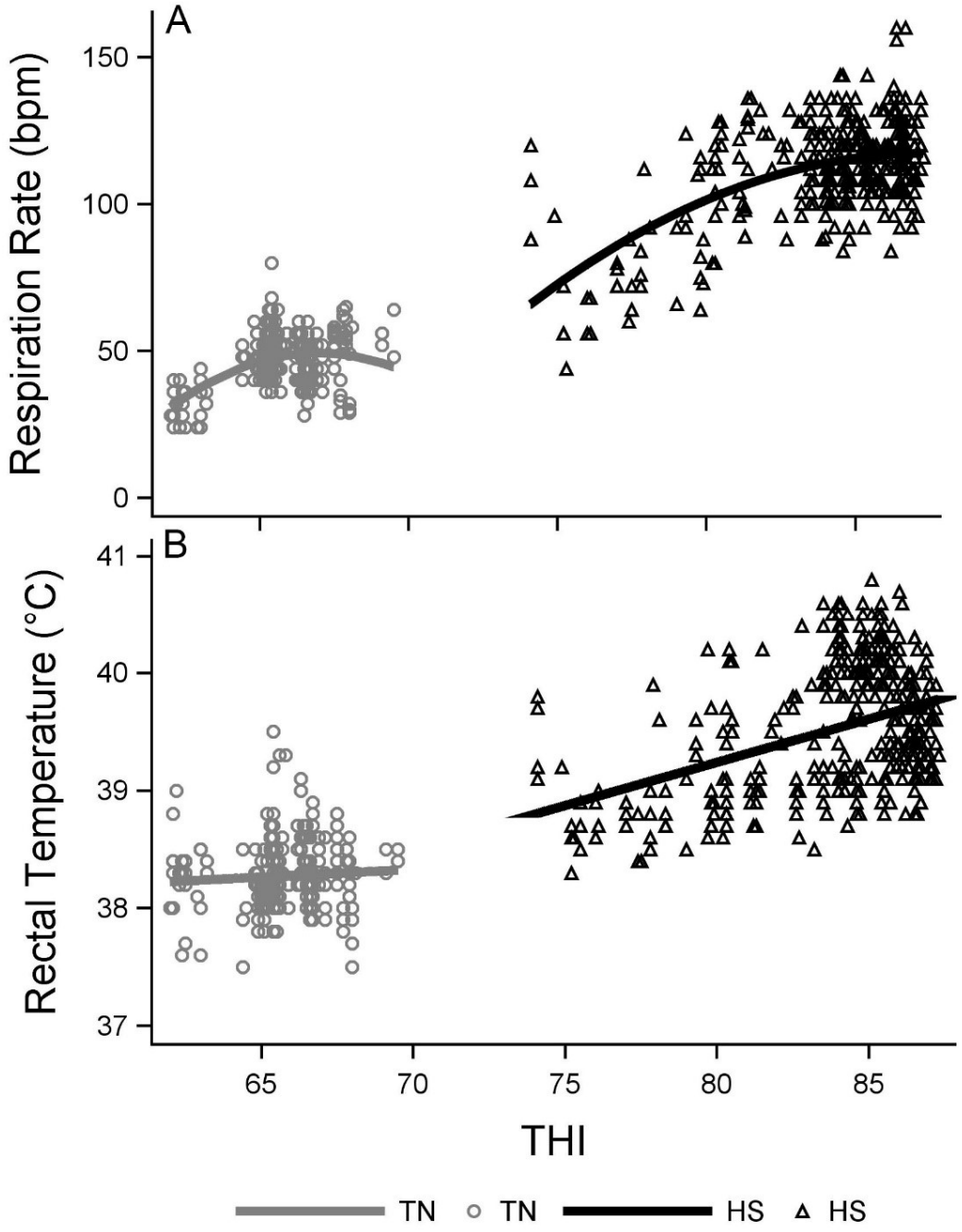

Figure 4. Changes in respiration rate (RR; Panel A) and rectal temperature (RT; Panel B) of thermoneutral (TN; grey line; circles) and heat-stressed (HS; black line; triangles) cows as a function of THI. A quadratic relationship was noted between RR and THI for thermoneutral ($RR = -0.9x^2 + 125.1x – 4429.1$) and heat-stressed ($RR = -0.8x^2 + 141.7x – 6216.4$) cows. A linear relationship with RT and THI was noted for thermoneutral ($RT = -0.01x + 38.9$) and heat-stressed ($RT = 0.04x + 34.3$) cows. bpm = breaths per minute, THI = temperature-humidity index

In face of an acute heat stress exposure whereby incremental changes in THI may occur in a continuum of several hours within a day, cattle may reach a point where they physically cannot breathe any faster. Reaching this “respiratory ceiling” (Gaughan et al., 2000) would explain why respiration rate increased in a quadratic fashion in our study and why RT increased in a linear fashion thereafter. Within the range of responses observed in our study, RR of lactating dairy cows was positively related to RT (P (linear) < 0.001; P (quadratic) < 0.001). Interestingly, a RR of 79 bpm corresponded to a RT that was ≥ 39.0°C (Figure 5). This finding is in agreement with others reporting that when cows reach ~75 bpm they lose the ability to maintain normal body temperature (Beatty et al., 2006). Information derived from examining relationship of RR with changes in RT is useful from a practical standpoint to determine when heat

