

Effects of ambient temperature and humidity on body temperature and activity of heifers, and a novel idea of heat stress monitoring

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Abstract

Context. Heat stress has led to a serious reduction in dairy cows production performance, thus increasing the stress of feeding and reproduction management.

Aims. Heat stress arises when cows are unable to dissipate excess body heat, we aimed to investigate the effects of ambient temperature (AT) and humidity on diurnal body temperature and activity.

Methods. For improving the technology for rearing dairy cows, the vaginal temperature (VT) and activity of 60 Holstein heifers in summer ($n = 20$), autumn ($n = 20$), and winter ($n = 20$) were measured using the oestrus monitoring system.

Key results. We found that VT fluctuated slightly (~ 38.22 – 38.32°C) when AT and temperature-humidity index (THI) were lower than 20°C and 68, respectively. However, when this threshold is reached, VT increased significantly with increasing AT and THI, whereas activity decreased significantly.

Conclusions. Heat stress may be caused when THI is above 68 and cow's VT reaches 38.32°C . Evidently, when the THI exceeds 68 and VT is more than 38.32°C , suitable measures for reducing the effect of heat stress on the productivity of dairy cows should be taken.

Implications. The combined monitoring of VT and THI might provide accurate guidance for preventing and controlling heat stress.

Keywords: activity, dairy cow, oestrus, heat stress, Holstein heifers, reproduction, temperature-humidity index, vaginal temperature.

Received 2 April 2020, accepted 1 June 2021, published online 27 July 2021

Introduction

The adverse effects of heat stress caused by high ambient temperature (AT) on dairy production are the greatest in summer (Bouraoui *et al.* 2002), and the effects of heat stress is intensifying and lasting longer because of global warming (Ammer *et al.* 2018). Many studies have investigated the relationship of ambient factors, such as temperature and humidity, and production, with the adverse effects of heat stress. When AT and humidity reach the condition at which heat stress is triggered, the body temperature of the cows change accordingly, increasing gradually with the severity of the heat stress. However, dairy cows usually maintain or restore thermal balance by sweating, increasing their respiratory rates, and decreasing their dry matter and nutrient intake (West 1999; West *et al.*

2003). Consequently, the intake (Gorniak *et al.* 2014), milk production (Gantner *et al.* 2011; Nasr and El-Tarabany 2011), and reproductive performance (Wolfenson *et al.* 1995; Cartmill *et al.* 2001) of cows are affected, thereby affecting the economic returns of dairy farms.

Body temperature is a physiological response to many factors. It is an important index to evaluate the state of health of dairy cows, and widely used in production. (Fan and Li 2012; Lee *et al.* 2016). With the successful development of devices for monitoring body temperature automatically, in addition to being used for disease monitoring, body temperature is also used for oestrus monitoring in dairy cows (Kyle *et al.* 1998; Talukder *et al.* 2014; Sakatani *et al.* 2016). Heat stress during summer affects the behaviour of cows, and indirectly affects their activity and

body temperature (Cook *et al.* 2007; Allen *et al.* 2015; Brzowska *et al.* 2014). However, the effects of heat stress on the diurnal variation in the activity and body temperature of cows, as well as the effects of heat stress on bovine body temperature and activity during oestrus remain unclear till date.

In the present study, the effects of AT and humidity on the activity of dairy cows were investigated, and fluctuations in vaginal temperature (VT) was studied because it is strongly related to rectal temperature. Our objective was to monitor heat stress in cows by measuring VT and temperature-humidity index (THI) jointly.

Materials and methods

Animals and management

All animal treatments and procedures were approved by and conducted according to the standard established by the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. The experiment was conducted at a dairy farm in Shijiazhuang, Hebei Province, China, in summer (July–August 2017), autumn (October–November 2017), and winter (December 2017–January 2018). In every season, different 20 Holstein heifers, aged between 19 and 24 months, and with normal oestrus, were randomly selected to collect data on VT and activity in a same herd of cows. All the cows were housed in a free stall barn and fed with automatic total mixed rations three times daily, and drinking water was provided *ad libitum*.

VT and activity collection

VT and activity data were collected automatically using the oestrus monitoring system (patent number: CN201620035605.9).

