



# Infrared thermography measured body surface temperature and its relationship with rectal temperature in dairy cows under different temperature-humidity indexes

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## Abstract

The aim of this study was to better understand the inflection point of RT and BSTs and measure different body surface temperatures (BSTs) under different temperature-humidity index (THI) conditions. A total of 488 Holstein dairy cows were chosen to manually measure rectal temperature (RT) and BSTs including left side of eye, ear, cheek, forehead, flank, rump, fore udder, and rear udder by infrared thermography for 14 times. Those measurements included six times under high THI (THI > 78), three times under moderate THI ( $72 \leq \text{THI} \leq 78$ ), and five times under low THI (THI < 72). Results showed that BSTs were affected by THI conditions ( $P < 0.01$ ). The THI conditions where mean and maximum forehead temperatures started to increase rapidly (71.4 and 66.8) were lower than that where RT started to increase rapidly (74.1). The correlation coefficients of mean and maximum forehead temperatures to THI were 0.808 and 0.740, and were 0.557 and 0.504 to RT, all showing the highest as compared to other region temperatures with THI and RT, respectively. Thus, we conclude that BSTs are more sensitive to thermal environment than RT, suggesting the variability of BST to reflect body core temperature. In addition, the forehead is a relatively reliable region to assess the heat stress reflecting RT compared to the eye, ear, cheek, flank, rump, fore udder, and rear udder regions.

**Keywords** Body surface temperature · Heat stress · Infrared thermography · Rectal temperature · Temperature-humidity index

## Introduction

With particular references to tropical and subtropical zones, heat stress is one of most recognized factors influencing livestock production and welfare. Large economic losses to the dairy industry (St-Pierre et al. 2003; Wang et al. 2010) are related to negative effects of heat stress on production (Joksimović-Todorović et al. 2011; Haqiya et al. 2017), reproductive performance (García-Ispierto et al. 2007), and immunity (Lacetera et al. 2005).

The temperature-heat index (THI), a widely used measure to assess heat stress in cattle (Gaughan et al. 2008), advises that exceeding a value of 72 may suggest that cows are suffering heat stress (Ingraham et al. 1976). However, genetic pressure for increased production performance has decreased heat tolerance in cattle due to the metabolic cost of increased milk yield and growth (West et al. 2003; Bryant et al. 2007; Oltenacu et al. 2010). Thus, it makes possible that considering 72 as a cutting-edge point to declare heat stress may underestimate the threshold of heat stress in cows. Consequently, inflection point of body temperatures under different THI conditions may reflect threshold of heat stress and help to ensure the accuracy of heat stress evaluation as well as improved management strategies for cows.

Rectal temperature (RT) increases whenever lactating cows are exposed to high environmental temperatures (Bourroui et al. 2002) causing concomitant changes to the homeostasis status of animals (Younes et al. 2011). Thus, RT is regarded as a common and practical tool to determine heat stress (Hicks et al. 2001). However, RT has limitations that include being

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time and labor consuming, requires direct contact with animals, and could cause stressful experiences to cows (Suthar et al. 2012; Ammer et al. 2016). On the other hand, due to its convenience, speediness, non-contact, and non-stress source, infrared thermography has been widely used in human medicine (Ng et al. 2004; Chiang et al. 2008) and animal science (Stewart et al. 2005; Ludwig et al. 2010; Do et al. 2013).

Due to the variation of skin vasomotor activity (Montanholi et al. 2008), variations of skin surface temperature can be assessed by using infrared thermography. Therefore, measuring BSTs has arisen as a stress indicator (Martello et al. 2016) and is of increasing importance (Adams et al. 2013). For instance, Collier et al. (2006) found that environmental temperature influenced the BST by heat exchange among the organism and environment. Nevertheless, despite many studies have reported the evaluation of body surface temperature (BST) on heat stress (Firk et al. 2002; Alzahal et al. 2011), there is a paucity of scientific information exploring which body region temperature reflects better the RT under differing THI conditions. Hence, the aim of this study was to better understanding of inflection point of RT and BSTs and depict the best body region reflecting RT to assess heat stress and under different THI conditions.

## Materials and methods

### Location

This study was carried out at a private dairy farm of breed for dairy (Zhongdi breeder farm for dairy) in Shunyi, Beijing, from July to October 2016. The zone of the farm is located at latitude between 40° 00' and 40° 18' and east longitude between 116° 28' and 116° 58' and belongs to warm temperate semi-humid continental monsoon climate. Chinese Holstein cows in one barn were used.

### Animals, feeding, and handling

Inclusion-exclusion criteria to select experimental cows were parity (two to four calving, at the beginning of the experiment), milk yield above 30 kg/day (the average of June,

July), and days in milk (DIM; at the beginning of the experiment) below 200 days (Table 1). A total of 488 cows were used. The barn (15 m × 260 m) having the capacity of 280 cows was concrete-floored without outdoor area, where every cow had more than one bed (1.45 m × 1.8 m) with litter of rice husk. Cows were fed a total mixed ration three times daily at 7:00 am, 14:00 pm, and 19:00 pm with free access to water and diet, and were milked three times daily at 8:00–8:40 am, 15:10–15:50 pm, and 21:40–22:20 pm, respectively. The barn used automatic manure scraper to remove feces. Besides, five auto-controlled axial fans and the spray of cooling system were installed, which were used after testing in the afternoon.

