Animal factors associated with core body temperature of nonlactating dairy cows during summer^{1,2}

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ABSTRACT: The primary objectives of the current study were to investigate animal factors associated with core body temperature (CBT) and to determine the time of the day in which CBT assessment best describes the magnitude of hyperthermia throughout the day of heat-stressed dry cows. The secondary objective was to develop a predictive model for CBT of dry cows. Nonlactating Holstein cows ($n = 105$) with 250 to 260 d of gestation from 2 commercial dairies were enrolled in the study during summer. During 4 consecutive days, CBT from all cows was recorded in 5-min intervals and average CBT was calculated for each cow. In addition, mean, maximum, minimum, and standard deviation of daily CBT were calculated and using these measures cows were categorized as having high temperature (HT) or low temperature (LT) based on the median values. Cows carrying twins had greater ($P < 0.01$) CBT than cows bearing singletons (39.07 ± 0.07 vs. 38.84 ± 0.03 °C). Average CBT decreased ($P < 0.01$) 0.015 ± 0.004 °C for each 1-d increase in gestation length. Cows in Dairy A tended ($P = 0.09$) to have lower CBT than cows in Dairy B (38.91 ± 0.04 vs. 39.00 ± 0.06 °C). Season of birth, lactation number, body condition

score category, previous projected 305-d mature equivalent milk yield, days in milk at dry-off, days after dry-off at enrollment, days of gestation at enrollment, and calf sex were not associated ($P > 0.12$) with CBT. Principal component analyses showed that 71% of the variance of CBT was explained by the first principal component alone, which was correlated with mean CBT ($r = 0.99$). Among all time points assessed, CBT recorded at 2215 h had the highest correlation with the first principal component ($r = 0.93$). The best agreement for classifying cows as HT or LT was between mean daily CBT and assessment at 2215 h ($k = 0.73$). The model that resulted in best predictivity (0.56) of average CBT included the following variables: dairy, gestation length, and twinning. In conclusion, findings from the present study suggest that CBT assessed between 250 and 260 d of gestation is negatively associated with gestation length and cows bearing twins have greater CBT than singletons. Our results indicate that the best time of the day to evaluate severity of heat stress in dry cows is 2215 h. Predictive models for CBT of dry cows should include dairy, twinning, and gestation length.

Key words: core body temperature, dairy cow, gestation length, heat stress, twinning

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INTRODUCTION

Dairy cows exposed to heat stress during the dry period have increased core body temperature (CBT) and reduced milk yield in the subsequent lactation (Collier et al., 2006). Although it has been demonstrated that the use of cooling systems reduces CBT of dry cows (do Amaral et al., 2009), association between thermoregulation of dry cows and animal-related factors, such as body condition score (BCS) and parity, is unknown. Body condition score, which is used to estimate energy requirement for dairy cattle (NRC, 2001), may affect heat dissipation. Furthermore, parity has been shown to be associated with CBT in early lactation cows (Suthar et al., 2011) and could potentially affect CBT of dry cows. Therefore, it is possible that BCS and lactation number, among other animal factors, are associated with thermoregulation capacity of dairy cows during the dry period. In most studies that evaluated the effects of heat stress during the dry period, CBT was assessed at 0730 to 0800 or 1400 to 1500 h (Adin et al., 2009; Tao et al., 2012; Karimi et al., 2015). Nonetheless, given the daily rhythm of CBT in dairy cows (Scanavez et al., 2016), it is not clear whether measurements obtained at those time points accurately represent severity of heat stress that dry cows undergo throughout the day.

We hypothesized that 1) BCS and lactation number, along with other factors, would be associated with CBT during the dry period and 2) time of the day when CBT is assessed would interfere with heat stress severity estimation in dry cows. Our primary objectives were to investigate which factors are associated with CBT in Holstein dry cows and to determine the time of the day in which CBT assessment best describes the magnitude of hyperthermia that heat-stressed dry cows undergo throughout the day. Our secondary objective was to develop and evaluate effectiveness of a predictive model for dry cows' CBT.

MATERIALS AND METHODS

Data of animals obtained from a previous experiment (Scanavez et al., 2017) were used in the present study. All procedures reported herein were conducted under protocols approved by the Kansas State University's Institutional Animal Care and Use Committee (IACUC).