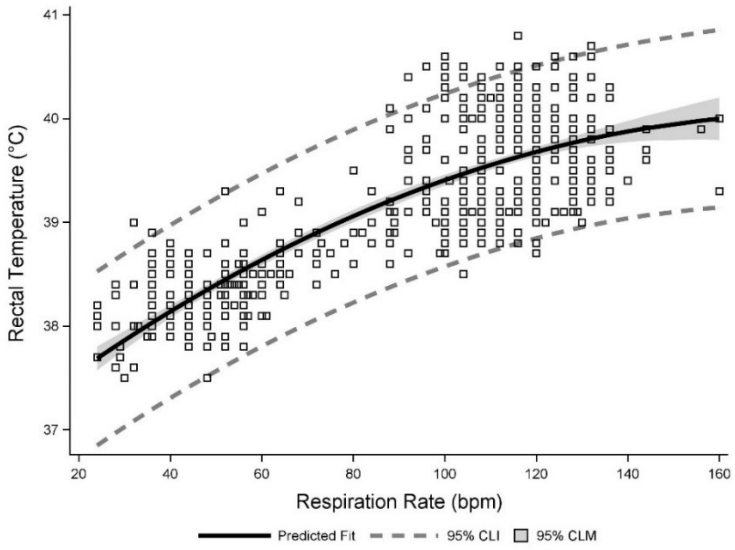

Figure 5. Relationship of respiration rate and rectal temperature in lactating dairy cows. Grey shaded area represents the 95% mean confidence interval and dashed lines represent the 95% prediction limits. bpm = breaths per minute

mitigation should be initiated. For example, to reduce incidence of RT approaching or exceeding 39.0°C, strategic cooling efforts would need to be implemented when RR approach 65 to 70 bpm; waiting any later may be problematic for milk yield (Collier et al., 2009, Spiers et al., 2004) and fertility (Gwazdauskas et al., 1973, Ulberg and Burfening, 1967).

The relationship between heat-induced changes in RR and RT after acute heat stress exposure has been reported by others (Collier et al., 2009, Ferrazza et al., 2017, Scharf et al., 2008) and is not unique to the dairy cow or even lactation status because similar outcomes have been observed in Angus crossbred heifers exposed to ambient temperatures up to 32°C (Beatty et al., 2006). Although response will likely vary depending on climate, other factors may also be influential. For instance, when 8-month old Angus crossbred steers were exposed to a moderate but acute heat stress with an average THI of 82, RR nearly doubled, RT did not change (Scharf et al., 2008).

Interestingly, RT of thermoneutral cows decreased at a rate of 0.05 ± 0.01°C per hour ($P < 0.001$; Figure 4, Panel B), even though RR rates remained constant ($P = 0.836$). At the time of GnRH administration, RT was 38.5 ± 0.3°C; 12 hours later they were 38.0 ± 0.2°C. The potential for RT to decrease during the time period after the LH surge is consistent with other reports showing that body temperature is often lowest closer to the time of ovulation (Mosher et al., 1990, Rajamahendran et al., 1989, Suthar et al., 2011). Basis for RT changes during this time period remains unclear but may be important for fertility. In the bovine, it has been suggested that follicles may be ~1.5°C cooler than surrounding tissues (Hunter et al., 2017). Depending on the extent to which

this is possible, a lower follicle temperature may obviate potential problems with estrus associated spikes in temperature. This, along with reductions in RT temperature after the LH surge and approaching the time period of ovulation, may be important to protect the maturing oocyte that is especially susceptible to hyperthermia. Because GnRH administration to induce the LH surge occurred around 10:00 pm and cow activity was restricted by housing in individual pens, diurnal variations in RT (Beatty et al., 2006, Piccione et al., 2003) may have also been contributive.

3.2 PREDICTING THERMOREGULATORY RESPONSES BASED ON PRIOR THI

Thermoregulatory responses were examined in treated cows between the months of February and May where THI ranged from 40 to 66 depending on when cows were assigned to treatment group. When examining the extent to which this prior THI (average THI 3 days before the initiation of treatments) was predictive of thermoregulatory responses, a treatment x THI x prior THI interaction was noted for RR ($P = 0.050$). Relevant for cows maintained at thermoneutral conditions, changes in RR were not influenced by a prior THI of 40, 56, 65, or 66 (P (linear) ≥ 0.5, P (quadratic) ≥ 0.6). However, exposure to a prior THI of 40 or 43 resulted in noticeable increases in RR when heat stress conditions were imposed ($RR = -0.5x^2 + 84.1x – 3522.4$; P (linear) < 0.001, P (quadratic) < 0.001; or $RR = -0.4x^2 + 62.9x – 2636.0$; P (linear) = 0.009, P (quadratic) = 0.013; Figure 6, Panel A). In contrast, RR of heat-stressed cows was slower to change when prior THI was 63 ($RR = -0.1x^2 + 20.2x – 742.3$; P (linear) = 0.226, P (quadratic) = 0.249; Figure 6, Panel A).

