Short communication: Relationships among temperature-humidity index with rectal, udder surface, and vaginal temperatures in lactating dairy cows experiencing heat stress

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ABSTRACT

The objective of this study was to evaluate relationships between measurements of temperature-humidity index (THI) and rectal, vaginal, and udder surface temperatures in lactating cows exposed to heat stress (HS). In experiment 1, 12 multiparous and 8 primiparous Holstein cows experienced a THI ranging from 69 to 76 at 2000 to 1000 h and THI from 74 to 82 at 1000 to 2000 h (peaked at 82 from 1400 to 1800 h). Cows were exposed to HS 10 h daily for 21 d. Measurements of rectal temperature (RT) and udder surface temperature were collected at 1000 and 1500 h (± 30 min). Vaginal temperature was monitored every 10 min using digital loggers, averaged over 1 h, and paired with corresponding rectal and udder surface temperature data. In experiment 2, 12 multiparous Holstein cows experienced a THI ranging from 60 to 76 at 2000 to 1000 h and THI from 69 to 83 at 1000 to 2000 h (peaked at 83 from 1600 and 1900 h), eliciting 10 h/d of HS for 7 d. Rectal and udder surface temperatures were analyzed at 0700 and 1500 h (± 30 min). Vaginal temperature was recorded and analyzed as indicated in experiment 1. Afternoon THI showed weak correlations with surface temperature ($r = 0.19$, $n = 420$ in experiment 1; $r = 0.23$, $n = 84$ in experiment 2), weak to moderate correlations with RT ($r = 0.34$, $n = 366$ in experiment 1; $r = 0.26$, $n = 84$ in experiment 2), and moderate correlations with vaginal temperature ($r = 0.34$, $n = 175$ in experiment 1; $r = 0.35$, $n = 40$ in experiment 2). Moreover, vaginal temperature increased 0.10 and 0.22°C per unit of THI ($R^2 = 0.15$ in experiment 1; $R^2 = 0.40$ in experiment 2). Afternoon vaginal temperature strongly correlated with RT ($r = 0.69$, $n = 131$ in experiment 1; $r = 0.63$, $n = 37$ in experiment 2) and explained 57 (experiment 1) and 68% (experiment 2) of variation in RT. Surface temperature showed moderate

to strong correlations with RT ($r = 0.57$, $n = 84$) and vaginal temperature ($r = 0.74$, $n = 37$) in experiment 2. In conclusion, THI showed a weak to moderate relationship with core body temperatures and explained the increase in rectal and vaginal temperatures experienced by HS cows. Compared with rectal temperature, vaginal temperature showed stronger relationships with THI and can be used to determine thermal load. Udder surface temperature showed a moderate to strong relationship with core body temperature, and this relationship may support the use of surface temperature data to manage thermal load in HS cows.

Key words: body temperature, dairy cow, heat stress

Short Communication

The temperature-humidity index (THI) is estimated using ambient temperature and relative humidity and is used to determine heat stress (HS) conditions for lactating dairy cows. A decline in the thermal gradient between an animal and its environment due to HS compromises the loss of metabolic heat and contributes to heat load. The THI correlates with rectal temperature (RT) and explains the increase in body temperature of HS cows in subtropical environments (Dikmen and Hansen, 2009). Measurement of RT is used to determine core body temperature in dairy cattle; however, this procedure disrupts animal behavior and can be time-consuming and labor intensive (Burdick et al., 2012). Alternatively, monitoring udder surface temperature by thermal imaging is a quick, noninvasive procedure (Berry et al., 2003; Montanholi et al., 2008). However, udder surface temperature and the daily pattern of udder surface temperature differ with that of RT (Montanholi et al., 2008). A weak relationship between RT and udder surface temperature might limit the use of thermal imaging to monitor changes of body temperature in HS dairy cattle (Cvetkovic et al., 2005). Recent work indicates that infrared thermography detected changes in temperature on the right and left flanks and showed strong correlation with heat production ($r = 0.62$ and 0.72 ; Montanholi et al., 2008). Thus, monitor-

Received September 7, 2017.

Accepted February 19, 2018.

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ing udder surface temperature is less laborious than the determination of RT and could be an effective method to determine HS in cattle.

