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Seasonal variation in body temperature as indicator of thermal stress in tropical dairy cows

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Abstract

Identifying suitable biomarkers in relation to climatic adversities appears important to minimise the harmful consequences of thermal stress (TS) on the health and productivity of farm animals. Accordingly, the association of body temperature (BT) with weather parameters and stress-associated serum factors were studied to assess the onset of TS in dairy cows. The diurnal pattern of BT among eight *post-partum* cows was recorded at biweekly intervals during the two seasons of highest and lowest temperature humidity index (THI). Daily recordings of weather parameters such as THI, ambient temperature (AT) and relative humidity, and weekly assessments of serum factors such as heat shock protein 70 (HSP 70), Malondialdehyde (MDA) and cortisol were carried out simultaneously. A highly significant ($p<0.01$) variation of BT was noticed between the two seasons, intervening months and across each time of daily recording. BT had a highly significant ($p<0.01$) association with the weather parameters, HSP 70 and MDA. However, the cortisol level showed no significant association with BT, indicating that it is regulated by other stress factors, in addition to TS. To conclude, BT can be used as an easily measurable indicator of TS in dairy cows,as it reflects AT reaching the upper limit for effective thermoregulation.

Keywords: Body temperature, Dairy cows, HSP 70, Thermal stress, Weather

Introduction

Thermal stress (TS) is a serious problem affecting lactating dairy cows, owing to their high rate of metabolism (Dikmen and Hansen, 2009). Increased secretion of milk causes excessive drainage of nutrients and necessitates intake of feed and water in large quantities to compensate for the nutrient loss, replenishment of the negative balance of the transition phase and additional needs for supporting pregnancy and/or growth as demanded by the management situations (Hansen, 2009). Intake of a large amount of ingesta adds to elevate the internal heat production consequent to digestion (including rumen fermentation) and associated metabolic processes, and precipitates the susceptibility of lactating animals to TS, especially when ambient temperature (AT) rises beyond the normal limits (Hansen, 2015).

The initial response of animals that are subjected to elevated thermal weather includes enhanced breathing and heart rate so that heat dissipation gets improved (Hansen, 2009). Further, an increase in surrounding temperature nearer to or beyond body temperature (BT), or reduced efficiency of heat dissipation mechanisms, causes an increase in BT. Subsequently, sweating and open-mouth breathing are initiated to enhance the evaporative heat loss, and long-term persistence of TS leads to suppression of the immune mechanism, making the animal susceptible to various diseases (Kadzere et al., 2002). Monitoring physiological indicators forms the preliminary step for assessing the approach towards the onset of TS-associated disruption of homeostasis and the consequences such as lowered production, impairment of reproduction and ill-health, even threatening the existence of animals depending upon the severity of temperature elevation (Mader et al., 2006; Allen et al., 2009).

The body temperature of the animal has been known to increase slightly in response to AT elevation (Marai et al., 2007). Understanding the exact pattern of diurnal variation in relation to weather parameters and association with biological indicators of TS appears important to understand the reliability of BT as the basis for management regulation, so that the harmful consequences of TS on the health and productivity of the animals can be minimized (Indu and Pareek, 2015). However, there is a dearth of studies describing the inter-relationship of BT with biological indicators of TS. Availability of such information may facilitate the usage of BT alone for the rapid assessment of

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TS in farm animals. Thus, the objective of the present study was to describe the diurnal pattern of BT variations across months and seasons of highest and lowest THI values and to understand its association with weather parameters and biological indicators of TS in cross-bred dairy cows under the prevailing climatic adversity of the region.

Materials and methods

The study was carried out at the Livestock Research Station, Thiruvazhamkunnu, under Kerala Veterinary and Animal Sciences University in India. The dairy farm of the station is located at an altitude of around 65 meters above mean sea level, positioned at 11°21' N and 76°21' E latitude and longitude, respectively. Cross-bred dairy cows managed under an intensive system with feeding and breeding as per standard recommendations (ICAR-NIANP, 2013) formed subjects for the study. Out of the cows calved each month and completed the early puerperal period uneventfully, four were randomly selected and subjected to the study for two months during their day 28 to 91 *post-partum.* Thus, eight animals were under study each month, of which four cows were sequentially replaced every month to ensure the prescribed *post-partum* period for the study.