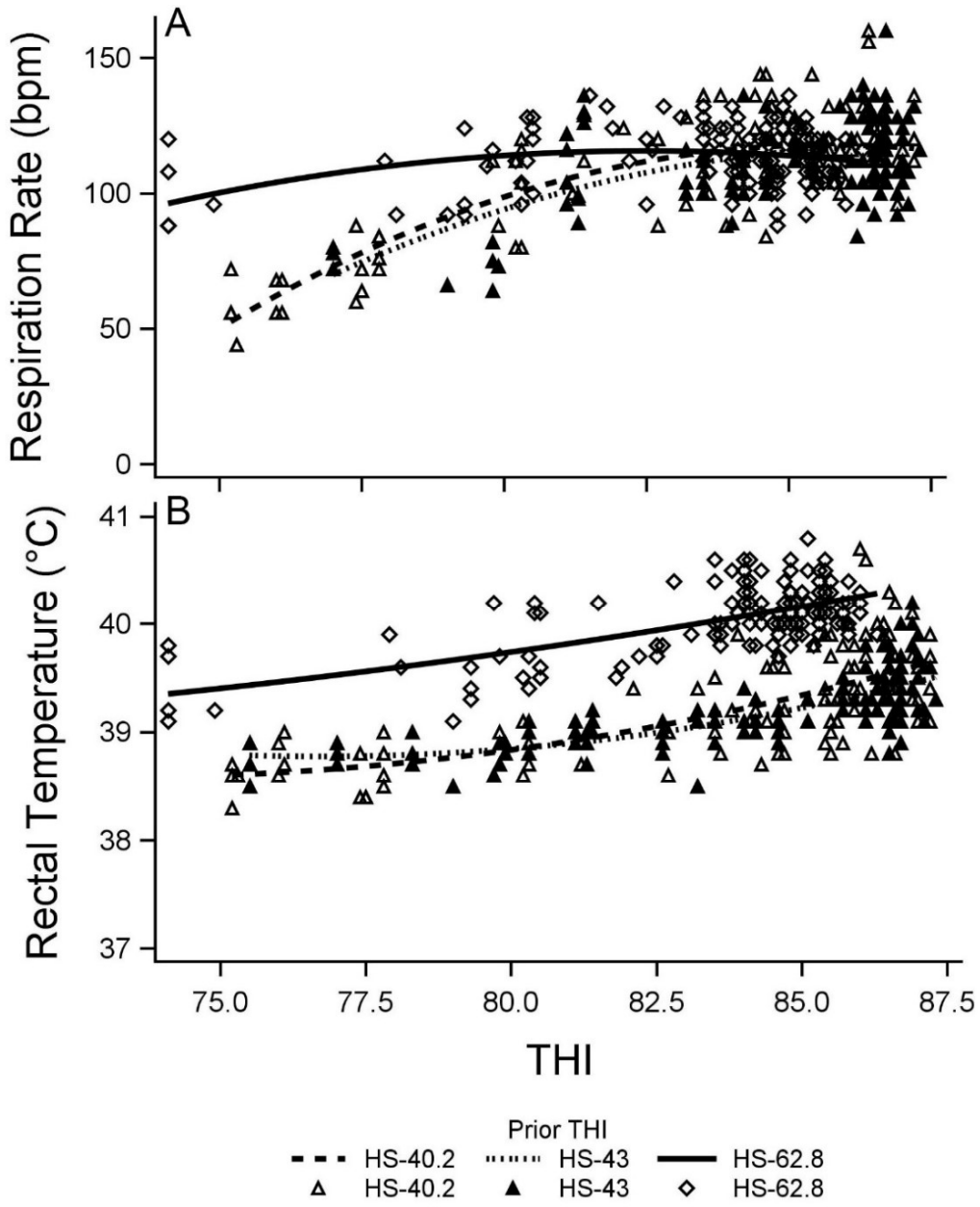

Figure 6. Changes in respiration rate (RR; Panel A) and rectal temperature (RT; Panel B) of heat-stressed cows as a function of THI. Thermoregulatory responses of cows exposed to an average THI of 40 (dashed line; open triangles), 43 (dotted line; closed triangles) or 63 (solid line; open diamonds) 3-days before the initiation of treatments are displayed. For RR, a treatment x THI x prior THI interaction was noted, and an interaction between treatment x prior THI for RT. bpm = breaths per minute THI = temperature-humidity index.