Compared with RT, the use of digital thermometers to monitor vaginal temperature can reduce the disruption of animal behavior (Collier et al., 2006; Montanholi et al., 2008) and provide continuous measurements of core body temperature (Collier et al., 2006). Vickers et al. (2010) demonstrated that rectal and vaginal temperatures were strongly correlated ($r = 0.81$) immediately postcalving and moderately correlated ($r = 0.46$) at peak lactation. In another study, rectal and vaginal temperatures were strongly correlated ($r = 0.89$) 48 h before calving (Burfeind et al., 2011). Although various physiological states alter surface and core body temperatures, the relationship of these parameters with THI have not been measured on lactating dairy cows experiencing thermal stress. The first objective of our study was to assess the relationship among THI and body temperatures in lactating dairy cows exposed to HS. The second objective was to evaluate the relationship between rectal, vaginal, and udder surface temperature. We hypothesized that THI would correlate and explain changes in rectal, vaginal, and udder surface temperatures in cows exposed to HS; likewise, vaginal and udder surface temperatures would display a strong relationship and explain changes in RT of cows exposed to HS.

All experimental methods were reviewed and accepted by the University of Tennessee, Institutional Animal Care and Use Committee. In experiment 1, Holstein lactating cows (144 ± 49 DIM; 12 multiparous and 8 primiparous) were housed in a sand-bedded freestall barn at the East Tennessee Research and Education Center-Little River Animal and Environmental Unit (Walland, TN). Cows were fed a TMR at 0900 h with a 50:50 forage-to-concentrate ratio on a DM basis containing varying amounts of CP and similar NE_L content (Kaufman et al., 2017). Cows were milked at 0900 and 1900 h and cooled during milking. Cows experienced prevailing east Tennessee climate for the months of July and August, and evaporative cooling was not provided to mimic diurnal changes of climate for 21 d. Measurement of ambient temperature (T) and relative humidity (RH) were used to determine THI using the following equation (Dikmen and Hansen, 2009):

$$\text{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T - 26)].$$

Temperature-humidity index ranged from 69 to 76 at 2000 to 2400 and 0100 to 1000 h and 74 to 82 at 1000 to 2000 h (peaked at 82 from 1400 to 1800 h).

Temperature-humidity index >68 was indicative of HS (Cook et al., 2007) and we imposed HS for an average of 10 h daily.

Rectal temperature ($n = 8$ multiparous and 8 primiparous cows) was measured at 1000 and 1500 h (± 30 min) throughout the study using a GLA M700 battery-operated digital read out thermometer (GLA Agricultural Electronics, San Luis Obispo, CA; accuracy $\pm 0.1^\circ\text{C}$). Vaginal temperature was monitored using an intravaginal data loggers (DS1922L Thermochron iButton Device; Maxim Integrated, San Jose, CA; size = 17.4×5.9 mm; weight = 3.3 g; accuracy $\pm 0.5^\circ\text{C}$) inserted into modified, blank (progesterone-free) internal drug release device (Zoetis, Florham Park, NJ) that was adapted as indicated previously (Dikmen et al., 2008; Burdick et al., 2012). The accuracy of intravaginal temperature loggers was tested using a 37.0°C water bath at 10-min intervals for 24 h as previously described (Burdick et al., 2012). Intravaginal loggers recorded temperature values every 10 min on d 11 to 15 ($n = 5$ multiparous and 2 primiparous cows) and d 16 to 20 ($n = 7$ multiparous and 6 primiparous cows). Vaginal temperature data were averaged by periods of 1 h (± 30 min) and paired with corresponding RT (i.e., 1000 and 1500 h on d 11 to 15 and d 16 to 20; Vickers et al., 2010). Udder surface temperature was measured at 1000 and 1500 h (± 30 min) using an infrared imaging gun (FLIR TG165 Imaging IR Thermometer; Wilsonville, OR; accuracy $\pm 1.5^\circ\text{C}$) on a clean area of the left rear quarter. Measurements were consistently taken on animals standing for longer than 5 min and at a distance of 15 cm from the udder. Air velocity was not measured, however, it may have influenced the relationship between udder surface and core body temperature. Udder composite milk samples were collected to determine SCC and evaluate inflammatory status of the mammary glands. Milk SCC did not change throughout the study (reported elsewhere; Kaufman et al., 2017).