### Experimental design and procedures

The dry bulb temperature ( $T$ ) and relative humidity (RH) of air inside the cowshed were obtained with handheld hygrothermograph (Testo-625, Germany), at intervals of 30 min during testing at sampling days from 11:00 am to 12:30 am, while recorded by automatic air detection machine at other days from 8:00 am to 20:00 pm (Fig. 1). The THI was calculated according to the below model:  $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$  (Rowell 1972) using 20 times values of  $T$  and TH during the daytime. As previously described (Ingraham et al. 1976), THI values were ranked as  $> 78$ ,  $72-78$ , and  $< 72$  to define high, moderate, and low THI, respectively. Six times with high THI in July and August, three times with moderate THI in September, and five times with low THI in September and October, totaling 14 days were chosen to measure RT and BSTs of cows (Table 2).

On the sampling days, cows were fixed by neck clip (MZX-001, Muzhixing livestock co, Ltd., Cangzhou, China), and tested at 11:00–12:30 am (neither the feeding nor the milking time). A portable infrared camera (IRC) VarioCAM® (InfraTec, Germany) was used to take infrared at least three images with temperature field of  $640 \times 480$  pixels as referring the previous studies used in pigs (Loughmiller et al. 2001) and dairy as well (Berry et al. 2003). Eight different regions were measured, which were eye (orbita plus surrounding), ear (inner side at the base of the ear), cheek, forehead, flank, rump, fore udder, and rear udder in the left side of the body (Fig. 2). These regions were prevented from direct

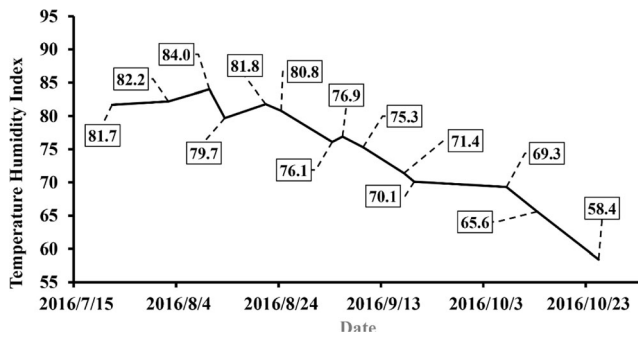
**Table 1** Parity, milk, and days in milk of high-, moderate-, and low-temperature humidity index (THI) cows

Group	<i>n</i>	Parity <sup>1</sup>	Milk yield <sup>2</sup> (kg/day)	Days in milk <sup>1</sup> (day)
High THI	198	3.01 ± 1.39	34.34 ± 4.12	122.21 ± 31.66 a
Moderate THI	111	2.97 ± 1.34	35.34 ± 4.23	140.17 ± 34.31 b
Low THI	179	3.08 ± 1.36	35.52 ± 3.93	159.35 ± 35.78 c

<sup>a-c</sup> Means within a column between the three groups with different superscripts differ ( $P < 0.05$ )

<sup>1</sup> The parity on the beginning days of the experiment (July 22, 2016)

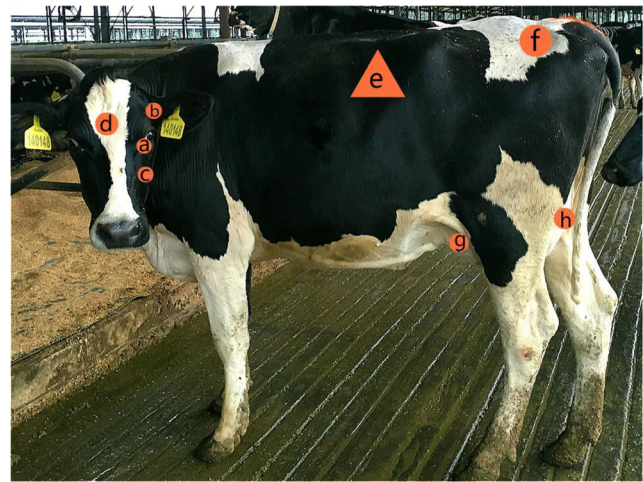
<sup>2</sup> The averaged milk yield in July



**Fig. 1** The temperature humidity index during the whole experiment, which was calculated based on data from 8:00 am to 20:00 pm

sunlight and dirt spot during the measurement. The camera was parallel to the height of determined regions and with 1.5-m distance to cow according to Montanholi et al. (2008). Cows kept standing and quiet during the measurements.