Heightened response in RR in our study with no prior exposure to heat stress is consistent to what Ferrazza et al. (2017) reported when examining thermoregulatory responses of non-lactating Holsteins in Brazil to acute versus chronic heat stress. Increases in RR were noted during acute and chronic conditions of heat stress; however, changes occurred at a slower rate during chronic exposure (Ferrazza et al., 2017) suggesting an adaptive response (reviewed by Nienaber & Hahn, 2007). Although the impact of previous day(s) on physiological responses has been documented (Ferrazza et al., 2017, Kabuga, 1992), the differential impact of prior THI in our study on RR was not entirely expected because 63 is less than the 68 expected to present mild heat stress conditions for the high producing dairy cow (Collier et al., 2009).

Changes in RT in our study differed depending on treatment and prior THI (treatment X prior THI interaction; $P < 0.001$). Average THI 3-days before the initiation of treatments did not influence changes in RT when cows were maintained at thermoneutral conditions ($P \geq 0.6$). In contrast, heat-stressed cows previously exposed to a prior THI of 40 and 43 consistently had a RT that was 0.8 ± 0.01°C lower than those previously exposed to a prior THI of 63 (slope = 0.04 ± 0.02°C per unit prior THI; $P < 0.001$; Figure 6; Panel B). The rate at which RT increased during heat stress treatment however, was similar regardless of prior THI (40, 42 or 63; slope = 0.03 ± 0.01, 0.04 ± 0.01, 0.05 ± 0.01°C per unit increase prior THI, respectively). Taken together, these results demonstrate that in instances where prior THI is low or approaching stressful conditions, magnitude of response (i.e., starting body temperature

and/or degree of hyperthermia) will likely differ. This may explain why body temperature of heat-stressed cows progressively increase during chronic heat stress. In support of this, Ferrazza et al. (2017) showed that during the first 3 days of exposure to acute heat stress (THI 88), RT were consistently lower than those obtained in cows that had been chronically exposed for 13 days. Interestingly, RT during chronic exposure was higher, and remained higher, even though rate of change was similar in cows exposed to an acute versus chronic heat stress. These along with our findings help to further document the impact of previous day(s) influences on RT (Kabuga, 1992), even in circumstances where prior THI is less than expected to induce mild heat stress.

3.3 RELATEDNESS OF AMBIENT TEMPERATURE AND RELATIVE HUMIDITY WITH THERMOREGULATORY RESPONSES

Dikmen and Hansen (2009) showed that THI explained much of the variation in RT in lactating cows maintained in a subtropical environment. Because THI was only slightly better at predicting RT than ambient temperature alone, models including current and prior ambient temperature (T_a) and relative humidity (RH) were also explored in our study to determine the extent to which thermoregulatory responses were appropriately indexed by THI as described for section 3.1. When this was performed, RR in heat-stressed cows was best predicted (R^2 = 0.90) by the equation $-0.3*(T_a^2) + 21.8*(T_a) - 0.4*(RH) – 1.7*(\text{prior } T_a) – 2.8*(\text{prior RH}) – 102.9$. Although RH was influential (P = 0.001), RR was most influenced by room T_a (P < 0.001) explaining why R^2 is almost identical to that obtained when examining relationship to THI in section 3.1. Rectal temperature was best predicted (R^2= 0.87) using the equation $0.07*(T_a)$ –

0.001*(RH) - 0.08*(prior T_a) - 0.2*(prior RH) + 46.9. Although inclusion of RH in the model was important to achieve a lower Akaike information criterion (AIC) value, changes in T_a ($P < 0.001$) and prior RH ($P = 0.062$) best explained changes in RT. The R^2 with these two variables (T_a and prior RH) in the model was 0.86; when only T_a was included the R^2 was 0.63. This supports the notion that relative humidity levels preceding heat stress exposure influences RT. Towards that end, Ferrazza et al. (2017) reported that the most important environmental variables influencing RT were previous day minimum and maximum temperature, previous day minimum THI and previous day minimum relative humidity, in that order.

For ambient temperature of the climate-controlled room to be more influential on thermoregulatory responses in lactating dairy cows is in agreement with Seath and Miller (1946) reporting that regression coefficients for ambient temperature were 13 to 15 times larger than those generated for percent humidity. Relative humidity during heat stress conditions in our study averaged 51.8 ± 0.8 and ranged from 30.0 to 81.8%; per each degree Celsius increase in ambient temperature, relative humidity decreased by 2.1% ($P < 0.001$). In a subtropical environment with higher levels of humidity, Dikmen and Hansen (2009) concluded that little is to be gained from using THI to predict RT because ambient temperature was so highly correlated with THI.

This is not to imply that relative humidity isn't influential on heat-induced increases in RT. In previous efforts aimed to defining ambient room conditions necessary to induce hyperthermia, incremental increases in ambient temperature from 22.0 to 40.0°C increased RR at a rate of 7.0 ± 3.2 bpm per hour ($P = 0.05$) but had

minimal impact on RT (P = 0.933; Figure 7). Heat-induced increases in RT were not noted until installation of misters which increased relative humidity from 18 to 67% (P =0.023 Figure 7). Similar findings have also been noted when using beef cows. Biggers et al. (1987) showed that a heat stress exposure of 33°C increased RR. However, changes in RT were only noted when humidity levels were 38 versus 27%.