Animals, Housing, and Feeding

The experiment was conducted in 2 commercial dairies located in southwest Kansas. Dry Holstein

cows with 250 to 260 d of gestation in their first, second, or third lactation with locomotion score of <3 (Sprecher et al., 1997) and 4 functioning quarters at dry-off were eligible to be enrolled in the study. Standard operating procedures at both dairies consisted of drying-off cows at 220 d of gestation to target a dry period length of approximately 60 d. Thus, cows were already dry at enrollment. On the day of enrollment, a list of eligible cows was generated from the on-farm management software (DairyComp 305, Valley Ag Software, Tulare, CA). Cows were blocked by lactation number and randomly selected from the list. A total of 105 cows (first-lactation = 37; second-lactation = 37; and third-lactation = 31) were enrolled in the study from June 2014 through July 2014. Four replicates were performed in Dairy A ($n = 90$), and 1 replicate in Dairy B ($n = 15$). Body condition score was assessed by 1 person upon enrollment using a scale from 1 (emaciated) to 5 (obese) using 0.25-point increments (Ferguson et al., 1994). For statistical analysis, cows were classified into low BCS (LBSC; ≤ 3.5) or high BCS (HBSC; > 3.5).

Cows were in the far-off pen at enrollment and during the entire period that CBT was assessed. Cows were moved to the close-up pen after 260 d of gestation. In both dairies, dry cows were housed in dry-lot pens with shaded areas and were fed once daily with a total mixed ration formulated to meet or exceed nutrient requirements (NRC, 2001). In the close-up pen, cows were fed a ration containing acidogenic salts.

CBT and Temperature–Humidity Index Assessment

At enrollment, 250 to 260 d of gestation, a calibrated temperature logger (iButton DS1922L, Embedded Data Systems, Lawrenceburg, KY) fitted in a blank controlled internal drug release device (CIDR) was inserted into the vagina to collect CBT measurements. Loggers were calibrated for precision of ± 0.13 °C (Thermodata Corporation, Milwaukee, WI) and were fixed in the CIDR device using a moisture-resistant flexible plastic (Parafilm, Oshkosh, WI) and silicone aquarium sealant (Loctite, Henkel Corporation, Rocky Hill, CT). CBT was assessed every 5 min during 4 consecutive days. Time of CIDR insertion and removal was documented. After CIDR removal, measurements recorded by loggers were downloaded to a computer. To ensure accuracy of temperature readings, body temperature measurements collected within 1 h after CIDR insertion were excluded.

During the time CBT was being monitored, ambient temperature and humidity were recorded

every 5 min from the pens where cows were housed using loggers (HOBO U23 Pro v2; Onset Computer Corp., Pocasset, MA) fixed under the shade structure at approximately 3 m above ground level. The following equation was used to calculate temperature–humidity index (THI): $THI = T - (0.55 - 0.55 RH/100) \times (T - 58)$, where T and RH are dry bulb temperature (°F) and relative humidity (%), respectively (NOAA, 1976).

Statistical Analyses

Descriptive statistics were generated using PROC MEANS, PROC FREQ, and PROC HPMIXED procedures of SAS 9.4 (SAS Inst. Inc., Cary, NC). Average CBT of all readings was calculated for each cow to explore its association with various factors. Multivariable linear regression analysis was performed using the PROC GLIMIX procedure of SAS using average CBT as the dependent variable. Fixed effects tested in the model included dairy, lactation number at enrollment (first, second, or third), body condition score category (BCSC; LBSC vs. HBSC), previous projected 305-d mature equivalent milk yield (P305ME), days in milk at dry-off (DIMD), days after dry-off at enrollment (DDE), days of gestation at enrollment (DGE), gestation length, twinning (yes vs. no), calf sex (male vs. female), and cow's season of birth (winter, spring, summer, or fall). Season of birth was defined according to the month that cows were born (winter: December to February; spring: March to May; summer: June to August; and fall: September to November). Interactions between dairy and lactation number, dairy and BCSC, lactation number and gestation length, and twinning and gestation length were also explored. For a second series of regression analysis, P305ME, DIMD, DDE, and DGE were transformed into categorical variables based on tertiles (T). These categorical variables (P305ME T, DIMD T, DDE T, and DGE T) replaced their corresponding continuous fixed effects in the initial model. All models included all fixed effects and interactions aforementioned. Variables and interactions with $P < 0.10$ were excluded from the initial model by backward elimination. Statistical significance was defined as $P < 0.05$ and tendencies as $0.05 \leq P < 0.10$.