The study was performed during the two seasons of highest and lowest THI prevailing in the locality (Kutty et al., 2019), such as post monsoon, falling from December to February and summer, comprised of March to May. The BT of the eight animals under the study were recorded manually by using a digital thermometer (Omron model MC 246®, Omron Health Care, Japan) at fortnightly intervals, collecting the data six times a day at an interval of 2.5 hours from 6.30 AM to 7 PM. There was no pathological BT elevation detected in the study animals during the study period.

Serum samples collected from the eight animals at weekly intervals throughout the study period were stored frozen until completion of the sample collection and thereafter subjected to ELISA for assessing the levels of stress factors such as HSP 70 and Cortisol using ELISA kits (Chongqing Biospes-China and Neogen-USA, respectively). In addition, the antioxidant level of serum was assessed through the estimation of Malondialdehyde by colorimetric assay using a spectrophotometer.

Daily ambient conditions of the study period were collected from a Hobo data logger (HOBO pro V2, Onset Computer Corporation, USA) installed within the barn. The data logger was set for hourly recording of AT and relative humidity (RH), and daily maximum and minimum values were arrived from the routine recordings. Daily average values of AT and RH were used for the calculation of temperature humidity index (THI) using the formula for livestock and poultry heat stress index (LPHSI, 1990) given as

$$THI(LPHSI) = T - \left(\left(0.55 - \frac{0.55 \times RH}{100}\right) \times (T - 58)\right)$$

Where T- Average temperature (in degrees Fahrenheit)
RH - Per cent relative humidity

Data were analysed using SPSS software for descriptive details, diurnal pattern, monthly and seasonal changes, and correlations between the study parameters, to understand the variation pattern of the BT (t-test) and its inter-relationship with weather parameters, as well as the stress indicators in serum and the results are discussed.

Results and discussion

The mean BT of the eight animals at six times of recording during the two seasons and the overall mean are given in Table 1. BT recorded at 9.00 am alone showed significant variation (p<0.05) between the two seasons. However, between animals, there was no significant variation for the mean values of BT in either of the two seasons, and the pattern of diurnal variation remained the same. Mean BT dropped from 6.30 am to 9.00 am, increased thereafter, and the peak was attained only by 4.30 pm before starting further decline. Bolocan (2009) also reported a highly significant variation of BT between 3 pm than at 7 am daily. The reduction of BT from 6.30 am to 9.00 am is contrary to the usual expectation of a steady increase of BT from morning to evening, corresponding to the diurnal variations of the weather parameters as reported by Guo et al. (2017) and Rashamol et al., (2018). This difference in the pattern appears to be due to milking operations between 5 am to 6 am and the associated increase in the activity in these animals. Elevation of BT occurs whenever internal heat production increases due to increased metabolism and /or affection of heat dissipation (Mishra and Palai, 2014).

Table 1. Mean ± SE (n=104) of body temperature at each time interval of recording compared between the two seasons

Recording time (Hours)	Seasons			t-value	p-value
	Post Monsoon	Summer	Both		
6.30	38.33 ± 0.05	38.72 ± 0.04	38.53 ± 0.03	1.525	0.218
9.00	38.16 ± 0.05	38.36 ± 0.03	38.26 ± 0.03	5.777*	0.017
11.30	38.44 ±0.04	38.83 ± 0.04	38.63 ± 0.03	0.183	0.669
14.00	38.74 ± 0.04	39.40 ± 0.05	39.07 ± 0.04	3.059	0.082
16.30	38.77 ± 0.06	39.59 ± 0.11	39.18 ± 0.07	3.130	0.078
19.00	38.54 ± 0.11	39.23 ± 0.05	38.88 ± 0.07	0.238	0.626
Mean	38.50 ± 0.04	39.02 ± 0.04	38.76 ± 0.03	0.209	0.648

Significant at 5 % level

The attainment of daily maximum BT during the afternoon can be due to the slow rate of heat dissipation when the outside temperature is higher, which is also aggravated by the enhanced breathing effort itself contributing to an increase in internal heat production. Since dairy animals are more comfortable at temperatures below 20 ºC (Kristyna et al., 2017), elevation of AT beyond the thermo-neutral zone leads to activation of breathing rate as the immediate response to maximise heat dissipation through exhaled air (Rashamol et al., 2018). Usually, the highest AT of the day is attained between 2 to 2.30 pm, irrespective of the region and declines thereafter (Kumar, 2013). Simultaneously, reduced heat dissipation together with increased breathing effort and associated metabolic processes cause the accumulation of internal heat. Even though heat dissipation improves when the AT starts declining after 2.30 pm, the highest body temperature recorded in the study was at 4.30 pm, attributable to the continuation of internal heat production out of enhanced breathing for a few more hours, as reported earlier by Macias-Cruz et al. (2016).