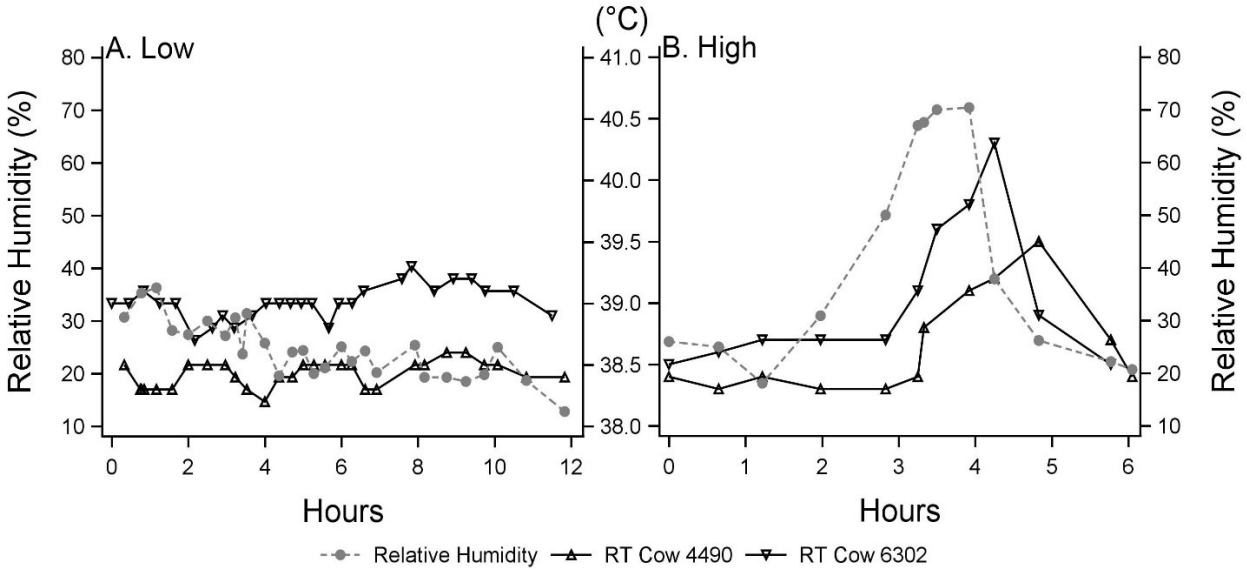

Figure 7. Changes in rectal temperatures of cow 4490 (solid black line; triangles facing up) and cow 6302 (solid black line; triangles facing down) during preliminary efforts to determine room conditions necessary for inducing hyperthermia. Ambient temperature ranged from 22.0 to 40.0°C both days with relative humidity (dotted grey line; circles) reaching a maximum of 36 (Panel A) or 67% (Panel B). Rectal temperatures did not change with low humidity (Panel A), but increased at a rate of 0.2 ± 0.06°C per hour when humidity was elevated (Panel B).

3.4 PREDICTING THERMOREGULATORY RESPONSES BASED ON SIZE AND GROWTH RATE OF THE OVULATORY FOLLICLE AND HORMONE CONCENTRATIONS PRIOR TO INITIATION OF TREATMENTS

Best models utilized in sections 3.1 & 3.2 were also used to examine the extent to which dominant follicle size and growth rate, along with levels of progesterone, estradiol, and luteinizing hormone and milk yield prior to the initiation of treatments were predictive of thermoregulatory responses. Follicle size and growth rate of the ovulatory follicle were similar in cows that were to be maintained at thermoneutral or heat stress conditions. Neither size nor growth rate of the ovulatory follicle were predictive of thermoregulatory responses of thermoneutral or heat-stressed cows. This was the case when THI or time was included in the statistical model ($P > 0.07$ and $P > 0.05$). It was also noted that there was no influence of milk yield, days in milk or parity on changes in RR ($P = 0.890$, $P = 0.613$, or $P = 0.456$, respectively) or RT ($P = 0.187$, $P = 0.370$ or $P = 0.224$, respectively). Lack of an effect of milk yield was not an expected outcome because of previous reports (Berman, 2005, Collier et al., 2009) but was similar to what had been previously reported by Dikmen and Hansen (2009).

Hormonal profiles of progesterone, estradiol, and LH in cows that were to be maintained at thermoneutral or heat stress conditions are shown in Figure 8. Effort to determine hormone levels prior to treatment initiation allowed for examining predictiveness on thermoregulatory responses after treatment initiation. For thermoneutral cows, none of the hormone levels at any of the times examined prior to treatment initiation were predictive of RR or changes in RT. This was the case when

THI or time were included in statistical models for RR (P4, P > 0.30 or P > 0.06; E2, P > 0.15 or P > 0.10; LH, P > 0.50 or P > 0.17) or RT (P4, P > 0.50 or P > 0.50, (E2, P > 0.20 or P > 0.50, LH, P > 0.30 or P > 0.40).