The system includes automatic VT and activity measuring devices (Fig. 1a) and a data collector (Fig. 1b) with an acquisition radius of 150 m. The collector was installed in the cowshed and connected with a computer. The devices were inserted via applicator into the vagina of cows (one device for each cow) after disinfection with potassium permanganate, and VT and activity data was measured every 30 min, transmitted wirelessly to the data collector every 2 h, then uploaded to the computer system. The cow's activity was accumulated every 30 min, and its VT were recorded simultaneously. After 2 days of preliminary experiments, the experiment was carried out when the system was able to collect data steadily and normally.

AT and relative humidity collection

AT and relative humidity (RH) were recorded using automatic recorders of AT and humidity (Anymetre Instrument Co. Ltd, Zhongshan, Guangdong, China), with a precision of $\pm 0.3^{\circ}\text{C}$ and measurement range from -40 to 125°C for AT recorder, and a precision of $\pm 2\%$ and measurement range of 0–100% for the RH recorder. Following the methods used in previous studies, the two recorders were placed 3 m above the bed and the feeding area (Suthar *et al.* 2012; Lambertz *et al.* 2014), and the temperature and humidity data collection frequency and time of recordings were consistent with that of the automatic VT and activity measuring devices. VT and activity before 7 days of oestrus were defined as VT and activity of diestrus. The average AT and RH of each time point from the two recorders were used as AT and RH of that time point, and the formula used for THI calculation was: $\text{THI} = (1.8 \times T + 32) - ((0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T - 26))$, where T is the dry bulb temperature ($^{\circ}\text{C}$) and RH is the RH (%)

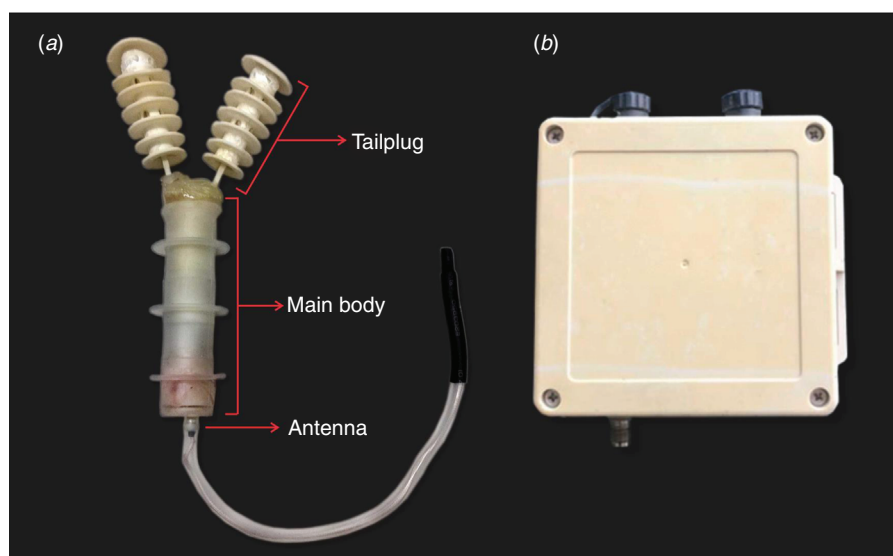


Fig. 1. The vaginal temperature and activity monitoring system. The sensor of temperature (ADT7320) and activity (ADXL362) were integrated into main body, the main body was attached with tailplug and antenna. Its size is 210 mm length and 20 mm in diameter, and the width of the tail plug is 80 mm. (a) The information of temperature and activity was sent by main body via antenna and collected by the data collector (b).

(Legrand *et al.* 2011). Daily minimum, maximum, and average AT, RH, and THI were measured for the different seasons.

Oestrus identification

In this study, a temperature increase of 0.3°C between the current VT and the average VT recorded at the same time point 7 days earlier, and lasting at least 2.5 h, was defined as oestrus. In addition, activity of more than 2.5 times higher than the average activity at the same time point 7 days earlier, which lasted at least 2.5 h, was also defined as oestrus. The end of oestrus was marked when the relative increase in VT or activity during oestrus between two consecutive time points was less than 0.3°C or 2.5-fold, respectively. However, observation on oestrus was performed four times a day from 08:00 to 20:00 hours for 15–20 min each time. When the automatic detection system sent alerts of a cow being in oestrus or we observed a cow was crawled, we measured the follicle size of the cow using a B-mode HS-101 V ultrasound scanner (Honda Electronics Co., LTD, Japan). When the presence of a preovulatory follicle larger than 10 mm in diameter, and absence of an active corpus luteum were detected, the cow was considered to be in true oestrus (Madureira *et al.* 2015).

