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Comparison of breathing rate in cross bred dairy cows across two seasons of highest and lowest temperature-humidity index as an indicator of thermal stress under the humid tropical climate

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Abstract

Understanding the variations of biomarkers in relation to weather parameters and biological indicators of thermal stress (TS) appears important to minimize the harmful consequences of climatic adversities on health and productivity of the animals. Hence, diurnal pattern of breathing rate (BR) among eight postpartum dairy cows were compared across two seasons of extreme THI variations. Association of BR with daily recording of weather parameters and weekly assessments of stress associated serum factors were analyzed to find out the usefulness of this basic biomarker in determining the extent of TS suffered by the animal. Highly significant (p<0.01) variations of BR were noticed between the two seasons, months and even across each time of daily recording. The BR was also found to be influenced by weather parameters causative of TS such as THI, AT and RH and there was highly significant (p<0.01) association with TS associated serum factors such as HSP 70 and MDA. However, cortisol level was found to have no significant association with BR indicating the difference of its regulatory mechanism. To conclude, BR can be used as an easily measurable indicator of TS in dairy cows as it reflects the thermo-regulatory effort upon exposure to TS factors

Keywords: *Breathing rate, HSP 70, MDA, Thermal stress, Weather*

Thermal stress (TS) is a serious problem affecting lactating dairy cows, owing to their high rate of metabolism (Badinga *et al*., 1985, Dikmen and Hansen, 2009). Increased secretion of milk causes excessive drainage of nutrients and necessitate excessive intake of feed and water to compensate the nutrient loss, replenishment of the negative balance of the transition phase and additional needs for supporting pregnancy and/or growth demanded by the management situations (Hansen, 2009). Large amount of ingesta adds to elevate the internal heat production consequent to digestion(including rumen fermentation) and associated metabolic processes, and precipitate the susceptibility of lactating animals to TS especially when ambient temperature (AT)rises beyond the normal limits (Hansen, 2015).

The initial response of animals subjected to elevated thermal weather include enhanced breathing and heart rate so that heat dissipation gets improved (Hansen, 2009). Further, increase of surrounding temperature nearer to or beyond body temperature, or reduced efficiency of heat dissipation mechanisms causes hike of body temperature. Subsequently, sweating and open mouth breathing are initiated to enhance the evaporative heat loss, and long-term

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persistence of TS leads to suppression of the immune mechanism thereby making the animal susceptible to various diseases (Kadzere *et al*., 2002).Thus monitoring physiological indicators forms the preliminary step for assessing the approach towards TS associated disruption of the homeostasis and associated consequences such as lowered milk production, impairment of reproduction and affection of well-being to the extent of even threatening existence of the animals depending upon the severity of temperature elevation (Allen *et al*., 2009; Mader *et al.,* 2006).

Breathing rate (BR) of the animal has been known to increase in response to AT elevation (Marai *et al.,* 2007). However, understanding the exact pattern of diurnal variation of BR in relation to weather parameters and its association with biological indicators of TS appears important to understand the reliability of this simple biomarker as the basis of management and to minimize the harmful consequences of TS on health and productivity of the animals (Indu and Pareek, 2015). However, there is dearth of studies describing the inter-relationship of BR with biological indicators of TS so as to utilize the same for rapid assessment of TS in farm animals. Hence, objective of the present study was to describe the diurnal pattern of BR variations across months and seasons of extreme THI variations and to understand their relationship 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 Livestock Research Station, Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University, Kerala, India. Dairy farm of the station is located at an altitude of 60-70 meters above mean sea level, positioned 11°21’ N and 76°21’ E latitude and longitude respectively. Cross bred dairy cows managed under intensive system with feeding and breeding as per standard recommendations (ICAR-NIANP, 2013) formed the subjects of the study. Out of the cows calved each month and completed early puerperal period uneventfully, four were randomly selected and subjected to the study from day 28 to 91post-partum so that eight animals were under the study each month.