In experiment 2, multiparous Holstein cows ($n = 12$; 120 ± 12 DIM) were housed in tiestalls in a temperature-controlled chamber at the East Tennessee Research and Education Center-Johnson Animal Research and Teaching Unit (Knoxville, TN). Cows were housed for a 3 d adaptation to facilities, followed by 7 d of HS. All cows were individually fed at 0600 and 1700 h a common TMR at 10% daily refusal (45% forage and 55% concentrate on DM basis) and milked at 0700 and 1800 h with no supplemental cooling. The HS treatment was designed to mimic a cyclical pattern of hot summer days in the southeastern United States. Temperature-humidity index was determined throughout the experiment and HS was determined as indicated in experiment 1. Temperature-humidity index

ranged from 60 to 76 at 2000 to 2400 and 0100 to 1000 h and 69 to 83 at 1000 to 2000 h (peak at 83 from 1600 to 1900 h) and elicited HS for an average of 10 h daily for 7 d (Kassube et al., 2017).

Rectal temperature was measured at 0700 and 1500 h (± 30 min) on all cows throughout the study using a Sharptemp V digital thermometer (Cotran Corporation, Portsmouth, RI; accuracy ± 0.1). Vaginal temperature was monitored, and data were averaged and paired with measurements of RT, as indicated in experiment 1. Udder surface temperature was measured in accordance with RT following the same procedures listed for experiment 1.

Statistical methods were conducted in SAS (version 9.4; SAS Institute Inc., Cary, NC). Hourly THI data were paired with corresponding measurements of body temperatures. Vaginal temperature values below 36.0°C (i.e., measurements during placement and removal of vaginal loggers) were considered artifacts and removed from the analysis (Vickers et al., 2010; Burfeind et al., 2011). Body temperature data were analyzed using the Mixed procedure, and least squares means and standard error of the means are reported for each experiment. The model included time period (i.e., morning and afternoon) as a fixed effect and animal as a random effect. The correlation procedure was used to determine Pearson correlation coefficient between THI and rectal, vaginal, and udder surface temperature data. Correlations of ambient temperature or relative humidity with body temperature data were conducted, but relationships were weaker compared with those between THI and body temperatures (data not shown); therefore, THI and body temperature correlations are presented. Positive relationships were examined at interest under moderate ($r \geq 0.30$) and strong correlations ($r \geq 0.60$). Significance was declared at $P \leq 0.05$ and a trend was declared at $0.05 < P \leq 0.10$. Regression analyses were conducted to model RT and vaginal temperature using THI and body temperature data. Regression analyses of udder surface temperature using THI were conducted (data not shown). A base model (BM) was included as an independent variable using data from morning or afternoon milk yield, DMI, parity, and DIM to address variation in the model

$$Y = \beta_0 + \text{BM} + \beta_1(X_1),$$

where Y = the dependent variable, β_0 = the intercept, BM = the base model, and $\beta_1(X_1)$ = the linear effect of predictor variables. To determine perfect agreement (i.e., slope = 1 then $P > 0.05$) among rectal, vaginal, and udder surface temperatures, slopes were statistically tested to be equal to 1 using the regression pro-

cedure and test statement. A slope of 1 indicates that the magnitude in the change in body temperature is directly associated with a change of equal magnitude on another body temperature. Moreover, slopes were statistically compared with 0 to unravel relationships among THI with body temperature measurements. Significance was declared at $P \leq 0.05$, and a trend was declared at $0.05 < P \leq 0.10$.