Principal component analysis was used to reduce dimensionality of CBT data. Principal components were calculated by singular value decomposition of the centered and scaled data matrix, using the prcomp function in the R package Stats (version 3.3.0; R Core Team, 2016), and eigenvectors

of the variance–covariance matrix of temperatures. Using the R package Stats, a K-means test was performed in the first 2 principal components (PC1 and PC2) to verify the presence of clusters in the data. Pearson correlation coefficients (r) were calculated between single-measurements of CBT and PC1 using the R package Stats.

Mean, maximum, minimum, and standard deviation of daily CBT were calculated for each cow. Using these measures, median values from the population of cows were calculated. Cows with values less than the calculated median were classified as low temperature (LT), and cows with values greater than or equal to the calculated median were classified as having high temperature (HT). Cohen's Kappa reliability coefficients (κ) were calculated using the R package Psych (Revelle, 2014) to evaluate agreement in classifying cows as HT or LT by using different CBT measures (e.g., single-measurement of CBT vs. mean daily CBT).

A linear model was fit using the lm function in the R package Stats to predict CBT based on observed parameters. Fixed effects and its interactions explored in the linear regressions were tested in the prediction models. In addition to lactation number, parity (primiparous vs. multiparous) was further explored in the analyses. To evaluate the quality of the model, predictivity was calculated. Quality of predictions was defined by the correlation between observed and estimated average individual temperatures for the testing population. Predictivity was calculated by cross-validation, which was performed 500 times for each model, randomly selecting 79 animals for training the model and 25 animals for evaluating the quality of predictions. Calculation of predictivity was only performed for the testing population.

RESULTS

One primiparous cow lost the temperature logger and was excluded from the study. The final study population consisted of 104 cows ($n = 36, 37$, and 31 , for first-, second-, and third-lactation cows, respectively).

Parturition and Ambient Descriptive Data

Forty-one cows (39.4%) had singleton male calves, 54 (51.9%) had singleton female calves, and 9 cows (8.7%) were bearing twins. Average ($\pm$ SD) CBT was $38.83 (\pm 0.23) ^\circ\text{C}$ (Table 1). Previous projected 305-d mature equivalent milk yield, BCS, and gestation length were $12,976 (\pm 1,780)$ kg, $3.60 (\pm 0.47)$,

and $277 (\pm 5.0)$ d, respectively. Proportion of cows born during winter, spring, summer, and fall seasons was 5.8%, 15.4%, 52.9%, and 26.0%, respectively.

During the period of enrollment, average daily THI was 73.4 for Dairy A and 74.2 for Dairy B. Additionally, average minimum and maximum THI were 63.9 and 81.5 for Dairy A, and 64.8 and 80.6 for Dairy B, respectively. Daily pattern of CBT for cows classified as having LT or HT is depicted in Figure 1. Further prepartum descriptive data comparing cows with LT or HT, and THI data for each replicate are reported in Scanavez et al. (2017). As expected, average daily CBT was greater ($P < 0.01$) for HT cows than LT cows (39.01 ± 0.03 vs. 38.72 ± 0.03 °C), given the criterion used to categorize cows as having HT or LT.

Factors Associated with CBT

In the first model, cows carrying twins had greater ($P < 0.01$) CBT compared with

Table 1. Descriptive data of nonlactating Holstein cows ($n = 104$) used in the study

Item	Average (SD)
Core body temperature, °C	38.83 (0.23)
Lactation number at enrollment	1.95 (0.81)
Previous projected 305-d mature equivalent milk yield, kg	12,976 (1,780)
Body condition score ¹	3.60 (0.47)
Days after calving at dry-off	325.6 (48.2)
Days after dry-off at enrollment	31.4 (11.1)
Days of gestation at enrollment	254.0 (2.8)
Gestation length, d	277.0 (5.0)

¹Body condition score was assessed by 1 person on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) using 0.25-point increments.

cows bearing singletons (39.07 ± 0.07 vs. 38.84 ± 0.03 °C). Gestation length was negatively associated ($P < 0.01$) with CBT. For each 1-d increase in gestation length, average CBT decreased 0.015 ± 0.004 °C. The interaction between twinning and gestation length was not ($P = 0.50$) associated with CBT. Cows in Dairy A tended ($P = 0.09$) to have lower CBT than cows in Dairy B (38.91 ± 0.04 vs. 39.00 ± 0.06 °C). Season of birth, lactation number, BCSC, P305ME, DIMD, DDE, DGE, and calf sex were not ($P > 0.12$) associated with CBT. The interactions between dairy and lactation number, dairy and BCSC, and lactation number and gestation length were not ($P > 0.12$) significant in the model.