The infrared images were interpreted using IRBIS 3 Standard software (Yashihengyuan, Beijing, China). Using the eye for example, 1134 pixels were applied to calculate mean and maximum temperatures of the specific area (Fig. 3). The minimum temperature is the easiest to be affected by external environment and smudge. The mean temperature represents the mean value of the specific area and reflects the overall situation, which however may be also affected by external environment and smudge. The maximum temperature



**Fig. 2** Illustrative images of each region of the body surfaces where the infrared thermography images are obtained. **a** Eye. **b** Ear. **c** Cheek. **d** Forehead. **e** Flank. **f** Rump. **g** Fore udder. **h** Rear udder

represents the highest temperature in the specific area, which is hardly affected by smudge. Thus, the mean and maximum temperature are usually used in the studies (Montanholi et al. 2008; Salles et al. 2016; Martello et al. 2016). Meanwhile, RT was manually measured with a rectal thermometer (Kelibo Co., Ltd., Wuhan, China) to a depth of 10 cm to minimize bias according to Burfeind et al. (2010) between individuals. After an acoustic signal emitted from the thermometer, each measurement was finished in 15 s.

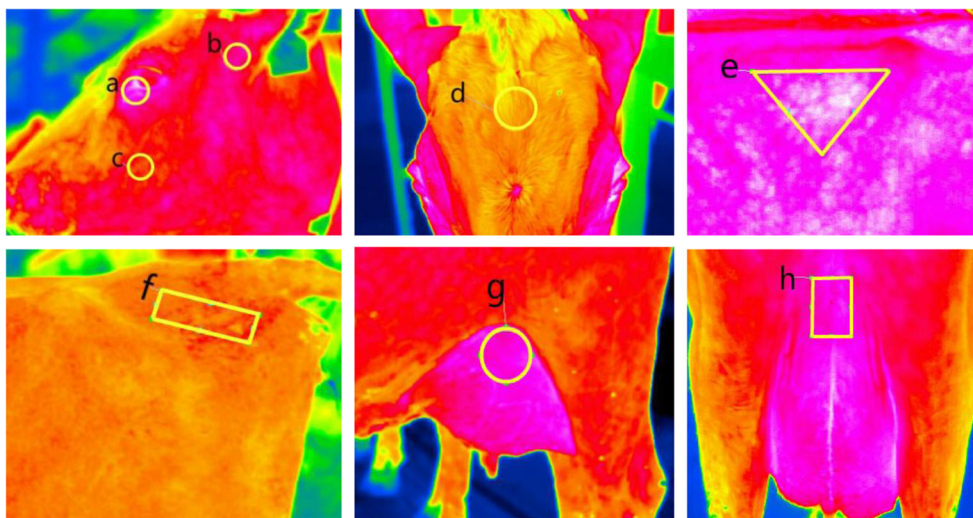
**Table 2** The temperature humidity index (THI) and rectal temperature (RT) during the 14 sampling times

THI conditions	Date (MM/DD/YY)	THI <sup>1</sup>	<i>n</i>	RT (°C)	
High THI	07/22/16	81.7 ± 0.14	34	39.00 ± 0.10	
	08/02/16	82.2 ± 0.27	24	39.32 ± 0.08	
	08/10/16	84.0 ± 0.18	37	39.21 ± 0.07	
	08/13/16	79.7 ± 0.10	35	38.99 ± 0.06	
	08/21/16	81.8 ± 0.79	32	38.99 ± 0.06	
	08/24/16	80.8 ± 0.36	36	39.19 ± 0.05	
	Average		81.8 ± 0.28 A	198	39.12 ± 0.03 A
Moderate THI	09/03/16	76.1 ± 0.30	38	38.57 ± 0.05	
	09/05/16	76.9 ± 0.26	37	38.74 ± 0.05	
	09/09/16	75.3 ± 0.12	36	38.73 ± 0.05	
	Average		76.0 ± 0.18 B	111	38.66 ± 0.03 B
Low THI	09/17/16	71.4 ± 0.69	31	38.70 ± 0.06	
	09/19/16	70.1 ± 0.58	39	38.61 ± 0.05	
	10/07/16	69.3 ± 0.20	33	38.40 ± 0.06	
	10/13/16	65.6 ± 0.46	38	38.65 ± 0.09	
	10/25/16	58.4 ± 0.94	38	38.68 ± 0.12	
	Average		67.6 ± 0.77 C	179	38.56 ± 0.03 C
	Total			488	38.86 ± 0.02

<sup>A-C</sup> Means within a column between the three groups with different superscripts differ ( $P < 0.01$ )

<sup>1</sup> The averaged 24 times THI data were calculated based on *T* and RH during the daytime from 8:00 am to 20:00 pm measured at intervals of 30 min

**Fig. 3** Illustrative infrared thermography images of the **a** eye, **b** ear, **c** cheek, **d** forehead, **e** flank, **f** rump, **g** fore udder, and **h** rear udder. The mean and maximum temperatures are calculated based on these regions



### Data analysis and statistics

Data were presented as mean  $\pm$  standard deviation (SD) and analyzed by SAS 9.3 (SAS Institute Inc., Cary, NC, USA). One-way ANOVA was used to analyze differences of THI values under high, moderate, and low THI conditions.