Measurements of intravaginal loggers in a 37.0°C water bath reported an average of $37.3 \pm 0.31^\circ\text{C}$ ($n = 3,876$) and a coefficient of variation of 0.83%. In experiment 1, data (d 11 to 15) from 3 intravaginal loggers were deemed erroneous and removed from the analysis; thus, loggers from 17 animals were included in the analysis. Afternoon measurements of rectal, vaginal, and udder surface temperature were higher than morning measurements ($P < 0.01$; Table 1). Morning and afternoon vaginal temperatures were 0.51 and 0.25% higher ($P < 0.01$) compared with RT, respectively, but udder surface temperatures were 4.4 and 3.3% lower ($P < 0.01$) compared with RT, respectively. Morning THI (74.5 ± 0.08) showed a moderate positive correlation ($r = 0.33$, $n = 336$; $P < 0.01$; explained 13.0% of the variation; Table 2) with RT ($38.9 \pm 0.06^\circ\text{C}$), a weak positive correlation ($r = 0.13$, $n = 420$; $P < 0.01$) with udder surface temperature ($37.2 \pm 0.10^\circ\text{C}$), and no correlation with vaginal temperature ($39.1 \pm 0.06^\circ\text{C}$). Morning vaginal temperature showed a moderate positive correlation ($r = 0.47$, $n = 131$; $P < 0.01$; explained 38% of the variation) with RT. Morning udder surface temperature showed a weak positive correlation ($r = 0.25$, $n = 336$; $P < 0.01$) with RT and no correlation with vaginal temperature. Afternoon THI (81.5 ± 0.09) showed a moderate positive correlation ($r = 0.34$, $n = 336$; $P < 0.01$) with RT ($39.7 \pm 0.06^\circ\text{C}$) and explained 14% of the variation in RT with a slope of $0.08 \pm 0.01^\circ\text{C}$ (slope, $P < 0.01$). Afternoon THI showed a moderate positive correlation ($r = 0.34$, $n = 175$; $P < 0.01$) with vaginal temperature ($39.8 \pm 0.06^\circ\text{C}$) and explained 15% of the variation in vaginal temperature (Table 3) with a slope of $0.10 \pm 0.02^\circ\text{C}$ (slope, $P < 0.01$). Afternoon THI showed a weak positive correlation ($r = 0.19$, $n = 420$; $P < 0.01$) with udder surface temperature ($38.4 \pm 0.10^\circ\text{C}$). Afternoon vaginal temperature showed a strong positive correlation ($r = 0.69$, $n = 131$; $P < 0.01$) with RT and explained 57% of the variation in RT ($P < 0.01$), with a 5 to 95 percentile prediction error of -0.52 to 0.43°C and a slope of $0.55 \pm 0.05^\circ\text{C}$ (slope, $P < 0.01$). Afternoon udder surface temperature showed a moderate positive correlation ($r = 0.34$, $n = 336$; $P < 0.01$) with RT and explained 14% of the variation in RT ($P < 0.01$). Afternoon udder surface temperature showed a moderate positive correlation ($r = 0.31$, $n =$

Table 1. Effect of time period (morning and afternoon) on body temperature measurements (°C; LSM and SEM)

Item	Morning	Afternoon	SEM	P-value ¹
Experiment 1				
Rectal	38.9	39.7	0.063	<0.01
Vaginal	39.1	39.8	0.061	<0.01
Udder surface	37.2	38.4	0.10	<0.01
Experiment 2				
Rectal	38.4	38.8	0.14	<0.01
Vaginal	38.8	39.4	0.15	<0.01
Udder surface	36.9	39.0	0.28	<0.01

¹Statistical difference between morning and afternoon temperature values.

175; $P = 0.03$) with vaginal temperature and explained 12% of the variation in RT ($P < 0.01$).

In experiment 2, afternoon measurements of rectal, vaginal, and udder surface temperature were higher than morning measurements ($P < 0.01$; Table 1). Morning and afternoon vaginal temperatures were 1.0 and 1.6% higher ($P < 0.01$) compared with RT, respectively. Morning and afternoon surface temperatures were 3.9%

Table 2. Regression analysis for predicting morning and afternoon rectal temperature using THI and vaginal and udder surface temperature measurements¹

Model	R ²	P-value ²
Experiment 1		
Base model (BM) ³		
AM	0.03	0.06
PM	0.03	0.03
BM + THI		
AM	0.13	<0.01
PM	0.14	<0.01
BM + vaginal		
AM	0.38	<0.01
PM	0.57	<0.01
BM + udder surface		
AM	0.07	<0.01
PM	0.14	<0.01
Experiment 2		
BM		
AM	0.27	<0.01
PM	0.24	<0.01
BM + THI		
AM	0.28	<0.01
PM	0.40	<0.01
BM + vaginal		
AM	0.32	0.02
PM	0.68	<0.01
BM + udder surface		
AM	0.36	<0.01
PM	0.57	<0.01

¹THI = temperature-humidity index, calculated from Dikmen and Hansen (2009).