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. BR of the eight animals under the study was recorded as the number of breaths per minute by counting the flank movements and the recording was done at fortnightly intervals, six times a day at an interval of 2.5 hours starting from 6.30 AM to 7 PM. Serum samples were collected at weekly intervals from the eight animals throughout the study period and were stored frozen immediately for assessing the levels of stress factors such as HSP 70 and cortisol at one time for all the samples, using ELISA kits (Chongqing Biospes - China and Neogen – USA respectively). In addition, antioxidant level of serum was assessed through estimation of Malondialdehyde by colorimetric assay of thiobarbituric acid (TBA assay) using spectrophotometer.

Daily ambient conditions of the study period were collected from 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). Daily maximum and minimum recordings of AT and RH were also collected from the routine recordings of the weather parameters. Daily average values of AT and RH were used for calculation of temperature humidity index (THI) using the formula for livestock and poultry heat stress index (LPHSI) given as

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

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

Data were analysed for descriptive details, diurnal pattern and variation between months and the two seasons, and the correlations between study parameters using SPSS software to understand the pattern of the biomarkers and their inter relationship with weather parameters and stress indicators in the serum and the results are discussed.

Results and discussion

Mean BR of the eight animals at six times of recording during the two seasons and the overall mean are given in Table 1. Variations of the BR was found to be significantly high during summer than post monsoon at 2 pm ($p<0.001$), 11.30 am, 4.30 pm and 7 pm ($p<0.05$). Mean values of BR across the two seasons showed significant variation ($p<0.01$) attributable to the increased physiological effort of the animals to maintain the BT more or less uniform while the AT increases. The ability of homeotherms to regulate BT relatively constant in spite of marked variations of AT is facilitated by enhanced breathing contributed by evolutionary adaptation (Marai *et al.,* 2007,Guo *et al*., 2018).

Pattern of diurnal variation of the BR irrespective of the seasons showed a decreasing trend from 6.30 am to 9.00 am, there after elevated continuously to reach the highest at 2.00 pm and declined thereafter. Between animals there was no significant variation for the mean values of BR showing similar pattern of diurnal variation in both the seasons. Bolocan (2009) also reported 3 to 3.5 times increase of BR at 3 pm than at 7 am with highly significant variation of BR between the two timings. The reduction of BR from 6.30 am to 9.00 am is contrary to the earlier reports of steady increase in BR from morning

Table 1. Mean ± SE (n=56) of breathing rate at each time interval of recordings compared between the two seasons

Recording time (Hours)	Seasons			t-value	p-value
	Post Monsoon	Summer	Both		
6.30	36.09 ± 1.16	52.58 ± 1.38	44.33 ± 1.07	2.358	0.126
9.00	32.3 ± 0.95	39.58 ± 1.03	35.94 ± 0.75	1.323	0.251
11.30	48.35 ± 1.24	66.89 ± 1.64	57.62 ± 1.21	4.161*	0.043
14.00	59.11 ± 1.22	81.08 ± 2.01	70.09 ± 1.4	29.11**	0.000
16.30	57.02 ± 1.52	76.19 ± 1.94	66.61 ± 1.4	6.202*	0.014
19.00	55.88 ± 1.28	73.6 ± 1.74	64.74 ± 1.24	6.412*	0.012
Mean	48.12 ± 0.94	64.99 ± 1.27	56.55 ± 0.98	9.253**	0.003

*Significant at 5 % level ** Significant at1% level

to evening corresponding to the diurnal variations of the weather parameters (Guo *et al*., 2018, 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 of the activity in these animals.