In contrast, LH levels (before luteolysis which was ~40 hours before acute heat stress exposure occurred) were predictive of heightened thermoregulatory responses of heat-stressed cows. When THI or time was included in the statistical model, RR increased by 11.0 ± 4.8 (P = 0.022) or 11.2 ± 5.0 bpm per ng/ml LH (P = 0.024). Rectal temperatures increased by 0.3 ± 0.2°C per ng/ml LH when THI (P = 0.031) and time (P = 0.024) were included in statistical models. Inability to find studies investigating relationship of LH levels during the luteal phase to impact thermoregulatory responses when heat stress occurs during the follicular phase of the estrous cycle poses a challenge for discussing the physiological significance of this finding. There may be something to this relationship because after cyclicity ceases, elevations in body temperature have been associated with higher LH levels in post-menopausal women (Casper et al., 1979, Fossum et al., 1995).

Progesterone levels at GnRH administration were predictive of reductions in thermoregulatory responses of heat-stressed cows. When THI or time was included in statistical model, RR decreased by 17.0 ± 7.7 (P = 0.027) or 16.8 ± 8.1 bpm per ng/ml P4 (P = 0.038). Rectal temperature decreased by 0.7 ± 0.2°C per ng/ml P4 with THI (P = 0.002) and time (P = 0.001) models. This finding was not expected and poses a challenge for discussion because cow (Lewis and Newman, 1984, Suthar et al., 2011) and human (Carpenter and Nunneley, 1988) derived data obtained during the luteal

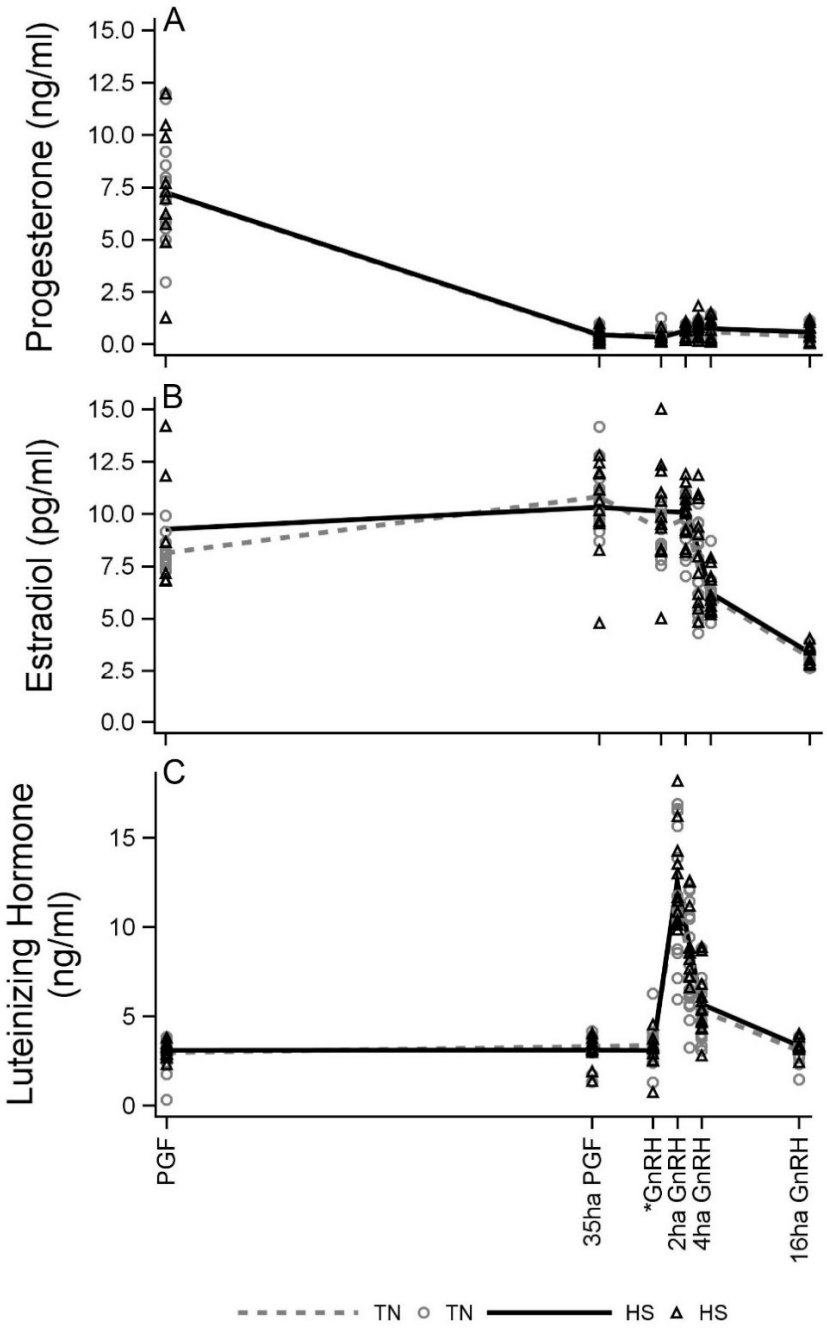

Figure 8. Hormone profiles of circulating progesterone (Panel A), estradiol (Panel B), and luteinizing hormone (LH; Panel C) from cows exposed to thermoneutral (TN; dashed lines, circles) or heat stress (HS; solid lines, triangles) conditions during the 12-hour treatment period. Blood samples were collected at the time of initial $PGF_{2\alpha}$ administration (PGF), 35 h after $PGF_{2\alpha}$ and CIDR removal (35ha PGF), at GnRH administration (* = treatment initiated), 2, 3, 4, and 16 hours after GnRH administration.