²Statistical significance of the variable explaining variation in the model.

³Base model includes a model adjustment for milk yield, DMI, parity, and DIM.

Table 3. Regression analysis for predicting morning and afternoon vaginal temperature using THI and rectal and udder surface temperature measurements¹

Model	R ²	P-value ²
Experiment 1		
Base model (BM) ³		
AM	0.04	0.16
PM	0.04	0.18
BM + THI		
AM	0.06	0.06
PM	0.15	<0.01
BM + rectal		
AM	0.36	<0.01
PM	0.52	<0.01
BM + udder surface		
AM	0.04	0.21
PM	0.12	<0.01
Experiment 2		
BM		
AM	0.15	0.17
PM	0.20	0.10
BM + THI		
AM	0.15	0.27
PM	0.40	<0.01
BM + rectal		
AM	0.21	0.16
PM	0.47	<0.01
BM + udder surface		
AM	0.28	0.09
PM	0.69	<0.01

¹THI = temperature-humidity index, calculated from Dikmen and Hansen (2009).

²Statistical significance of the variable explaining variation in the model.

³Base model includes a model adjustment for milk yield, DMI, parity, and DIM.

lower and 0.52% higher ($P < 0.01$) compared with RT, respectively. Morning THI (67.7 ± 0.25) showed no correlation with RT ($38.4 \pm 0.14^\circ\text{C}$), vaginal temperature ($38.8 \pm 0.15^\circ\text{C}$), and udder surface temperature ($36.9 \pm 0.28^\circ\text{C}$). We noted a trend for morning vaginal temperature to show a weak negative correlation ($r = 0.29$, $n = 40$; $P = 0.07$) with RT. Morning surface temperature showed a moderate positive correlation with RT ($r = 0.44$, $n = 72$; $P = 0.01$) and explained 36% of the variation in RT (Table 2). Morning surface temperature showed no correlation with vaginal temperature. Afternoon THI (76.4 ± 0.21) showed a weak positive correlation ($r = 0.26$, $n = 84$; $P < 0.02$) with RT ($38.8 \pm 0.14^\circ\text{C}$). Afternoon THI showed a moderate positive correlation ($r = 0.35$, $n = 40$; $P < 0.03$) with vaginal temperature ($39.4 \pm 0.15^\circ\text{C}$) and explained 40% of the variation in vaginal temperature (Table 3), with a slope of $0.22 \pm 0.06^\circ\text{C}$ (slope, $P < 0.01$). Afternoon THI showed a weak positive correlation ($r = 0.23$, $n = 84$; $P < 0.04$) with surface temperature ($39.0 \pm 0.28^\circ\text{C}$). Afternoon vaginal temperature showed a strong positive correlation ($r = 0.63$, $n = 37$; $P < 0.01$) with RT and explained 68% of the variation in RT ($P < 0.01$),

with a 5 to 95 percentile prediction error of -0.45 to 0.71°C and a slope of $0.50 \pm 0.14^{\circ}\text{C}$ (slope, $P < 0.01$). Afternoon surface temperature showed moderate positive correlations with RT ($r = 0.57$, $n = 84$; $P < 0.01$; explained 57% of the variation in RT; $P < 0.01$) and a strong positive correlation with vaginal temperature ($r = 0.74$, $n = 37$; $P < 0.01$; explained 69% of the variation in vaginal temperature; $P < 0.01$).