The difference in the timings of attaining the daily maximum of BR during the afternoon can be due to increased breathing effort itself contributing to elevation of BT and the slow rate of heat dissipation when the outside temperature is more. Since dairy animals are more comfortable at temperature below 20 ºC, elevation of AT beyond thermo neutral zone leads to activation of breathing rate as the immediate response to maximize heat dissipation through exhaled air (Rashamol *et al.,* 2018).Usually, the highest AT of the day is attained between 2 pm to 2.30 pm and declines thereafter (Kumar, 2013). Simultaneously, reduced heat dissipation together with increased breathing effort and associated metabolic processes cause accumulation of internal heat and the active breathing effort is reduced when the AT starts to decline (Macias-Cruz *et al.,* 2016).

The pattern of BR variations across the six months and diurnal variations across the two seasons are shown in Fig. 1 and 2 respectively. The BR showed reduction from December to January followed by continuous increase upto April and declined thereafter. The pattern of monthly variation showed striking similarity with the diurnal variation showing a reduction in the morning, followed by continuous increase until 2 pm and decline thereafter. It is evident that BR closely followed the pattern of AT

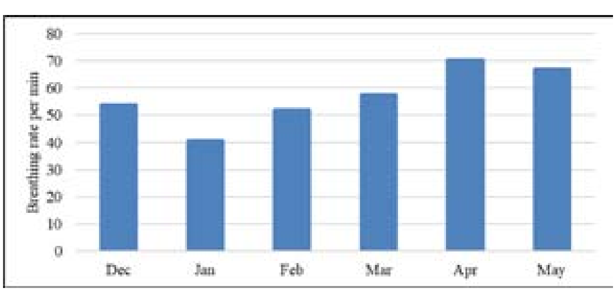

Fig. 1. Monthly variations of breathing rate across the six months

variation with the highest recorded in April on a monthly basis and around 2 pm on daily basis and declined thereafter. Slow rate of heat dissipation during elevated AT increases breathing activity contributing to the elevation of internal heat through increased metabolic activity as well as accumulation through slow dissipation pushing the animal into a vicious circle of thermal stress. Further, mean values of BR recorded at weekly intervals showed highly significant ($p<0.01$) variation between months and seasons with marked elevation in summer as reported by Morris *et al.* (2011).

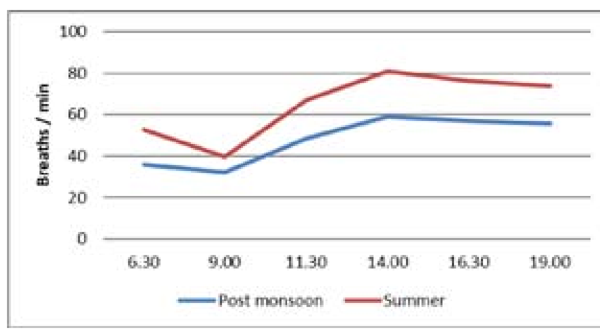

Fig. 2. Daily mean breathing rate at the six times of recording during the two seasons

The pattern of diurnal variation of BR compared between the two seasons in Fig. 2. Even though there was significant elevation of BR ($p<0.01$) from post monsoon to summer, the pattern of variation was similar during both the seasons with the lowest at 9 am and highest at 2 pm concurring the report of De-Souza *et al.* (2016). Strikingly, variation pattern of BR across 6 times of the day and across 6 months of the two seasons under the study were similar and is attributable to the similarity of AT variation brought about by the rise and fall in the hours of sunshine across the day and increase and decrease of effective day length across six months of the study. Even though day length in the northern hemisphere continue to increase from September 22 onwards until June 21, shrinking of the effective day length is caused by the onset of summer showers in the study locality during April or May causing fall of AT (Kutty, 2013) and is reflected in the monthly recording of BR.

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 highest AT and THI were April, while RH was highest during May attributable to onset of rainfall during the month. THI and its constituent variables AT and RH were highest during summer months causing extreme stress to the animals. Relatively high AT of the locality with daily maximum temperature (MxT) of the summer months reaching around 40ºC and daily minimum temperature (MnT) also at a comparatively higher level with wide diurnal variation makes the micro climate highly stressful to the animals. Lowest of AT and THI were during January while RH was lowest during February because of further drying of the atmospheric air by the elevation of AT. Thus, 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

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 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 high ($p<0.001$) 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, 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. Antioxidant level 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 in agreement with the earlier report of Mapham and Vorster (2013).