To analyze effects of THI on RT and BSTs, the following model of PROC GLM was used:

$$Y_{ij} = \mu + T_i + b_1X_j + b_2X_j + C_j + \varepsilon_{ij},$$

where  $Y_{ij}$  is the dependent variable (RT and BSTs) of cow  $j$  at THI  $i$ ,  $\mu$  is the overall mean,  $T_i$  is the fixed effect of THI ( $i$ : 1, 2, and 3 represent high, moderate, and low THIs, respectively),  $b_1X_j$  is the covariant factor of DIM,  $b_2X_j$  is the covariant factor of milk yield,  $C_j$  is the fixed effect of cow ( $j$ : 1, ..., 488), and  $\varepsilon_{ij}$  is the random residual error. When significant interactions ( $P < 0.05$ ) were detected, adjustments to the level of significance of the  $P$  value were used to account for the comparisons among different THI conditions using Tukey-Kramer adjustments. Pearson's partial correlation by counting the DIM, milk yield, and cow into considerations was used to calculate the correlation between BSTs and THI, and RT, respectively.

The segment regression analysis, which integrates the inflection point into the regression and obtains the most optimum value when the residual sum of squares showing lowest, was used to calculate the inflection point of RT and the mean and maximum forehead temperatures under different THI conditions.

The inflection points on RT, mean, and maximum forehead temperatures were calculated by the following formula, respectively:

$$y_{RT} = \begin{cases} -0.00677x + 39.0580, & 58.4 \leq x \leq 74.1 \\ 0.07271x + 33.1668, & 74.1 \leq x \leq 84.0 \end{cases}, R^2 = 0.83$$

$$y_{\text{mean}} = \begin{cases} 0.1059x + 22.4909, & 58.4 \leq x \leq 71.4 \\ 0.3448x + 5.4335, & 71.4 \leq x \leq 84.0 \end{cases}, R^2 = 0.89,$$

$$y_{\text{max}} = \begin{cases} 0.00556x + 29.9646, & 58.4 \leq x \leq 66.8 \\ 0.2732x + 12.0762, & 66.8 \leq x \leq 84.0 \end{cases}, R^2 = 0.84$$

where  $y_{RT}$ ,  $y_{\text{mean}}$ , and  $y_{\text{max}}$  are the RT, mean, and maximum forehead temperature, respectively ( $^{\circ}\text{C}$ ), and  $x$  is the THI.

Significance and great significance were declared as  $P < 0.05$  and  $P < 0.01$ , respectively.

## Results

### Rectal temperature

As shown in Table 1, THI ranged from 58 to 84 with significant differences ( $P < 0.01$ ) among high ( $81.8 \pm 1.339$ ), moderate ( $76 \pm 0.901$ ), and low ( $67.6 \pm 1.234$ ).

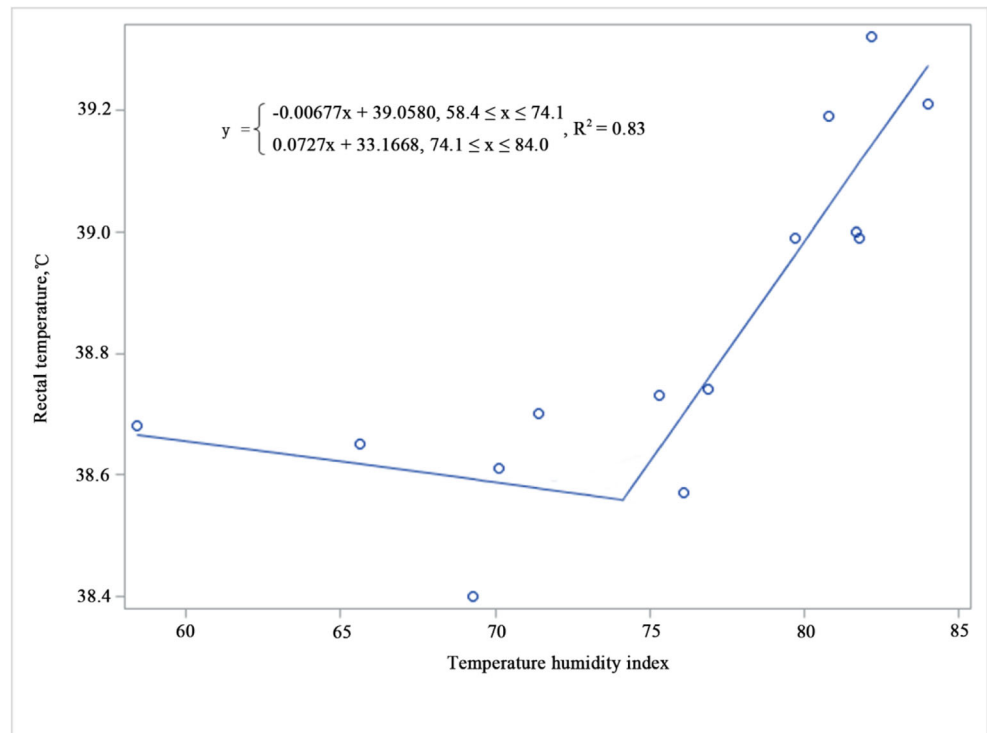
In relation to the inflection point, RT changed rapidly when THI was above 74.1 than below (Fig. 4).