Statistical analyses

The number of cows in oestrus detected based on VT was 18, 17, and 16 in summer, autumn, and winter, respectively; the number of cows in oestrus detected based on activity was 10, 10, and 11 in summer, autumn, and winter, respectively. Seasonal variation in VT and activity of diestrus, and variation in AT, RH, and THI were analysed using one-way analysis of variance. The same method was used to analyse seasonal variation in the relative increase in VT and activity during oestrus. Linear regression analysis was performed with VT of oestrus and diestrus as the dependent variables, and AT and THI as the independent variables. The differences were considered to be significant at $P < 0.05$, unless otherwise indicated. Data were processed with SPSS ver. 19.0 (IBM Corp., Armonk, NY, USA).

Results

Environmental conditions, VT, and activity during diestrus

Table 1 shows that the average RH in summer and autumn are not significantly different from each other, but both are significantly higher than that in winter ($P < 0.05$). The average AT in summer was 28.8°C, which was significantly higher than that in autumn (12°C) and winter (1.6°C) ($P < 0.05$). The average THI in summer (80.1) was significantly higher than that in autumn (53.8) and winter (39.5) ($P < 0.05$), and the average minimum THI in summer was 74.5, which was significantly higher than that in autumn (45.2) and winter (33.2) ($P < 0.05$), indicating that dairy cows were susceptible to heat stress in summer. Moreover, Fig. 2a shows that THI decreased gradually from the evening, and reached a minimum value at ~05:00, 07:00, 08:00 hours in summer (75.6), autumn (47.4) and winter (34.9), respectively. Subsequently, THI increased gradually, and reached a maximum value at ~14:00 hours in all three seasons. In

Table 1. Statistics of ambient temperature (AT), relative humidity (RH), and temperature-humidity index (THI) in summer, autumn, and winter

Data shown are means \pm s.d. Different lowercase letters within columns indicate significant differences ($P < 0.05$)

Variable	Period	Minimum	Maximum	Mean
AT(°C)	Summer	24.1 \pm 2.2a	34.9 \pm 5.1a	28.8 \pm 3.4a
	Autumn	6.7 \pm 3.0b	17.9 \pm 2.5b	12.0 \pm 2.1b
	Winter	-1.9 \pm 2.4c	5.5 \pm 2.0c	1.6 \pm 1.9c
RH (%)	Summer	58.3 \pm 15.5a	94.5 \pm 5.7a	78.6 \pm 10.4a
	Autumn	46.1 \pm 19.2b	91.1 \pm 6.1a	71.1 \pm 13.0a
	Winter	43.8 \pm 13.5b	77.9 \pm 13.2b	61.5 \pm 12.5b
THI	Summer	74.5 \pm 3.6a	86.2 \pm 4.6a	80.1 \pm 4.0a
	Autumn	45.2 \pm 4.9b	62.4 \pm 2.8b	53.8 \pm 3.0b
	Winter	33.2 \pm 4.2c	46.2 \pm 3.0c	39.5 \pm 3.1c

addition, the diurnal variation in THI was the largest in autumn and the smallest in summer.

The average VT of diestrus in summer was significantly higher than that in autumn and winter ($P < 0.05$) (Fig. 2b; Table 2), indicating that THI greatly affected VT during diestrus in summer. However, the changes in VT of diestrus were similar in autumn and winter, and only fluctuated slightly (~38.22–38.32°C), which indicated that VT of diestrus was less affected by THI in autumn and winter. Additionally, Fig. 2a, 2b shows that the average VT of diestrus decreased with THI and reached the lowest value (38.3°C) at ~07:00 hours in summer, and then increased with THI and reached a maximum (39.0°C) value at ~16:00 hours, and decreased gradually thereafter with THI. Moreover, Table 3 shows that the average activity in summer was significantly lower than that in autumn and winter ($P < 0.05$), but the difference between average activity in autumn and winter was not significant. The results indicated that dairy cows were more affected by heat stress in summer, but less affected by environmental conditions in autumn and winter.