Correlation of HSP 70, Cortisol and MDA with weather parameters are shown in Table 4. While HSP

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 ($p<0.05$)

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 ($p<0.01$)

Table 5. Correlationcoefficient of breathing ratewith stress associated climatic and biological variables

Biomarker	Stress associated climatic and biological variables							
	AT	RH	THI	MxT	MxRH	HSP 70	Cortisol	MDA
Mean BR	0.553**	0.329**	0.636**	0.058	-0.135	0.291**	0.062	0.363**
(p-value)	(< 0.01)	(< 0.01)	(< 0.01)	(0.450)	(0.052)	(< 0.01)	(0.375)	(<0.001

**. Significant ($p<0.01$)

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 TS only with the elevation of AT, as already reported by Kadzere et al. (2002) and Kristyna *et al*. (2017). Mean value of daily maximum temperature 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 with the exhaled moist air at moderate elevation of AT. At the same time, there was significant negative correlation of MxRH with Cortisol, indicating the difference in the regulation of cortisol secretion by other factors rather than TS. Prasad (2014) also reported no statistical difference in the average plasma cortisol values in cows from different THI zones of Kerala.

Correlation coefficients of BR with the biological indicators of TS and climatic variables are compared in table 5, to find out the reflection of TS on this physiological indicator. Mean BR showed highly significant correlation ($p<0.01$) with all the stress associated climatic and biological parameters except cortisol. The difference in the association between cortisol and the physiological markers indicates the involvement of factors other than TS in the regulation of cortisol secretion as mentioned earlier. SinceBRis an easily measurable biomarker, having significant correlation with TS associated climatic variables and serum factors, it can be used as apparent indicators of TS in dairy animals. Indu and Pareek (2015) also reported BR to bea useful indicator of TS in animals.

As depicted in the figure 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 the months of April (Fig. 1). Even during the post monsoon months, the THI is beyond 78 except during January, indicating the exposure of these animals to TS throughout the year (Kutty *et al.,* 2019, Kutty, 2021). Correspondingly the BR is more than 50/min, except during the fore noon hours of post monsoon season and throughout the summer days except around 9 am. Further, the breathing rate went beyond 70/minute to the level of absolute panting during the afternoon hours throughout the summer months indicating TS exposure of the study animals. Rhoads *et al*., (2009) observed that cows under TS were exposed to persistent elevation of AbT for the entire day so that BR showed peak differences during the afternoon hours ranging from 46 to 82 breaths/min.

Cardio- respiratory systems of the animals were influenced by weather parameters such as AT, RH, solar radiation and day timings. Consequent to the elevation of AT, BR is increased as the first mechanism for dissipation of extra heat load through increasing evaporative cooling and thus to maintain homeostasis (El-Tarabany *et al.,* 2017). Increase of THI has been found to cause elevation of BR in dairy cows (Sailo *et al.,* 2017) irrespective of the breeds, since cows relied more on respiration as the means for heat dissipation (Rashamol *et al.,* 2018).Hence, BR appears to be an ideal biomarker for assessing TS influence in dairy cows as it reflects the thermoregulatory effort in response to elevation of AT.

Conclusion

In the present study, BR of the animals showed highly significant ($p<0.01$) variation between the two seasons, months and even between each time of recording. BR was found to be regulated by climatic variables causative of TS such as THI, AT and RH. Also, there was highly significant association of this biomarker with

TS associated serum factors such as HSP 70 and MDA. Hence BR can be used as easily measurable indicators of TS in dairy cows since BR reflects the thermoregulatory effort to enhance evaporative cooling by the animal in response to the elevation of TS prone climatic factors

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Conflicts of interest

The author has no conflict of interest to declare.

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