phase suggest a pyrogenic effect of progesterone. For instance, Sakatani et al. (2016) showed that vaginal temperatures of Japanese black cows decreased by up to 0.3°C after administration of a luteolytic dose of $PGF_{2\alpha}$; insertion of a CIDR impregnated with progesterone prevented decreases in vaginal temperatures.

Luteolysis (i.e., regression of the corpus luteum) is important for initiation of the follicular phase; progesterone levels decrease markedly thereafter to less than 0.5 ng/ml (Wiltbank et al., 2014). Progressive increases in follicle-derived estradiol levels provide the endocrine environment important for GnRH-induced LH surge. Hormonal differences occurring during this major and important endocrine transition in the estrous cycle may explain why relationship of progesterone and RT in our study differs from that described by others during the luteal phase. Because decreases in body temperature occur after the LH surge and have been associated with estradiol levels in humans (Buxton and Atkinson, 1948, Tankersley et al., 1992), the possibility for high levels at the time of GnRH administration to be influential cannot be precluded. In support of this, Bayliss and Millhorn (1992) suggested that respiratory responses to P4 are dependent on E2 mediated neurons in the hypothalamus.

3.5 THERMOREGULATORY RESPONSES AFTER SUDDEN COOLING OF HS COWS

After a total of 12 hours, heat stressed cows were suddenly cooled; thermoregulatory responses were examined thereafter. Sudden cooling decreased RR at a rate of 0.6 ± 0.1 bpm ($P < 0.001$). Rectal temperatures decreased at a rate of 0.02 ± 0.002°C per min ($P < 0.001$). After a total of 45 min, RR and RT of heat-stressed

versus thermoneutral cows were similar (59.0 bpm and 38.6°C versus 43.2 bpm and 38.2°C). Effectiveness of sudden cooling in the manner described herein highlights how quickly dairy cows experiencing moderate to severe hyperthermia may be cooled. Although experimentally-induced, efficacy of short-term on farm cooling strategies utilizing evaporate cooling has been demonstrated. Valtorta and Gallardo (2004), with the use of fans and sprinklers 20 to 30 min before milking, reduced RR and RT in heat-stressed cows by 17 bpm and 0.3°C. Relevant for pasture-based cows, benefits of using shade and sprinklers 90 min before milking were greater when THI > 69 (Kendall et al., 2007).

It is interesting to note that thermoregulatory response of individual cows to heat stress may differ. In our study, two had to be strategically cooled to prevent RT exceeding 41°C. Use of a small fan aimed at shoulder and applying a water mist decreased RR from 124 to 108 bpm and RT from 40.6 to 40.0°C, within 36 min (Figure 9). Cooling in this manner was stopped after 1 hour. When heat-induced elevations in RT approached 41°C again, strategic cooling was implemented for a second time with efforts continuing until the end of heat stress treatment period. Although RR remained constant (116 bpm), strategic cooling reduced RT from 40.8 to 40.2°C within 25 min and prevented any further heat-induced increases. Whatever the approach, the need to strategically cool hyperthermic dairy cows is real to maximize production (Chen et al., 2016, Perano et al., 2015) and reproductive (Ealy et al., 1994, Moghaddam et al., 2009) efficiency and especially during extreme environments where heat stroke may be problematic (Bhojani et al., 2015).