### Body surface temperatures

Table 2 shows the BSTs under high, moderate, and low THI conditions. Both the mean and maximum temperatures of cheek and forehead as well as mean ear and rump temperatures were higher under high THI, followed by moderate THI, than low THI condition (all,  $P < 0.05$ ). Both the mean and maximum temperatures of eye, flank, and rear udder, as well as maximum temperatures of ear and rump, were higher under high and moderate THI conditions, than low THI condition



**Fig. 4** The change of rectal temperature (RT) with the temperature humidity index (THI). The 14 points of RT are the average temperatures of experimental cows measured under different THI conditions



(all,  $P < 0.05$ ). Referring to fore udder, the mean temperature was higher under high and moderate THI conditions, than low THI condition ( $P < 0.01$ ), while the maximum temperature was higher under moderate THI than high and low THI conditions ( $P < 0.05$ ).

Under high THI condition, the mean temperatures of eye, fore, and rear udder showed relatively high value with  $36.30 \pm 0.665$ ,  $36.08 \pm 0.967$ , and  $35.81 \pm 0.900$  °C, respectively, while the forehead showed a lower value (32.80 °C), whereas the maximum temperatures were also seen high values in eye, fore, and rear udder ( $37.30 \pm 0.718$ ,  $36.80 \pm 0.976$ ,  $36.44 \pm 0.890$  °C) and a lower value in forehead ( $33.66 \pm 1.024$  °C). These results are consistent with the region temperatures under moderate and low THI conditions.

The correlation coefficients of BSTs with THI were shown in Table 3. Both the mean and maximum temperatures of forehead showed the highest correlation coefficients (0.808 and 0.740) with THI. The mean and maximum forehead temperatures started to increase rapidly when THI was 71.4 (Fig. 5) and 66.8 (Fig. 6), respectively.

### Correlations between BSTs and RT

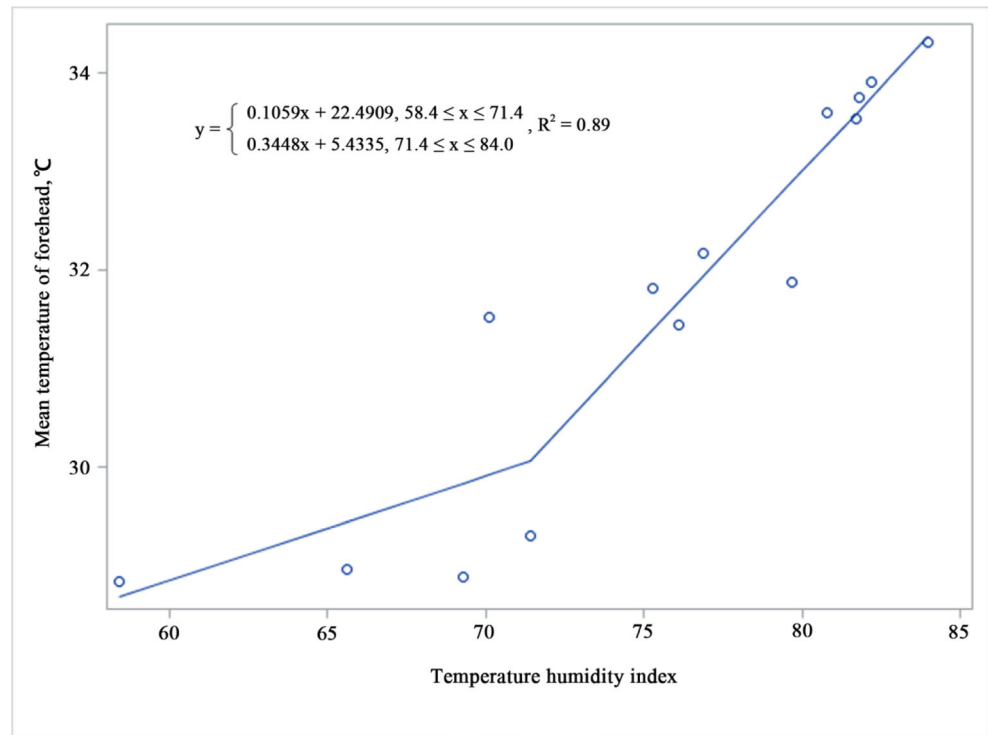
As compared to averaged RT ( $38.86 \pm 0.255$  °C; seen in Table 1), the BSTs showed relatively low temperature (31.65–36.11 °C; seen in Table 4). Body temperatures were significantly correlated with RT ( $P < 0.01$ ; Table 3). In relation to the mean temperatures, the forehead showed the highest correlation coefficient with RT (0.557), while the rear udder

showed the lowest (0.343). And on the maximum temperatures, still the forehead had the highest correlation coefficient with RT (0.504), while the eye showed the lowest (0.259). Both the mean and maximum ear, cheek, flank, and rump temperatures showed relatively high coefficients with RT, while relatively low in both the mean and maximum eye, fore udder, and rear udder temperatures.