Relationship of VT with AT, RH, and THI

Ambient temperature below 20°C had little effect on VT (Fig. 3a). However, when AT exceeded 20°C, VT was greatly affected, and VT gradually increased with increasing AT. Similarly, little effect on VT was found when THI was below 68 (the lowest THI in summer), but when THI was above this range, VT was greatly affected, and it also gradually increased with increasing THI (Fig. 3b). However, VT was less affected by RH (Fig. 3c).

Effects of AT and THI on VT in summer

Effect on VT of diestrus

AT and THI greatly affected the VT of diestrus in summer. Linear regression analysis of VT with AT and THI showed that VT during diestrus increased significantly with AT and THI ($P < 0.001$, Fig. 4). VT increased by 0.075°C for every 1°C increase in AT and 0.063°C per unit increase in THI.

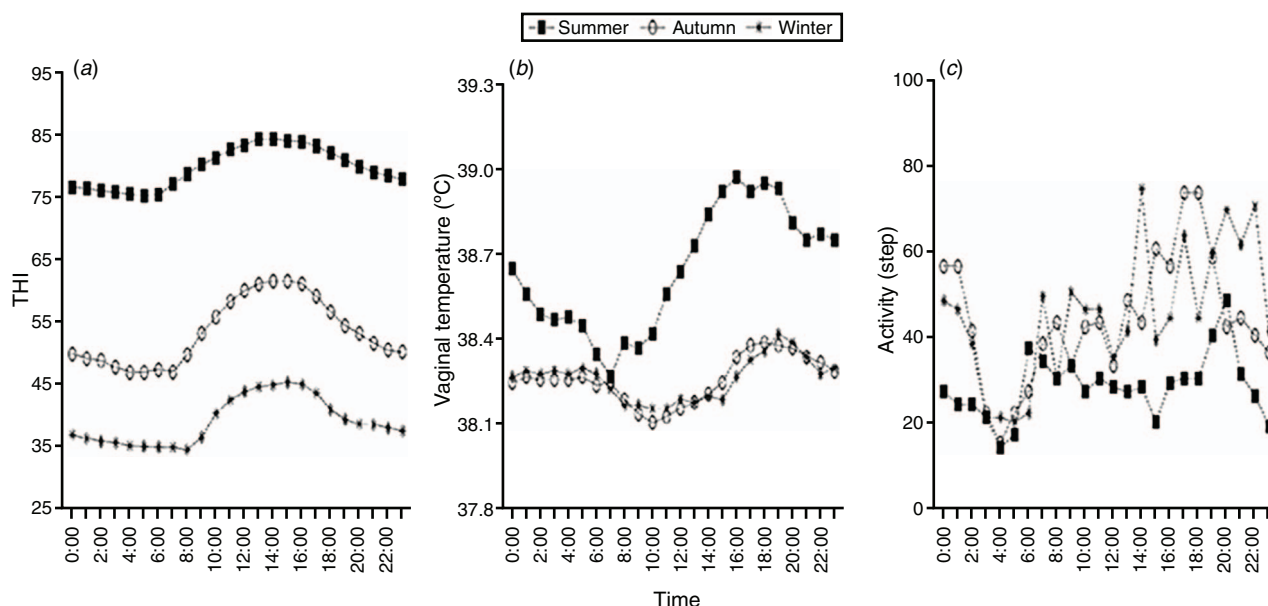


Fig. 2. (a) Daily changes in THI in summer (July–August 2017), autumn (October–November 2017), and winter (December 2017–January 2018); (b) daily changes in VT of dairy cows in diestrus during summer ($n = 18$), autumn ($n = 17$), and winter ($n = 16$); (c) daily changes in activity of dairy cows in diestrus during summer ($n = 18$), autumn ($n = 17$), and winter ($n = 16$).