The monthly pattern of BT (Fig. 1) showed a decrease from December to January, followed by a continuous increase up to May, with highly significant ($p<0.01$) variation of the biweekly recordings between months and seasons. Such an increase from January onwards can be attributed to the marked elevation of AT during summer months as reported by De-Souza et al. (2016). Similarly, diurnal variations of BT across the two seasons (Fig.2) showed a reduction in the morning, followed by continuous increase up to 4.30 pm, extending beyond the period of highest AT reached at 2.30 pm, contributed by increased panting (Morris et al., 2011; Maibam et al., 2017). Despite similar diurnal patterns, the variation between the two seasons was highly significant, attributable to the marked elevation of AT during summer months, concurring with the earlier report of De-Souza et al. (2016).

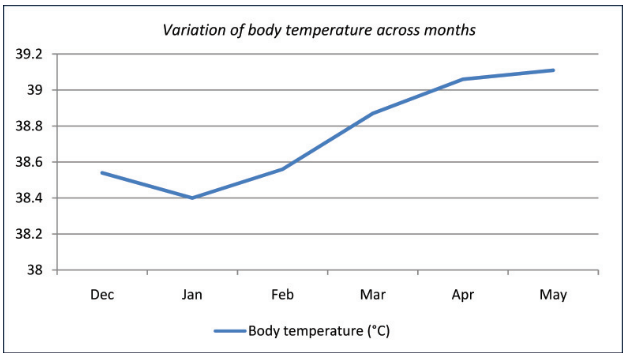

Figure 1. Monthly variations of mean body temperature

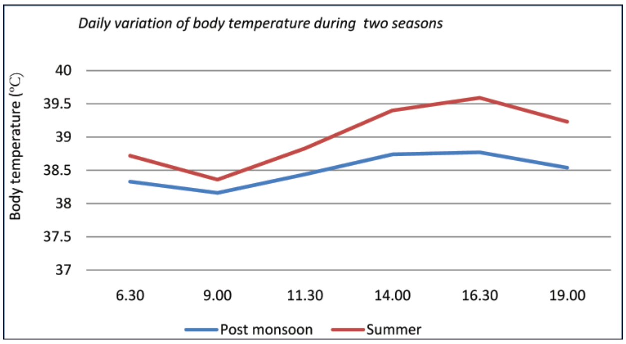

Figure 2. Daily variations of mean body temperature at the six times of recording during the two seasons

Strikingly, BT variation across 6 times of the daily recordings (Fig. 2) during the 6 months falling under the two seasons showed similarity of the pattern with that of monthly variation (Fig. 1). This can be attributed to the similarity of AT variation brought about by the rise and fall in the intensity of sunshine across the day and increase and decrease of day length across six months of the study respectively. Even though day length in the northern hemisphere continues to increase from December 21 onwards until June 21, the shrinking of the effective day length is caused by the onset of summer showers in the study locality during April or May, causing AT fall (Kutty, 2013) and is reflected in the recording of BT.

Weather Parameters:

Monthly and seasonal averages of AT, RH and THI recorded during the study period are shown in Table 2. Between the two seasons, RH ($p<0.05$) and THI ($p<0.001$) varied significantly with higher values during summer. The months of the highest AT and THI were April, while RH was the highest during May, attributable to the onset of rainfall during the month. THI and its constituent variables, AT and RH, were the highest during summer months, causing extreme stress to the animals. The relatively high AT of the locality with daily maximum temperature (MxT) of the summer months reaching around 40ºC and daily minimum temperature (MnT) remaining at a comparatively higher level, manifesting wide diurnal variation, makes the microclimate highly stressful to the animals. The lowest of AT and THI were during January while the lowest of RH was delayed to February because of the progressive drying of the atmospheric air with the elevation of AT. Thus post post-monsoon forms the season of minimal TS to the exposed animals. However, even during the post-monsoon, THI values of the study locality were beyond the comfort level (more than 72), indicating TS exposure of the animals throughout the year (Kutty, 2021).