### Discussion

Rectal temperature is a major physiological indicator to evaluate the impact of environment on growth and reproduction in dairy cows (West 1999). Our study also revealed that RT was higher under high THI than moderate and low THI conditions, which was consistent with a previous study (Bouraoui et al. 2002). The RT, however, varied when the environment temperature was beyond the regulation range of dairy cows (Bouraoui et al. 2002; Brown-Brandl et al. 2003), and the measurement of RT is time- and labor-consuming, and requires direct contact with cows, which may generate stressful experiences in animals (Suthar et al. 2012). Thus, the application of infrared thermography to measure BSTs is of increasing importance (Schaefer et al. 2007), especially referring to detect disease in dairy cows (Schutz and Bewley 2009; Adams et al. 2013). However, BST is influenced by various external and internal factors such as breed, milk production, parity, season, and climatic conditions (Bewley et al. 2008; Suthar et al. 2012; Adams et al. 2013; Liang et al. 2013). Despite the

**Fig. 5** The change of mean temperature of forehead with the temperature humidity index (THI). The 14 points of mean temperatures of forehead are the average temperature of the experimental cows measured under different THI conditions

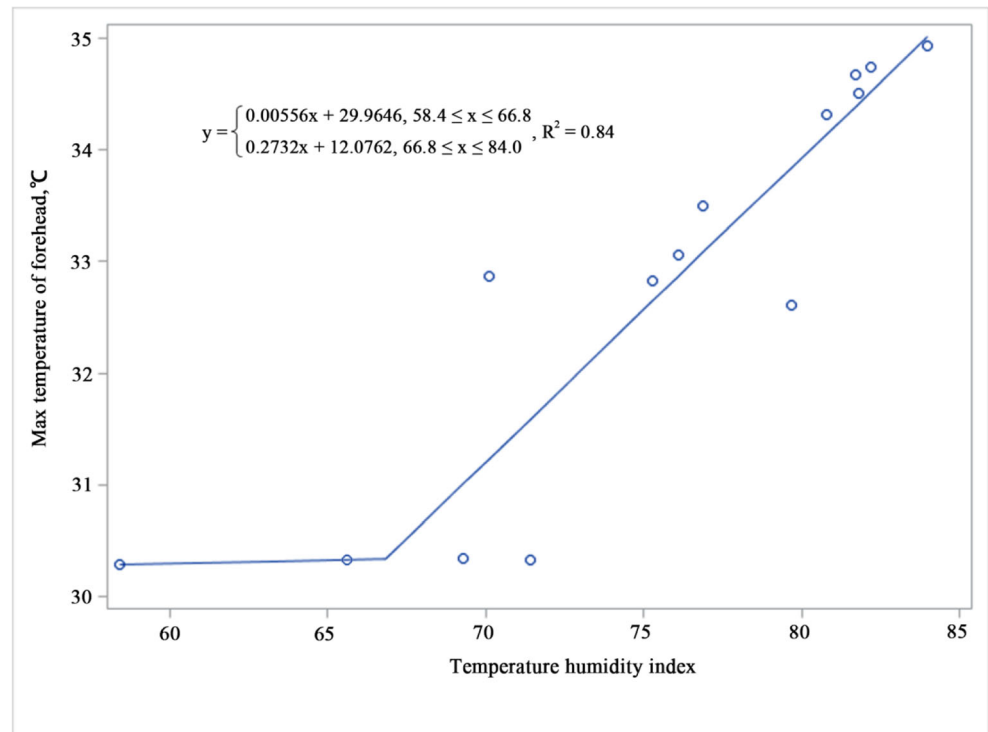


limitation of BST to reflect body core temperature, it is still practical for the early assessment of heat stress because of easy to use and requires minimal manpower as compared to the measure of RT and ruminal temperature as well.

In this study, all BSTs positively correlated with the THI; that is to say, the higher the THI is, the higher the BSTs are.

Further, the mean BSTs were lower (2.75–7.21 °C) than RT, which was similar with the study (2–7 °C) of Martello et al. (2016). The inflection point of RT (THI = 74.1), at which the temperature began to surge, was higher than that of the mean and maximum temperatures of forehead (THI = 71.4 and 66.8, respectively). Since RT is still regarded as the most common

**Fig. 6** The change of maximum temperature of forehead with the temperature humidity index (THI). The 14 points of maximum temperatures of forehead are the average temperature of the experimental cows measured under different THI conditions



**Table 3** The body surface temperatures (BSTs) under high-, moderate-, and low-temperature humidity index (THI) conditions, and their correlation coefficients with the THI ( $n = 488$ )