Table 2. Statistics of vaginal temperature (VT) during diestrus in summer, autumn, and winter
Data shown are means \pm s.d. Different lowercase letters within columns indicate significant differences ($P < 0.05$); n = number of cows used in the study

	02:01–07:00 hours	07:01–18:00 hours	18:01–02:00 hours	n
Summer (°C)	38.40 \pm 0.09a	38.70 \pm 0.24a	38.71 \pm 0.14a	18
Autumn (°C)	38.26 \pm 0.01b	38.23 \pm 0.10b	38.31 \pm 0.05b	17
Winter (°C)	38.28 \pm 0.03b	38.22 \pm 0.07b	38.32 \pm 0.06b	16

Table 3. Activity during diestrus in summer, autumn, and winter
Data shown are means \pm s.d. Different lowercase letters within columns indicate significant differences ($P < 0.05$); n = number of cows used in the study

	02:01–07:00 hours	07:01–18:00 hours	18:01–02:00 hours	n
Summer (step)	24.5 \pm 10.2a	28.3 \pm 3.2b	29.7 \pm 9.5b	18
Autumn (step)	24.8 \pm 8.8a	49.8 \pm 14.1a	46.7 \pm 8.5a	17
Winter (step)	26.7 \pm 12.4a	46.5 \pm 12.5a	53.9 \pm 12.4a	16

Effect on VT of oestrus

VT during oestrus increased significantly with AT and THI ($P < 0.001$) (Fig. 5), and that VT increased by 0.06 and 0.051°C with every unit change in AT and THI, respectively.

Effects of seasons on relative increase in activity and VT during oestrus

The relative increase in VT during oestrus gradually decreased from summer to winter, and was significantly higher in summer than in autumn and winter (Fig. 6b, $P < 0.05$), but the difference between autumn and winter

was not significant. However, the relative increase in activity during oestrus was significantly lower than that in autumn and winter (Fig. 6a, $P < 0.05$), but the difference between autumn and winter was not significant.

Discussion

Presently, studies on the effects of AT and humidity on the body temperature of cows have mainly focused on summer and winter AT and humidity (Nabenishi *et al.* 2011; Sakatani *et al.* 2012) to the best of our knowledge. There are no studies reporting the effects of these environmental parameters

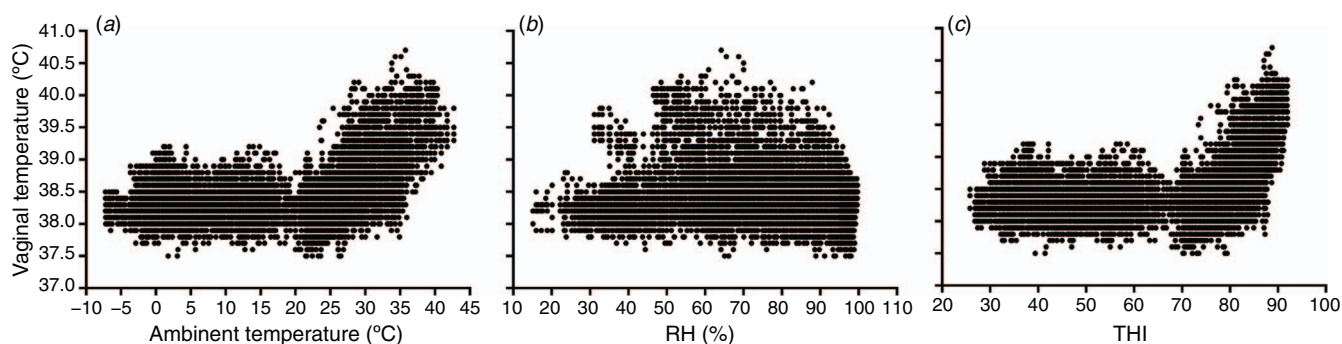


Fig. 3. Scatter plot of the relationship of VT with (a) AT, (b) RH, and (c) THI. A total of 51 cows (18 in summer, 17 in autumn, and 16 in winter) were used for this analysis.