TS indicators:

The occurrence of physiological stress in response to weather variations was assessed by comparing the weather parameters across the months and seasons with stress indicators in serum

Table 2. Mean + SE of monthly and season wise figures of ambient temperature, relative humidity and THI

Months	Monthly mean ± SE of			Seasons	Season wise mean ± SE of		
	AT (ºC)	RH (%)	THI		AT (ºC)	RH (%)	THI
Dec	28.70± 0.11	72.30± 0.48	79.54±0.17	Post monsoon	28.35± 0.16	66.53± 0.64	78.44± 0.26
Jan	26.82± 0.18	64.01± 0.97	75.90± 0.33				
Feb	29.90± 0.16	63.89± 1.07	80.30± 0.30				
Mar	31.41± 0.15	64.60± 0.98	82.60± 0.28	Summer	31.14 ± 0.14	71.30 ± 0.85	83.21± 0.16
Apr	32.07± 0.16	68.56± 0.67	84.21± 0.16				
May	30.16± 0.26	78.84± 1.23	82.89± 0.26				
Mean	29.74 ± 0.14	68.91± 0.55	80.83± 0.22	Mean	29.74± 0.14	68.91± 0.56	80.83± 0.22
F-value	105.78 **	40.85**	133.22**	t-value	2.63	5.68*	27.10**
p-value	< 0.001	< 0.001	< 0.001	p-value	0.106	0.018*	<0.001

** Significant (p<0.001) * Significant (p<0.05)

Table 3. Mean ± SE of HSP 70, Cortisol and MDA levels in the serum across months and seasons

Months	Monthly mean ± SE of			Seasons	Season wise mean ± SE of		
	HSP 70	Cortisol	MDA		HSP 70	Cortisol	MDA
Dec	2.73 ± 0.09	5.5 ± 0.36	2.46 ± 0.01	Post monsoon	3.11 ± 0.10	6.68 ± 0.29	2.46 ± 0.07
Jan	3.02 ± 0.11	7.08 ± 0.53	2.29 ± 0.08				
Feb	3.60 ± 0.27	7.37 ± 0.54	2.65 ± 0.19				
Mar	5.25 ± 0.84	11.67 ± 0.62	4.13 ± 0.23	Summer	5.59 ± 0.44	9.4 ± 0.29	3.85 ± 0.15
Apr	6.30 ± 0.92	8.99 ± 0.37	3.55 ± 0.31				
May	5.30 ± 0.62	7.94 ± 0.30	3.88 ± 0.25				
Mean	4.35 ± 0.24	8.05 ± 0.23	3.15 ± 0.09	Mean	4.35 ± 0.24	8.05 ± 0.22	3.15 ± 0.09
F- value	6.50**	18.87**	14.15**	t-value	15.57**	0.36	34.51**
p-value	<0.001	<0.001	<0.001	p-value	<0.001	0.55	<0.001

** Significant (p<0.001) * Significant (p<0.05)

such as HSP 70, Cortisol and MDA. Month-wise and season-wise averages of HSP 70, Cortisol and MDA levels in serum are shown in Table 3.

HSP 70 and MDA levels were significantly higher (p<0.001) during summer than post-monsoon, while the variation of Cortisol was non-significant between these two seasons. Even though cortisol elevation is associated with various forms of biological stress, the non-significant variation of cortisol level between the two seasons of significant THI variation (p<0.001), indicates that Cortisol is not a specific indicator of TS in cattle concurring the earlier reports of Prasad (2014) and Zarina (2016). Antioxidant level of serum indicated by MDA appears to be a better indicator, and HSP 70 is more specific as an indicator useful to assess the extent of TS suffered by the animal.

Correlation of HSP 70, Cortisol and MDA with weather parameters are shown in Table 4. While HSP 70, Cortisol and MDA levels were significantly (p<0.001) influenced by AT (p<0.001) and THI (p<0.001), there was no significant association of RH with any of the three biological indicators of stress. This implies that RH alone is not problematic and contributes to TS only with the elevation of AT. Mean value of MxT showed significant positive correlation with stress-associated biological factors, indicating the possibility of contributing TS even by warm dry air, while daily maximum relative humidity (MxRH) showed a minimal negative association with HSP 70 and MDA, may be attributable to the heat dissipation through exhaled moist air at moderate

Table 4. Correlation coefficient of HSP 70, Cortisol and MDA with weather parameters