Regions	°C <i>n</i>	High THI 198	Moderate THI 111	Low THI 179	Correlation coefficient <sup>1</sup> Total = 488
Eye	Mean	36.30 ± 0.07 A	36.17 ± 0.08 A	35.65 ± 0.08 B	0.386*
	Maximum	37.30 ± 0.07 a	37.34 ± 0.08 a	37.07 ± 0.07 b	0.141*
Ear	Mean	35.20 ± 0.09 a	34.96 ± 0.10 b	34.13 ± 0.09 c	0.613*
	Maximum	35.85 ± 0.09 A	35.80 ± 0.10 A	35.08 ± 0.09 B	0.479*
Cheek	Mean	35.15 ± 0.09 A	34.58 ± 0.09 B	33.92 ± 0.09 C	0.582*
	Maximum	35.56 ± 0.09 A	35.12 ± 0.09 B	34.49 ± 0.09 C	0.535*
Forehead	Mean	32.80 ± 0.12 A	31.63 ± 0.12 B	30.00 ± 0.12 C	0.808*
	Maximum	33.66 ± 0.12 A	33.05 ± 0.13 B	31.44 ± 0.13 C	0.740*
Flank	Mean	35.28 ± 0.10 A	35.06 ± 0.11 A	34.23 ± 0.11 B	0.543*
	Maximum	35.92 ± 0.10 A	35.90 ± 0.11 A	35.21 ± 0.11 B	0.421*
Rump	Mean	35.19 ± 0.12 a	34.87 ± 0.13 b	33.95 ± 0.13 c	0.516*
	Maximum	35.84 ± 0.12 A	35.73 ± 0.13 A	34.96 ± 0.12 B	0.442*
Fore udder	Mean	36.08 ± 0.09 A	36.09 ± 0.10 A	35.42 ± 0.10 B	0.412*
	Maximum	36.80 ± 0.09 b	37.02 ± 0.10 a	36.52 ± 0.10 c	0.202*
Rear udder	Mean	35.81 ± 0.11 A	35.57 ± 0.12 A	35.05 ± 0.12 B	0.248*
	Maximum	36.44 ± 0.11 A	36.44 ± 0.11 A	35.99 ± 0.11 B	0.155*

A–C Means within a row between the three groups with different superscripts differ ( $P < 0.01$ )

a–c Means within a row between the three groups with different superscripts differ ( $P < 0.05$ )

<sup>1</sup> The correlation coefficients between BSTs and THI with 488 cows

\* $P < 0.01$

temperature to reflect body core temperature (Sarkar et al. 2013). Thus, BSTs were more sensitive to thermal environment than RT, suggesting the variability of BST to ambient environment.

Eye temperature as a stress indicator has been investigated in both human (Pavlidis et al. 2000) and animal studies (Stewart et al. 2008) and was suitable for indicating core temperature as not affected by environment (Gloster et al. 2011). In our study, the variation of eye temperature was smaller compared with other regions under high, moderate, and low THI conditions. The correlation coefficient between the mean temperature of eye and RT was 0.379, which was similar with a previous study (0.430) (Martello et al. 2016). The coefficient of the maximum temperature of eye with RT was much lower (0.259) than other studies (0.520 and 0.300) (Martello et al. 2016; Salles et al. 2016). This inconsistent result may be caused by two main reasons: (1) different selected eye areas in different studies. In this experiment, we used the specific circles to analyze the infrared thermal images of the eye and selected the mean and maximum temperature for analysis; (2) different environment in different studies. In this experiment, THI ranged widely (58.4–84.0) covering from non- to moderate heat stress conditions (Ingraham et al. 1976), since eye temperature hardly changed under a non-stress condition, which may be the reason resulting in a lower value of correlation coefficient. Furthermore, cows move their eyes frequently, which made it difficult to photograph, and accurate eye temperature data could only be obtained when IRC was

vertically aligned with the eye. Therefore, we consider that the eye area may be not an ideal region to assess heat stress to reflect RT.

Compared with other regions, the mean temperature of ear (0.613), cheek (0.582), and rump (0.516) had relatively higher correlation coefficients with THI, which suggested that these three regions could reflect the changes of BSTs. Our finding agreed with the study of Hoffmann et al. (2013), showing that the back of the ear would be the preferable region for applying infrared thermography to detect the changes of BST. The correlation coefficients between the mean temperature of BSTs and RT were varied from 0.343 to 0.557, among which the correlation between the ear, cheek temperatures, and RT were not at higher values ( $r = 0.484, 0.472$ , respectively). These results were consistent with the study of Martello et al. (2016). The correlation coefficients of mean and maximum rump temperatures with RT also presented an intermediate value (0.476 and 0.430). Therefore, due to the low correlation coefficients, we consider that these regions are not the ideal regions for evaluation of heat stress to reflect RT by infrared thermography.