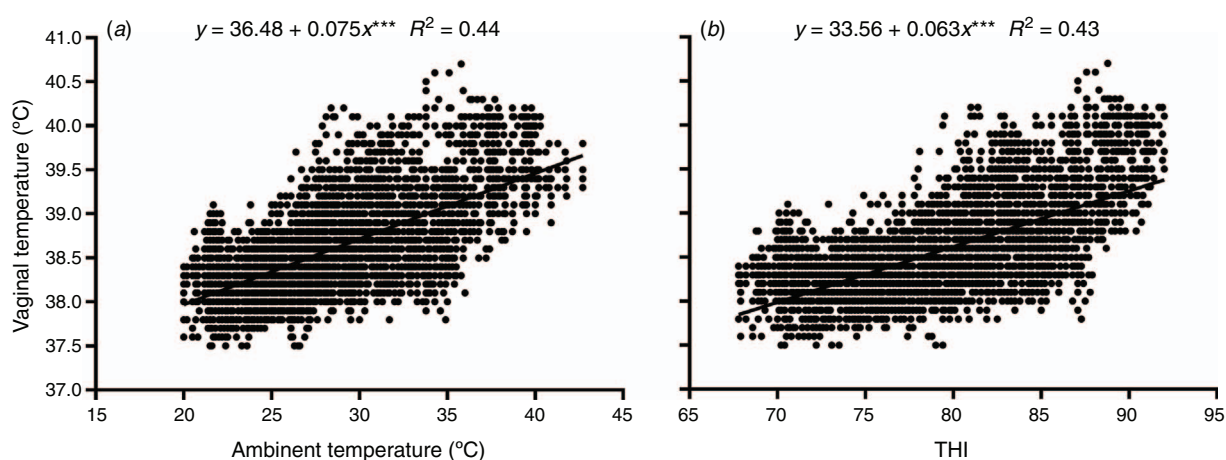


Fig. 4. Effects of (a) AT and (b) THI on VT during diestrus in summer. ***, $P < 0.001$ for the regression coefficients.

in autumn. To investigate the effects of continuous changes in AT, RH, and THI on the VT and activity of cows, we considered the effect of autumn season in the present study. It is necessary to understand the effects of environment as a whole on the physiology and behaviour of dairy cows, and to identify the patterns of changes in VT and activity in response to environmental conditions.

In order to establish a convenient method for body temperature monitoring, milk temperature has been measured, and was expected to give a better indication of the body temperature of dairy cows (West *et al.* 2003). However, milk temperature not only increased with THI, but was also easily affected by other factors, such as environmental conditions and diseases. As such, its application in monitoring bovine body temperature is rather unsuitable (West *et al.* 2003). In addition, our team also monitored the body surface temperature of cows by measuring ankle temperature; however, its application is also unsuitable as it is affected by uncontrollable environmental factors (Kou *et al.* 2017). The temperature correlation between rectum and vagina is 0.79, which is higher than that of uterine body, cervix, uterine angle and rectal temperature, and shows that long-term automatic

monitoring VT to indicate core temperature is more scientific and reasonable than rectum (El-Sheikh Ali *et al.* 2013). The real-time VT monitoring technique, based on sensor technology, is relatively new, and the automatically measured VT can serve as a strong indicator of bovine rectal temperature, since it is less affected by external factors (Collier *et al.* 2006; Kendall *et al.* 2007). Based on this technique, Nabenishi *et al.* (2011) and Sakatani *et al.* (2012) used semi-automated methods to study VT differences in cattle during summer and winter. Although a small number of cows were used, their studies revealed a trend in VT variation. On the basis of these studies (West *et al.* 2003; Nabenishi *et al.* 2011; Sakatani *et al.* 2012), we explored the effects of AT, humidity, and THI on VT of dairy cows in the present study. We found that AT and THI hardly affected the circadian rhythm of VT in autumn and winter. However, their effects on VT in summer were significant, and VT during both oestrus and diestrus increased significantly with increasing AT and THI ($P < 0.001$). However, the effects of AT and THI on VT during diestrus were greater than that during oestrus. This phenomenon could not be explained by the fact that greater quantities of heat are produced in reproductive organs with the development of follicles