In the second model, in which P305ME, DIMD, DDE, and DGE were transformed into categorical variables, similar findings were observed. Twinning and gestation length were ($P < 0.01$) and dairy tended ($P = 0.09$) to be associated with CBT. Other variables or interactions were not ($P > 0.12$) significant in the model.

Principal Component Analysis and Reliability of a Single Measurement of CBT

The first principal component (PC1) explained 71% of the variance of CBT, and the second principal component (PC2) explained 13% (Table 2). Less than 17% of CBT variance was explained by the combination of all other principal components. Moreover, the *K*-means test showed that clustering the observations in 2 groups accounted for only 45.9% of the total variance in the data, denoting that no groups of cows explained variance in CBT. Correlation between PC1 and mean daily CBT was 0.99. Among the 288 time points assessed, CBT

Figure 1. Pattern of CBT measured in 5-min intervals during 4 consecutive days in 104 nonlactating dairy cows. Error bars represent SEM. Lines represent cows with average CBT greater (solid line—HT) or lower (dotted line—LT) than the median value within parity. Average daily CBT was greater ($P < 0.01$) for HT cows than LT cows (39.01 ± 0.03 vs. 38.72 ± 0.03 °C).

recorded at 2215 h had the highest correlation ($r = 0.93$) with PC1 (Figure 2).

Kappa statistics indicated substantial agreement ($k = 0.73$) between mean daily CBT and assessment at 2215 h when classifying cows into LT or HT (Table 3). Classification based on maximum, minimum, or standard deviation of daily CBT resulted in suboptimal agreement ($k < 0.43$) with assessment at 2215 h (Table 3). Agreement between mean daily CBT and single measurements across the day to classify cows into LT or HT is depicted in Figure 3. Among all the time points evaluated, CBT recorded at 0715 h resulted in the poorest agreement ($k = 0.23$).

Predictive Models of CBT

Linear models tested for predictivity of CBT are presented in Table 4. Variables with best predictivity of average daily CBT were gestation length and twinning (0.50 and 0.47, respectively). BCSC, lactation number, and parity were poor predictors of average daily CBT (predictivity < 0.21). Transforming projected milk yield and days after dry-off into categorical variables (tertiles) provided better adjustment to the models and increased predictivity by 0.07 and 0.08, respectively. Inclusion of interactions increased predictivity only marginally (data not shown). The model with best predictivity (0.56) consisted of including

dairy ($P = 0.09$), gestation length ($P = 0.01$), and twinning ($P = 0.01$). Estimated average daily CBT based on the model with best predictivity is illustrated in Figure 4.

DISCUSSION

Besides having a major economic impact to the dairy industry (St-Pierre et al., 2003), heat stress considerably affects well-being of dairy cattle (Polsky and von Keyserlingk, 2017). Hyperthermia caused by high ambient temperature induces metabolic imbalances, predisposing cows to health disorders. Considering that most health disorders in dairy cows are diagnosed early postpartum (LeBlanc et al., 2006), research regarding the effects of heat stress during the dry period on the periparturient cow is fundamental to improve animal welfare and health during summer months. In a recent study conducted by our research group, we demonstrated an association between CBT during the dry period and postpartum health in the subsequent lactation (Scanavez et al., 2017). Nonetheless, it is unknown which factors are associated with CBT during the dry period. Identifying some of these factors may increase our understanding of underlying processes involved in temperature regulation of nonlactating cows during periods of heat stress. In addition, determining which factors are associated with CBT may contribute to the development of strategies to

Table 2. Principal component (PC) analysis coefficients, standard deviation of PC, and cumulative proportion of variance from PC1 to PC8 of core body temperature

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of variance	0.71	0.13	0.04	0.02	0.01	0.01	0.01	0.01
Cumulative proportion of variance	0.71	0.84	0.88	0.90	0.92	0.93	0.94	0.94
Standard deviation	14.29	6.15	3.57	2.20	2.07	1.75	1.59	1.48

Figure 2. Pearson correlation coefficients (r) between single measurements of CBT and the principal component that explained the greatest proportion of the variance in CBT (PC1) of nonlactating Holstein cows. Among the 288 single measurements, CBT assessed at 2215 h had the highest correlation with PC1 ($r = 0.93$).

manage subpopulations of cows more prone to be affected by heat stress.