The head region (i.e., brain), which is near the central nervous system regulating body temperature (Weschenfelder et al. 2013), was a suitable indicator of core body temperature because of its proximity to the brain (McCafferty 2007; Kessel et al. 2010). Our study revealed that the forehead temperature started to increase rapidly when THI was at 71.4 (mean) and 66.8 (maximum). Given that THI reaching at 72 indicated no

**Table 4** The mean and maximum body surface temperatures, and their correlation coefficients with rectal temperature ( $n = 488$ )

Regions	Mean temperature (°C)	Correlation coefficient <sup>1</sup>	Maximum temperature (°C)	Correlation coefficient <sup>2</sup>
Eye	36.11 ± 0.03	0.379*	37.28 ± 0.03	0.259*
Ear	34.82 ± 0.05	0.484*	35.61 ± 0.05	0.434*
Cheek	34.62 ± 0.05	0.472*	35.11 ± 0.05	0.452*
Forehead	31.65 ± 0.10	0.557*	32.74 ± 0.09	0.504*
Flank	34.95 ± 0.06	0.455*	35.77 ± 0.05	0.386*
Rump	34.74 ± 0.06	0.476*	35.55 ± 0.06	0.430*
Fore udder	35.98 ± 0.05	0.398*	36.81 ± 0.04	0.296*
Rear udder	35.55 ± 0.05	0.343*	36.31 ± 0.04	0.284*

<sup>1</sup> Correlation coefficient between mean temperature of the region and RT

<sup>2</sup> Correlation coefficient between maximum temperature of the region and RT

\* $P < 0.01$

stress or relatively slight heat stress in cows (Ingraham et al. 1976), the inflection points of forehead occurred during a non- or slight heat stress condition. Further, the non-susceptibility to smudge of forehead (Hoffmann et al. 2013) made it easy to photograph. More importantly, the correlation coefficient between mean forehead temperature and THI was up to 0.808, which was consistent with a recent result (0.810) (Salles et al. 2016). Whereas that between the mean forehead temperature and RT was also the highest (0.557), which was similar with the previous study (0.600) (Martello et al. 2016). This above suggests that the forehead was sensitive in the thermal environment and suitable for monitoring early heat stress by infrared thermography, and showed a relatively high correlation coefficient between forehead temperature and RT.

The fore and rear udder showed relatively higher temperatures than other regions (except for the eye), which was consistent with Montanholi et al. (2008). This finding may be due to the thinner skin thickness and lower hair density (Arkin et al. 1991). The fore udder temperatures were higher than the rear udder temperatures, which we assumed that fore udder was shielded by the side legs leading to less heat emission. Nevertheless, it is not clear why the maximum temperature of fore udder was higher under moderate THI than high THI condition. Further, the measurement of both fore and rear seemed difficult in placing the infrared thermography to photograph and needed additional labor to lift the tail when measured rear udder temperature. Besides, the tail easily contacts feces especially when cows were laying, which resulted in an inaccurate measurement, as proven in our previous study that the degree of smudge had a great influence on udder surface temperature (Peng et al. 2016). Moreover, the coefficients of fore and rear udder with THI and RT were low. Therefore, we consider that the fore and rear udder are not the ideal regions for evaluation of heat stress to reflect RT by infrared thermography.

The correlation coefficient of the mean flank temperatures to THI ( $r = 0.543$ ) was inconsistent with the study of Salles

et al. (2016) ( $r = 0.810$ ). The infrared images of the flank were easy to obtain, which was the advantage for using infrared thermography to monitor. However, due to its close distance to rumen, the flank temperature was considered as a good indicator to reflect the rumen temperature (Getty 1975; Montanholi et al. 2008), and thus not reliable to reflect RT measured by IRT. However, since rumen generated heat in ruminant animals (Czerkawski 1980; Beatty et al. 2008), the flank temperature reflecting rumen temperature may probably be useful to evaluate heat stress, however, a low correlation coefficient (0.430) with RT.

As aforementioned, measurements of reflecting body core temperature have various limitations. Such as, the BST is easily influenced by external and internal factors (Bewley et al. 2008; Suthar et al. 2012; Adams et al. 2013; Liang et al. 2013), the ruminal temperature is affected by water and feed intake (Adams et al. 2013), while the measurement of RT is time-consuming and requires manpower (Suthar et al. 2012; Ammer et al. 2016). Despite the limitation, our study would provide more specific information of BST to measure body core temperature.

## Conclusions

The averaged BST was lower than RT under the same THI conditions. The THI conditions where mean and maximum forehead temperatures started to increase rapidly (71.4 and 66.8) were lower than that where RT started to increase quickly (74.1), suggesting the variability of BST to reflect body core temperature. The correlation coefficients of mean and maximum forehead temperatures to THI were 0.808 and 0.740, and were 0.557 and 0.504 to RT, all showing higher values than other coefficients of region temperatures with THI and RT, respectively. Thus, we conclude that BSTs were more sensitive to thermal environment than RT, suggesting the potential of using IRT-measured BSTs to early detect heat stress



in cows. In addition, the forehead is a relatively reliable region to early assess the heat stress reflecting RT using IRT compared to the eye, ear, cheek, flank, rump, fore udder, and rear udder regions.

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## Compliance with ethical standards

**Ethics statement** The experimental protocols were approved by the Experimental Animal Care and Committee of Institute of Animal Science, Chinese Academy of Agricultural Sciences (approval number 2016IAS018).

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