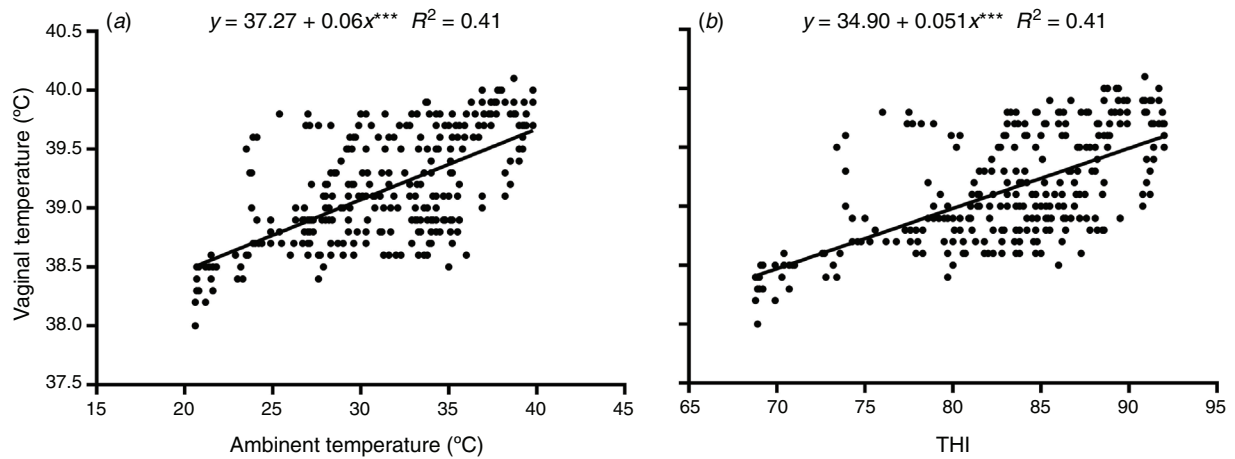


Fig. 5. Effects of (a) AT and (b) THI on VT during oestrus in summer. ***, $P < 0.001$ for the regression coefficients.

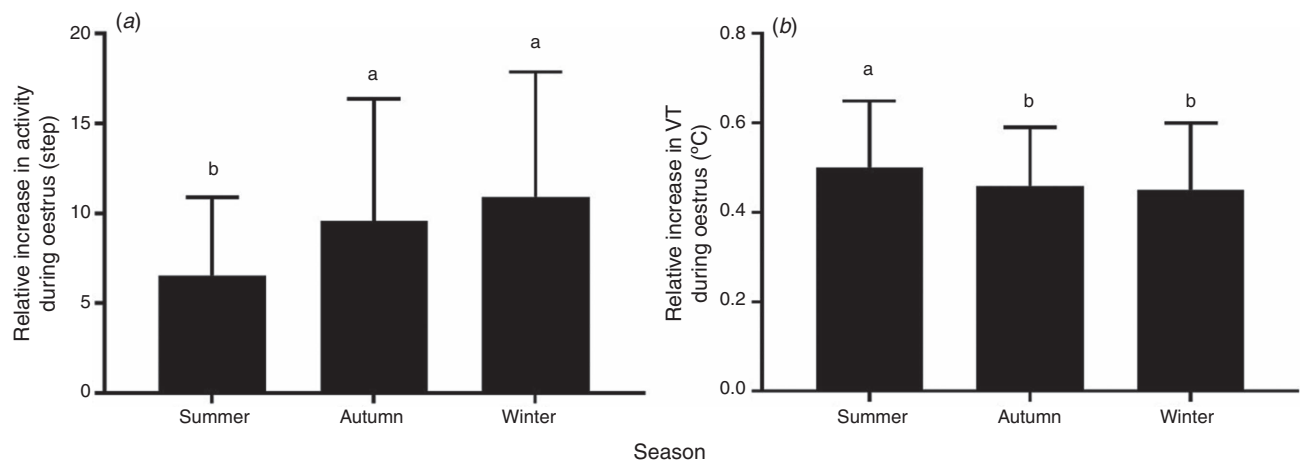


Fig. 6. Relative increase in (a) activity and (b) VT during oestrus in different seasons. Data are means \pm standard deviations (mean \pm s.d.). Different letters indicate significant difference ($P < 0.05$).

(Bowman *et al.* 2007), which may have caused a higher VT increase during oestrus. The mechanism should be explored further in the future.