Various methods have been used to evaluate magnitude of heat stress in dry cows. The most common methods consist of assessing CBT rectally (do Amaral et al., 2011; Karimi et al., 2015) or vaginally (Chen et al., 2016; Scanavez et al., 2017). Although rectal and vaginal temperatures are highly correlated (Suthar et al., 2013), vaginal temperature assessments enable monitoring of CBT in multiple time points across the day (e.g., 1 measurement every 1 min). In fact, one of the objectives of the current study was to investigate the ideal time of the day to assess CBT in dry cows. Moreover, the authors explored other potential animal factors associated with CBT during the dry period. To increase its external validity, the present study was conducted at large commercial dairies, which prevented assessment of certain animal factors of interest, such as body weight and DMI.

We hypothesized that CBT would be associated with lactation number because previous experiments have shown differences in CBT between lactating primiparous and multiparous cows under

conditions of heat stress. In studies conducted with early-lactation Holstein cows, it was reported that primiparous cows have greater temperature than multiparous cows (Suthar et al., 2011; Wenz et al., 2011). Wenz et al. (2011), however, did not exclude cows with metabolic and infectious disorders, limiting major conclusions because health disorders may affect CBT. Nonetheless, Suthar et al. (2011) reported similar findings in an experiment that excluded cows diagnosed with clinical disorders. Even though several studies recorded CBT in mid-lactation cows, comparisons between primiparous and multiparous CBT have not been described in the literature. One could speculate that midlactation multiparous cows likely have greater CBT than primiparous cows due to their increased feed intake and milk yield, but studies are necessary to test this hypothesis. In a study conducted with beef cows focusing on predicting time of parturition based on CBT (Aoki et al., 2005), no differences were observed between parities. Aoki et al., however, did not include primiparous cows in the experiment. Our results suggest that CBT does not differ between primiparous and multiparous cows in a nonlactating state under conditions of heat stress. To the best of our knowledge, no other studies have evaluated whether lactation number is associated with CBT during the dry period. Understanding whether number of lactation is associated with CBT of dry cows is critical to avoid bias when designing and interpreting research experiments and field trials.

Similar to lactation number, BCS was not associated with CBT of dry cows. Because dairy cows with high BCS have increased caloric demands for maintenance (NRC, 2001), we hypothesized that

Table 3. Cohen's Kappa reliability coefficients for classifying non-lactating Holstein cows into low- or high-core body temperature (CBT) using measurements at 2215 h and mean, maximum, minimum, or standard deviation of daily CBT

Item	Kappa
Mean daily CBT	0.73
Maximum daily CBT	0.42
Minimum daily CBT	0.27
Standard deviation of daily CBT	0.23

Figure 3. Agreement between using a single measurement of CBT and mean daily CBT for classifying cows into LT or HT groups. Greatest and poorest agreement between single measurements and mean daily CBT were at 2215 h ($\kappa = 0.73$) and 0715 h ($\kappa = 0.23$), respectively. Kappa coefficient agreement (Landis and Koch, 1977): <0.00 = poor, 0.00 – 0.20 = slight, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial, and 0.80 – 1.00 = almost perfect agreement.

Table 4. Predictivity mean and standard deviation of models to predict average core body temperature over 500 cross-validation samples

Model	Predictivity	Standard deviation
Dairy	0.26	0.19
Lactation number	0.14	0.14
Parity	0.21	0.19
BCSC	-0.11	0.13
P305ME	0.09	0.20
P305ME T	0.16	0.17
DIMD	0.07	0.13
DIMD T	-0.17	0.14
DDE	0.15	0.15
DDE T	0.23	0.19
DGE	-0.10	0.15
DGE T	-0.05	0.16
Gestation length	0.50	0.12
Twins	0.47	0.20
Calf sex	0.18	0.18
Season	0.14	0.16
Dairy + parity	0.31	0.20
Parity + gestation length	0.48	0.13
Dairy + parity + gestation length	0.51	0.12
Dairy + parity + DIMD + gestation length	0.51	0.12
Gestation length + P305ME T + DDE T	0.52	0.12
Gestation length + DDE T	0.52	0.11
Dairy + gestation length + twins	0.56	0.14

The following variables were tested: dairy, lactation number, parity, body condition score category¹ (BCSC), previous projected mature milk yield (P305ME), days in milk at dry-off (DIMD), days after dry-off at enrollment (DDE), days of gestation at enrollment (DGE), gestation length, twin pregnancy (twins), calf sex, and cow's season of birth (season). Continuous variables not significant in the linear regression model (P305ME, DIMD, DDE, and DGE) were transformed into categorical variables based on tertiles (T). Models with more than 1 variable and predictivity below 0.31 were omitted.

