Thermal Stress Associated Serum Factors and the Influence of Weather Parameters in Crossbred Dairy Cows Under Humid Tropics

Cholakkal Ibrahem Kutty, Hiron Meloor Harshan, Bibin Bahuleyan Becha, Chirakkal Puthurmadathil Abdul-Azeez, Kanjirakkuzhiyil Promod and Chulliparambil Sunanda

ABSTRACT

Assessment of the level of thermal stress (TS) in animals exposed to adverse weather helps to formulate and implement alleviation measures. Present study was to identify the presence and seasonal pattern of serum factors to be used as biological indicators for early detection of TS in animals. The year-round study involved eight cows each at a time, belonging 28 to 91 days postpartum, and replaced two cows every month. Analyzed the levels of Heat shock protein 70 (HSP 70) and cortisol in serum samples collected at weekly intervals and assessed the seasonal pattern and correlation with weather parameters. While minimum temperature (MinT) of the region varied significantly (P<0.01) between seasons (lowest mean 22.09±1.19 °C), maximum temperature (MaxT) was consistently high across seasons (yearly mean 33.64±0.77 °C). Moderate to high (66 to 85 %) relative humidity (RH) was prevalent year-round, mainly contributed by the extended rainy season. Temperature-humidity index (THI) of the locality exceeded 78, exposing the animals to moderate to severe TS throughout the year. HSP 70 levels varied significantly (P<0.01) between seasons, with a significant hike (6.24±0.51 ng/mL) during summer (THI 83.20±0.53) and lesser elevation during other seasons of moderate THI, indicating its association with TS. Cortisol levels varied significantly (P<0.01) between seasons, however there was no significant correlation with THI and other weather parameters. To conclude, while cortisol forms a general indicator of physiological stress, HSP 70 appears more specific to TS, and its usefulness for early detection of TS needs further studies.

Keywords: Climate, HSP 70, Thermal stress, Weather

I. INTRODUCTION

Thermal stress forms one of the major impediments to increasing animal productivity, particularly in tropical regions with high ambient temperatures (AT). The IPCC has projected an increase in atmospheric temperature up to 0.2 °C per decade, reaching 1.5 °C above the level of the pre-industrial period between 2030 and 2050 [1]. At the same time, the hike in milk production over the years has made dairy animals more vulnerable to stress factors [2], [3].

In high-yielding dairy animals, while extremes of TS even threaten their survival, less severe stress affects the productivity and well-being of these animals and is manifested in the first instance as infertility [4], [5]. Many fertility issues affect crossbred dairy cattle in the tropical climate [5]–[7]. Such issues get precipitated by ongoing global warming and adverse weather conditions, resulting in a drastic increase in TS issues [8]–[10].

Even though well-defined seasons comparable to those in temperate countries are lacking in the tropical region, the seasonal pattern of reproductive performance has been well-established among farm animals [7], [11]. Photoperiodicity is the well-established regulator of reproductive seasonality, acting mainly through the pineal gland secretion [12]. Various other factors work indirectly [13], the major one being TS [8], [14], and are regulated by ambient temperature (AT) and RH [11], [15].

Various stress alleviation measures are incorporated into the management to minimize the drop in milk production and to restore normal physiological parameters [16], [17]. However, usual stress alleviation measures are of little benefit for avoiding infertility, owing to its early onset and long-standing consequences of TS on reproductive processes [18], [19]. Accordingly, interventions to minimize the upcoming and persistence of infertility due to weather adversities demand a precise understanding of the onset, severity, and pattern of TS, and necessitate suitable biological indicators for its early detection and management. Accordingly, the present study intends to identify salient determinants, seasonal patterns, and
biological indicators for early detection of TS in crossbred dairy cows reared in the humid tropical climate with more emphasis on the unique pattern of seasons [20] prevailing in the state of Kerala in India.

II. MATERIALS AND METHODS

A. Study Setting

The study was carried out at Livestock Research Station (LRS), Thiruvazhamkunnu belonging to Kerala Veterinary and Animal Sciences University in India. The location of the farm is at an altitude of 60-70 m above the mean sea level, with latitude and longitude positioning denoted by 11°21’ N and 76°21’ E, respectively. Due to adjacent mountains, the area has a warmer climate than the rest of the state. The dairy farm of the station maintains around 300 crossbred dairy cattle under an intensive system of management as per standard recommendations [21] formed the study settings. The year-round study involved a group of eight cows at any time, belonging to Day 28 to Day 91 postpartum (PP). The study group was maintained more or less uniform throughout the period, replacing two cows every month with recently calved cows.

B. Weather Parameters

Hourly recordings of AT and RH were obtained periodically from a data logger (HOBO Pro v2) installed within the animal house. Weather parameters such as maximum (Mx), mean (Av), and minimum (Mn) recordings of AT and RH were obtained, and THI values were calculated using the formula for Livestock and poultry heat stress index (LPHSI) given as:

\[
\text{THI (LPHSI)} = \frac{T - (0.55 - 0.55 \times \frac{\text{RH}}{100}) \times (T - 58)}{\text{where}}
\]

\[T – \text{Average temperature (in Degree Fahrenheit);}\]

\[\text{RH – Percent relative humidity.}\]

C. Seasonal Pattern

Divided the study period into four seasons based on the pattern of rainfall and day length occurring in the region such as Northeast monsoon, Post monsoon, Summer, and Southwest monsoon [5], [20]. The seasons arrived corresponded to four quarters of three months each comprised of September to November (SON), December to February (DJF), March to May (MAM), and June to August (JJA), respectively.