The effect of environment on cow's VT also varies from different breeds. Sakatani *et al.* 2016 reported that an average increase of VT in Japanese black cows during oestrus was ~ 0.30 – 0.33°C in both hot and cool seasons. Differences in the average VT during oestrus and non-oestrus, and the increase of VT during oestrus between hot and cool seasons, were not significant. Our present study showed that the increases of VT during oestrus were all over 0.4°C in the three tested seasons. Both the average VT during non-oestrus and the increase of VT during oestrus in summer were significantly different from the values in autumn and winter. We postulate that the discrepancy is probably attributed to the distinct cattle breeds. The Holstein cows displayed a bigger increase of VT than that of beef cattle in summer, especially during oestrus may be explained by the fact of more vigorous metabolism of the former breed than the latter used in the study of Sakatani

and co-authors (Sakatani *et al.* 2016; Randi *et al.* 2018). We supposed that dairy cattle were more prone to heat stress than beef cattle, and should be given priority to protect against heat stress in summer.

Heat stress not only affects cow's VT, but also their oestrus performance. Rodtian *et al.* (1996) showed that the percentage of cows with clearly detectable oestrus in the cool season was 90% (63/70) and 92% (55/60) in two separate experiments. They were significantly higher than the values observed during the hot season (73% (44/60) and 74% (49/66) ($P < 0.05$), respectively). The results of the experiment indicate that the activity during oestrus was significantly reduced in the hot season. In our present study, the cows with less activity during oestrus were found in all three seasons, and were particularly higher during summer. Simultaneously, the activity of dairy cows during summer was lower than that in autumn and winter, and the relative increase in activity during oestrus in summer was significantly lower than that in autumn and winter ($P < 0.05$). However, the difference between autumn and

winter was not significant. The results indicated that heat stress weakened the dairy cows' oestrus performance in summer, and inhibited the increase in activity, thereby decreasing the oestrus detection rate. This result was consistent with those reported by López-Gatius *et al.* (2005), who speculated that oestrogen acts to induce oestrus, the heat stress reduces the secretion of oestradiol from cow follicles, then reduces the intensity of oestrus behaviour (López-Gatius *et al.* 2005). However, both the higher VT and increase of VT during oestrus in summer than in other seasons in our study showed that, using VT for oestrus identification will achieve better results.

As in the present study, the THI value has been used as a standard in many studies to determine whether animals are in a state of heat stress and to determine the degree of heat stress. Sometimes, THI over 68 was used as an index of heat stress in some studies (Bouraoui *et al.* 2002; Xue *et al.* 2010), whereas THI over 72 was used in other studies (Ravagnolo *et al.* 2000; West *et al.* 2003). However, superior facilities may alleviate the effects of heat stress on the animals, and judging whether the cows are under heat stress based only on environmental index is not an optimal method. The present study showed that the mean VT during diestrus in summer was significantly higher than that in autumn and winter ($P < 0.05$), but the activity of cows from 02:01 to 07:00 hours in summer was not significantly different from that in autumn and winter, during which the average VT was 38.4°C and the minimum THI was 68 (the AT was 20°C). At other times of the day during summer, the average VT and THI all exceeded 38.4°C and 68, respectively, whereas these values were recorded only in a few observations during autumn and winter. Therefore, it appears that cows are only heat stressed during the day when their body temperatures are elevated. The study indicates that combining VT and THI could reflect the state and degree of heat stress of cows more scientifically, which means that heat stress may be caused when THI is above 68 and VT of cows reaches 38.32°C. Evidently, when the THI exceeds 68 and VT is more than 38.32°C, measures suitable for reducing the effect of heat stress on the productivity of dairy cows should be taken. There are also other parameters related to heat stress, and the introduction of more parameters in future studies may lead to more scientific conclusions.

In conclusion, heat stress significantly affects VT and activity of dairy cows, further affecting dairy cattle production. Our findings provide the novel idea of combining *in vivo* measurements and environmental index calculations for monitoring heat stress, which might provide an accurate guidance for preventing and controlling heat stress.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

This work was supported by the National Key R&D Program of China (grant numbers 2017YFD0501903) and the Beijing Dairy Industry Innovation Team (grant numbers BAIC06–2017); we thank Editage (www.editage.cn) for English language editing and Beijing Le Dian Xinnan Technology Co., Ltd for technical support.

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Handling editor: John Gaughan