¹Body condition score was assessed by 1 person on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) using 0.25-point increments. Cows were classified into low BCS (LBCS, ≤ 3.5) or high BCS (HBCS, > 3.5).

BCS would influence thermoregulation. Indeed, in humans, increased body mass index is associated with increased body temperature (Obermeyer et al., 2017). It is possible that different DMI preceding parturition of high- and low-BCS cows influenced CBT. Cows with greater BCS have more pronounced decrease in DMI before calving (Hayirli et al., 2002; Grummer et al., 2004), which may have resulted in similar CBT between high- and low-BCS cows via reduced caloric intake.

Recent studies showed evidence that season of conception may be linked to thermal homeostasis and productive and reproductive performance during adulthood in dairy cows (Ahmed et al., 2017; Pinedo and de Vries, 2017). Calves that experienced in utero heat stress in the last 6 wk of gestation had better capacity of thermoregulation at maturity (Ahmed et al., 2017). In addition, cows exposed to heat stress in utero presented reduced CBT in response to a heat stress challenge compared with cows that did not experience heat stress in utero. Based on these findings, we investigated the association between season of cow's birth and CBT. We classified cows based on season of birth as a proxy for heat stress exposure in utero. Our results contrast with previous findings because there was no association between season of birth and CBT. Considering that cows not exposed to heat stress in utero are more likely to complete first lactation (Monteiro et al., 2016), the authors recognize the possibility of selection bias, given the enrollment criteria used in the present study. In addition, a small number of cows enrolled in the study were born during winter and spring, which resulted in an uneven distribution of cows born per season, limiting even further our inferences.

Herds used in this study were selected based on similar characteristics, such as breed, milk yield,

Figure 4. Estimated average daily CBT based on the final predictivity model. Fixed effects included in the model were dairy ($P = 0.09$), gestation length ($P < 0.01$), and pregnancy type ($P < 0.01$). Black lines represent cows from Dairy A carrying singleton (solid line) or twin (dashed line) pregnancies. Gray lines represent cows from Dairy B carrying singleton (solid line) or twin (dashed line) pregnancies.

housing system for dry cows, and geographic proximity. Despite a careful herd selection, dairy tended to be associated with CBT. Given that heat tolerance has a genetic component (Garner et al., 2016), differences in genetics may be the reason for cows from Dairy B to have greater CBT than cows from Dairy A. Regardless of geographic proximity of herds, differences in exposure to heat stress should not be neglected. In the experiment that used the same cows as the current study (Scanavez et al., 2017), no difference in CBT was detected between Dairy A and Dairy B. It is likely that classifying cows as having high or low CBT reduced the statistical power of the analysis. In the present study, CBT was included as a continuous variable in the analyses, which increased statistical power to detect differences.

Our results indicate that CBT is negatively associated with gestation length. Reduced gestation length has previously been reported in cows exposed to heat stress during the dry period (Adin et al., 2009; Tao et al., 2012). Nonetheless, such effects were observed in noncooled dry cows compared with cows that had access to an evaporative cooling system, whereas in the current study, all cows were managed under similar environmental conditions. Unfortunately, causality cannot be established with the current dataset. It is intriguing to speculate whether CBT affects gestation length, or gestation length alters CBT. It is well documented that CBT of beef and dairy cows is reduced in the last 24 to 48 h before parturition (Lammoglia et al., 1997; Aoki et al., 2005; Burfeind et al., 2011). Nevertheless, little is known about daily rhythm of body temperature 2 to 3 wk before calving. Calf sex, which is associated with gestation length, was another factor explored in this study to investigate whether it influences CBT. Although gestation length of cows pregnant with female calves is approximately 1 d shorter than cows pregnant with male calves (Silva et al., 1992; Vieira-Neto et al., 2017), calf sex was not associated with CBT. In agreement with our findings, Aoki et al. (2005) did not detect differences in CBT between cows calving male or female calves in the last 6 d of gestation. In contrast, Lammoglia et al. (1997) reported that CBT was approximately 0.3 °C greater from 2 to 6 d before parturition for cows carrying male calves compared with cows carrying female calves. Nevertheless, a type I error should not be discarded in the latter study. Twinning is another risk factor for shorter gestation length. Occurrence of twinning reduces gestation length by approximately 6 d (Echternkamp