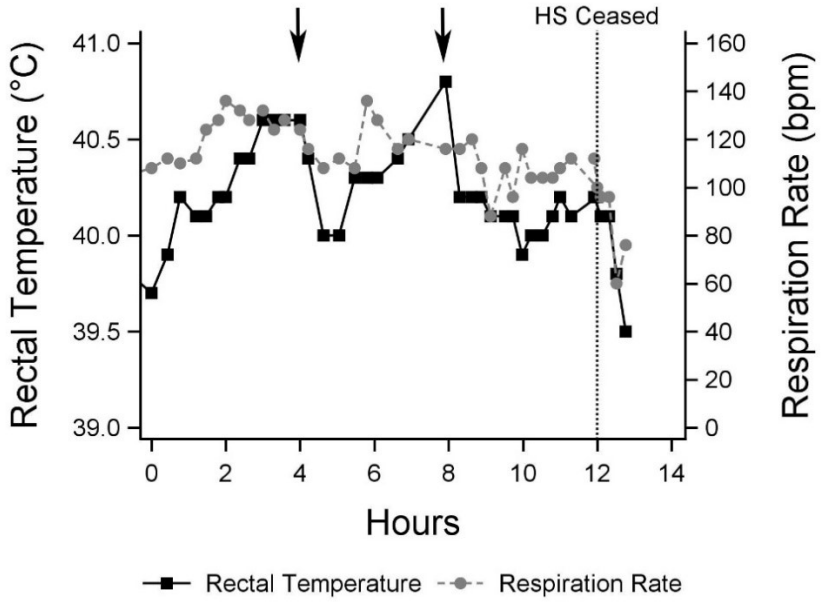

Figure 9. Representative data for respiration rate (dotted line, circles) and rectal temperature (solid line, squares) of heat-stressed cow # 4576 during strategic evaporative cooling (arrows) and sudden cooling at 12 hours (vertical dashed line). First strategic cooling was initiated at 4 hours and reduced RR (124 to 108 bpm) and RT (40.6 to 40.0°C) within 36 min. A second strategic cooling at ~8 hours did not change RR (116) but reduced RT (40.8 to 40.2°C) within 25 min. At 12 hours, sudden cooling effectively reduced RR and RT to thermoneutral levels within 45 min. HS = heat stress bpm = breaths per minute

CHAPTER FOUR

SUMMARY AND CONCLUSIONS

Assessing heat load of periovulatory dairy cows after an acute heat stress event is important because during this time period the oocyte is highly susceptible to hyperthermia. To that end, exposure to incremental increases in THI that may occur during the onset of an unexpected heat wave increased RR within 30 to 40 min of initial exposure. Differences in RT temperature were noted within 110 min of steadily increasing THI. The speed and magnitude of resultant changes in heat load not only demonstrate feasibility of the approach described herein for use as an in vivo model to study the impact of hyperthermia on different components important for fertility but help to re-emphasize the need to implement on farm cooling strategies sooner than later if milk yield and fertility are to be maximized. Moreover, additional efforts to document the rapidness for which hyperthermic cows may be cooled provide foundational steps towards the development or modification of existing strategies aimed at mitigating the negative impact of heat stress when occurring at or near the time of estrus.

Interestingly, the magnitude of thermoregulatory responses differed in our study when conditions before the acute heat stress event (prior THI) was less than that expected to pose stressful conditions for the lactating dairy cow (i.e., heat-stressed cows coming from a prior THI in the low 40s had RT that were 0.8 ± 0.01°C lower than those coming from a prior THI in the low 60s). Because the rate of change was not affected, previous ambient conditions are more influential on the degree of hyperthermia achieved in response to an acute heat stress event. Heat stress effects are dependent

on severity and duration of hyperthermia; these data suggest the need to be mindful of the previous days' conditions when intent is to make decisions about when to implement on farm cooling strategies.

In circumstances where THI steadily increased from the mid-70s to mid-80s in our study, increases in RR were positively related to increases in RT. Because heat-induced changes in RR were noticeable soon after incremental changes in THI occurred and were mostly affected by ambient conditions during the acute heat stress event, effort was also put forth to determine the extent to which RR, THI and ambient temperature recorded in our study was predictive of RT changes in lactating cows acutely exposed to an otherwise unexpected heat stress (Table 1). To that end, when the prior THI is set at 40, a respiration rate of 80 is predictive of a RT of 38.9 C. When the prior THI is set at 60, a respiration rate of 80 is predictive of a RT of 39.2 C. Although experimentally-derived, others have reported that 80 bpm corresponded to a RT of 39.0 C (Beatty et al., 2006). Moreover, a THI of 80 in our study is predictive of a RT of ≥ 39.0 C which is similar to what was presented by Dikmen and Hansen (2009) using data from lactating cows managed in a subtropical environment. Taken together, these data document the usefulness of RR, THI and ambient temperature to predict degree of hyperthermia in animals experiencing an acute heat stress event. Information conveyed in a manner to highlight possible differences in outcomes based on previous conditions, regardless of which variable is to be used to predict thermal load, support the need for producers to be more aggressive with strategic cooling, especially when previous days have been approaching levels expected to be stressful.

Table 1: Use of respiration rate, THI and ambient temperature to predict rectal temperature of heat-stressed lactating cows when coming from prior conditions where the THI was 40 or 60.

	Predicting rectal temperatures using respiration rate		Predicting rectal temperatures using THI		Predicting rectal temperatures using ambient temperature	
*Prior THI	Respiration Rate (bpm)	Rectal Temperature (°C)	THI	Rectal Temperature (°C)	Ambient Temperature (°C / °F)	Rectal Temperature (°C)
40	40	38.3	70	38.5	24 / 75	38.5
40	80	38.9	80	39.0	29 / 85	38.9
40	120	39.3	85	39.2	35 / 95	39.4
40	150	39.6	90	39.5	38 / 100	39.6
60	40	38.1	70	38.6	24/ 75	38.7
60	80	39.2	80	39.6	29 / 85	39.4
60	120	40.0	85	40.0	35 / 95	40.1
60	150	40.5	90	40.5	38 / 100	40.4

* Prior THI: Average of ambient conditions of the preceding 3 days prior to acute heat stress exposure

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