Results showed weak or no relationships between morning THI and body temperatures in experiments 1 and 2. Body temperature data were collected at different times in experiment 1 relative to experiment 2. This may partially explain the inconsistency between experiments reported herein. Afternoon THI showed weak to strong correlations with surface, rectal, and vaginal temperature. The relationship was consistent in experiments 1 and 2 despite different THI (THI averages = 81.7 and 76.6) and experimental conditions. Afternoon THI predicted vaginal temperature with small error, and vaginal temperature increased by 0.10 ± 0.02 and $0.22 \pm 0.06^{\circ}\text{C}$ per unit of THI in both experiments. However, the use of the relationship between THI and RT to explain changes in core body temperature showed more variability. In comparison to the present study, variability in RT was reported in cows exposed to severe HS conditions (Dikmen and Hansen, 2009). Rectal temperature only provides a snapshot and does not reflect the circadian rhythm of body temperature or the capacity to adapt to HS conditions (Vickers et al., 2010). Compared with RT, continuous monitoring of vaginal temperature may be an alternative method to determine core body temperature and its relationship with THI (Kendall et al., 2007) and is less labor intensive and disruptive to animal behavior. In previous studies, vaginal temperature was monitored in 12 mixed-age cows (Kendall et al., 2007) and 21 multiparous and 8 primiparous cows (Vickers et al., 2010). In the current work, we analyzed vaginal temperature data from 17 cows in experiment 1 and 12 cows in experiment 2; thus, a greater number of animals could have strengthened the relationship reported herein. Therefore, the present data indicates that THI might be used to predict vaginal temperature and thermal status of HS lactating cows.

In the current work, data showed a moderate to strong relationship between afternoon rectal and vaginal temperature [experiment 1 ($r = 0.69$) and experiment 2 ($r = 0.63$)]. Vaginal and rectal temperatures reached similar values and vaginal temperature predicted RT. Vaginal temperature was slightly greater in both studies, suggesting that this organ is more sensitive to changes in core body temperature relative to the rectum (Burdick et al., 2012). Results indicated a greater circulatory

blood flow in the vagina of women compared with the rectum and could explain, in part, the results reported in the current study (Emmanuel et al., 2000). Depth of controlled internal drug release insertion was not recorded; however, deeper placement of the vaginal temperature can result in greater temperatures compared with RT (Burfeind et al., 2010; Vickers et al., 2010). In addition, the increase of afternoon core body temperature reduced the magnitude of the difference between rectal and vaginal temperatures, resulting in stronger association between them. As indicated by the slope, a 1.0°C increase in vaginal temperature increased RT by 0.55 (experiment 1) and 0.50°C (experiment 2). In agreement with the current results, Burdick et al. (2012) reported a strong relationship between rectal and vaginal temperatures ($r = 0.97$); thus, the results presented herein support the use of digital loggers to monitor vaginal temperature and determine increases in rectal body temperature in lactating cattle exposed to HS.

Lactating mammary glands are metabolically active and reflect surface temperature (Cvetkovic et al., 2005; Lin et al., 2008). Monitoring udder surface temperature is a less disruptive method than measuring RT and can be determined with less labor and at a low cost. However, udder surface temperature is affected by ambient conditions of temperature, humidity, air velocity, bedding material, and lying behavior (Cook et al., 2007; Marcillac-Embertson et al., 2009; Avendaño-Reyes et al., 2010). In the present work, THI showed weak correlations with surface temperature in HS cows (experiments 1 and 2). The results indicate that ambient conditions (e.g., air velocity and bedding material) could have affected surface temperature and altered the strength of the relationship with THI. In addressing the relationship between surface and core body temperature, udder surface temperature showed a strong correlation and explained changes in rectal and vaginal temperature in cows housed in chambers (experiment 2). Thus, surface temperature may be used to determine and predict thermal load in HS cows housed in controlled environments.

In conclusion, THI showed a weak to moderate relationship with core body temperatures and predicted the increase in afternoon rectal and vaginal temperatures in lactating dairy cows exposed to HS. Compared with RT, vaginal temperature showed stronger relationships with THI and can be used to determine thermal load. Vaginal temperature predicted RT and was an effective method to determine thermal load. Udder surface temperature revealed thermal load and may be a measurement assessed to determine HS in cows housed in confinement and to manage HS on-farm.

ACKNOWLEDGMENTS

The authors acknowledge funding for this project provided by University of Tennessee Institute of Agriculture. The authors acknowledge the assistance of East Tennessee Research and Education Center, Little River Animal and Environmental Unit staff in Wal-land, Tennessee.

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