and Gregory, 1999). In the current experiment, cows carrying twins had greater CBT than cows calving singletons. It has been reported that pregnant dairy cows have greater vaginal temperature than nonpregnant cows (Suthar et al., 2012), and it was suggested that increased concentration of progesterone in plasma was associated with higher vaginal temperature (Suthar et al., 2012). Given that cows carrying twins have greater progesterone concentration compared with cows carrying singletons (García-Ispierto et al., 2016), it is plausible to speculate that thermoregulation of cows bearing twins was influenced by progesterone levels. Furthermore, it is possible that greater uterine blood flow required to support the additional calf results in increased heat production, and consequently, greater CBT. Conversely, Aoki et al. (2005) did not detect differences in CBT between cows carrying twins or singletons. In that study, however, CBT was monitored during the last week of gestation, which differs from the current study. Nevertheless, it is important to acknowledge that a small number of cows carried twins in the current study and further research is necessary to investigate the association between twinning and CBT during late gestation.

We investigated whether time of day at CBT assessment reflects the variation in the daily rhythm of body temperature. Understanding the daily oscillation of CBT of dry Holstein cows is of great importance because researchers commonly use CBT assessments to determine efficiency of cooling strategies (do Amaral et al., 2009; Karimi et al., 2015). In previous experiments, CBT of dry cows was assessed in the morning, afternoon, or both. Despite the practical aspect of assessing CBT once or twice daily in commercial settings, limited assessments may lead to inaccurate representation of exposure to heat stress throughout the day because of daily patterns of CBT (Scanavez et al., 2016). Among all CBT assessments across the day, measurements recorded at 2215 h resulted in the greatest correlation with PC1. This indicates that CBT recorded at 2215 h was a fairly precise proxy to assess variance in CBT during the entire day under the conditions of the study. In addition, our findings revealed a poor correlation between PC1 and CBT assessed early-morning or mid-afternoon. Therefore, estimating severity of heat stress to which dry cows are exposed throughout the day by recording CBT in the morning or afternoon may result in misleading conclusions. In fact, in studies that evaluated effectiveness of evaporative cooling, CBT differed between treatment groups in

the afternoon, but not in the morning (do Amaral et al., 2009; Tao et al., 2012). This reinforces the importance of our findings, indicating that CBT recorded at these times (e.g., morning and afternoon) leads to conflicting results and potentially, equivocal interpretation. The authors of the present study recognize the difficulty in implementing CBT monitoring programs at 2215 h in commercial dairies. For research purposes, however, recording CBT at this time of the day should be considered in studies conducted in a similar geographic area that use herds with comparable production systems as the ones described herein. These findings may be considered when designing and interpreting results of future research projects that involve heat-stressed cows, and particularly for studies aiming to evaluate efficacy of heat abatement systems. Nonetheless, more research experiments must be conducted using a larger number of herds to confirm our results.

A secondary objective of the present study was to develop and test accuracy estimates of statistical models to predict CBT of dry Holstein cows under heat stress. These models were developed using training and validation datasets. Accuracy of the models were evaluated by comparing actual CBT values from the original dataset with values obtained from the statistical models. A similar approach has been used to evaluate models to predict carcass traits in beef cattle (Soulat et al., 2016) and methane emission levels in dairy cattle (Castro-Montoya et al., 2017). Interestingly, variables in the model with best predictivity were the same variables retained in the reduced regression analysis that evaluated factors associated with CBT. Dairy tended to influence CBT, whereas gestation length and twinning had a significant effect on predictivity of CBT.

In conclusion, the present study demonstrated that CBT assessed between 250 and 260 d of gestation is greater for cows carrying twins and is negatively associated with gestation length. In addition, findings from this study suggest that 2215 h is the most appropriate time of the day to evaluate severity of heat stress of dry cows during warm months. Lastly, CBT of dry cows can be estimated using statistical models that include dairy, twinning, and gestation length of dry Holstein cows. These findings are of utmost importance for the development of strategies focused on improving overall performance of subpopulations of cows affected by heat stress, which may result in reduced economic losses and improved animal well-being in dairy operations.

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