Parameter	Weather parameters				
	AT	RH	THI	MxT	MxRH
HSP 70	0.280**	0.014	0.271**	0.211**	-0.057
(p-value)	(< 0.001)	(0.845)	(< 0.001)	(0.002)	(0.416)
Cortisol	0.261**	-0.082	0.215**	0.217**	-0.229**
(p-value)	(< 0.001)	(0.241)	(< 0.001)	(0.002)	(0.001)
MDA	0.389**	0.113	0.404**	0.144*	-0.158*
(p-value)	(< 0.001)	(0.103)	(< 0.001)	(0.038)	(0.022)

*. Significant (p<0.05);**. Significant (p<0.01)

elevation of AT. Rashamol et al. (2018) also reported the possibility of more passive heat transfer at ambient temperature within the thermo-neutral range. At the same time, there was a significant negative correlation of MxRH with Cortisol, further supporting the difference in the regulation of cortisol secretion by other factors rather than TS mentioned earlier.

Assessed values of BT were compared with the biological indicators of TS and climatic variables to find out the reflection of TS on this vital parameter. Mean BT showed a highly significant correlation (p<0.01) with all the stress-associated climatic and biological parameters except Cortisol. The difference in the association of cortisol with BT indicated the involvement of factors other than TS in the regulation of Cortisol secretion, as mentioned earlier. However, BT can be used as a preliminary indicator of TS in dairy animals, being an easily measurable biomarker having a significant correlation with TS-associated climatic variables and serum factors included in the study.

Table 5. Correlation of mean body temperature with stress associated climatic and biological variables

Physiological indicator	Stress associated climatic and biological variables							
	AT	RH	THI	MxT	MxRH	HSP 70	Cortisol	MDA
Mean BT	0.423**	0.318**	0.513**	0.148	-0.106	0.228**	0.086	0.226**
(p-value)	(< 0.01)	(< 0.01)	(< 0.01)	(0.033)	(0.127)	(< 0.01)	(0.215)	(<0.001)

**. Significant (p<0.01)

As depicted in Figs. 1 and 2, the animal suffers maximum TS between the periods of 2.00 to 4.30 pm on all the days, and the seasonal peak is during April and May during the year of study. Even during the post-monsoon months, THI is beyond 78 except during January, indicating the exposure of the study animals to TS throughout the year (Kutty et al., 2019; Kutty, 2021). Rhoads et al. (2009) observed that cows under TS were exposed to persistent elevation of AT for the entire day, even though the BT showed its peak during the afternoon, and the variation ranged from 38.7° to 40.2°.

Elevation of BT occurs whenever the heat production increases due to increased metabolism and /or affection of heat dissipation (Mishra and Palai, 2014). Marked elevation of BT has been reported to be associated with TS by Torres-Junior et al. (2008). A highly significant positive correlation of BT with HSP 70, MDA, THI, AT and RH shown in Table 5 indicates direct regulation of BT by the TS factors. On the contrary, Cortisol did not show a significant correlation with BT, showing its regulation predominantly by stress factors other than TS. Torres-Junior et al. (2008), Gaughan et al. (2013) and Maibam et al. (2017) also reported a moderate correlation of BT with HSP 70.

Mammals, being homeotherms, regulatetheir BT relatively constant and function well in spite of marked variations in AT. Accordingly, marked elevation of BTsimultaneous with the rise of surrounding temperature happens only in case of exceeding the limit for thermoregulation,leading to the disruption of homeostasis (Marai et al., 2007;Guo et al., 2017). Whenever increased breathing and peripheral blood flow fail to dissipate excessive heat load, elevation of BT gets initiated (Cain et al., 2006). Bolocan (2009) reported a BT rise of 1.5 to 2 degrees above the normal value in response to increasing AT, indicating high level of TS. Thus, BT forms a vital biomarker for assessing the onset of TS in animals, since BT elevation beyond the upper limit for effective thermoregulation indicates the onset of disturbances to homeostasis (Kadzere et al., 2002;Rashamol et al., 2018).

Conclusion

To conclude, BT of the animals showed highly significant (p<0.01) variation between the two seasons, months and even between each time of daily recording and was found to be regulated by climatic variables causative of TS such as THI, AT and RH. There was a highly significant association of BT variation with TS-associated serum factors as well such as HSP 70 and MDA. Hence, BT can be used as an easily measurable indicator of TS in dairy cows as it denotes the elevation of surrounding temperature beyond the limit for effective thermoregulation, disrupting homeostasis.

Declaration: The work was carried out as per the approval from the institutional committee of Kerala Veterinary & Animal Sciences University, Pookode.

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