D. Stress Factors in Serum

Blood samples were collected (4 ml each) from the animals under the study at seven-day intervals. In cows exhibiting oestrus during the period, blood collection was delayed to the 7th day post oestrus and continued at weekly intervals. The serum samples were separated and stored (in duplicates) at -20 °C and analyzed using ELISA kits specific for HSP 70 (Chongqing Biospes Co Ltd, China) and cortisol (Neogen – USA). Accessories used for ELISA included a Microplate washer (Immuno wash - model 1575, Bio-Rad Laboratories, CA), Microplate reader (iMark- Bio-RAD laboratories), and microplate manager software (Bio-Rad Laboratories, CA). Estimated the concentration of HSP 70 in 544 serum samples. Cortisol was estimated from 445 serum samples (excluding 99 samples post oestrus) after performing ether extraction of each sample as per the kit instruction [22].

E. Data Analysis

The weather parameters and stress indicators in serum samples were analyzed for descriptive details, monthly, quarterly, and half-yearly variations, and correlation between the parameters using SPSS software (SPSS V. 24.0.). More emphasis was on identifying the determinants and seasonal pattern of TS and the usefulness of the HSP 70 and cortisol levels in the serum to predict the intensity of TS affecting the animal.

III. RESULTS AND DISCUSSION

A. Weather Features

Recorded maximum, minimum, and mean values of AT and RH, and the composite index THI showed considerable variation between days and months. The interaction of different weather parameters with determinants of seasonality in the region, such as day length and rainfall, is synergistic or antagonistic [5]. Hence, the combined influence of all these atmospheric factors in producing TS was assessed by comparing the patterns of AT, RH, and THI across seasons with more emphasis on THI.

All three weather parameters exhibited significant variation (P<0.05) between seasons (Table I). While MAM was the period of maximum THI contributed by the highest AT, DJF had the lowest THI attributable to lower quarterly mean AT and the lowest mean RH, being the period of shorter day length and minimum rainfall [20], [23]. Even though JJA formed the period having maximum day length and highest AT in the northern hemisphere [24], the quarter had the highest mean value for RH and the lowest AT in this study, since the period coincides with maximum rainfall in the locality [20], [25]. At the same time, SON maintained a moderate level for all the three weather parameters under consideration, contributed by lesser rainfall and declining day length [12], [23].

B. THI as an Indicator of Thermal Stress

Proportionate contribution to THI appears to be more from AT, indicated by the highest THI (83.20) during MAM with the highest AT, even though RH was not high enough during the period. In addition, high THI (>80) was also evidenced during the periods of moderate AT with moderate RH (SON) and lower AT with the highest RH (JJA).

<table>
<thead>
<tr>
<th>Weather parameters</th>
<th>Daily mean values of the season</th>
<th>F value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT (°C)</td>
<td>28.66 *</td>
<td>28.40 *</td>
<td>31.12 *</td>
</tr>
<tr>
<td>RH (%)</td>
<td>78.90*</td>
<td>66.22 *</td>
<td>71.17*</td>
</tr>
<tr>
<td>THI</td>
<td>80.61 *</td>
<td>78.47 *</td>
<td>83.20 *</td>
</tr>
</tbody>
</table>

TABLE I: DAILY MEAN VALUES OF AMBIENT TEMPERATURE (AT), RELATIVE HUMIDITY (RH), AND THI OF FOUR SEASONS RECORDED WITHIN THE ANIMAL HOUSE
Increased contribution of AT to THI is also evidenced by the higher correlation coefficient of THI (Table II) with the daily average of AT (0.930, P < 0.001) compared to RH (0.412, P = 0.015). Across seasons, the variation pattern of mean RH was opposite to that of THI, while the pattern for AT and THI was similar [20]. Further, during all the seasons, THI was above the upper limit (68-72) of the comfort level [26], indicating exposure of animals in the locality to TS throughout the year, which was also evidenced in the retrospective study already reported [5].

Table II shows the correlation of THI values with weather parameters such as daily mean, minimum, and maximum values of AT and RH. Besides daily mean AT, MnT showed a highly significant (P<0.001) positive correlation with THI, indicating more influence of MnT on THI, thus affecting the thermal comfort of animals in the tropical climate. Similar to the observation, the elevation of MnT forms one of the major determinants of TS-interfering heat dissipation, as reported by [7]. Elevation of MnT occurs during morning hours, affecting the animals through reduction of evaporation to eliminate the excess heat load during the days of high AT. However, the absolute elevation of MnT alone may not produce a major hike in TS unless accompanied by the elevation of MxT without or with RH.

The highest MnT occurred during MAM, coinciding with the period of maximum day length and the highest AT, widening the range and duration of exposure to elevated AT daily, leading to more TS, concurring with the report by Orihuela [27]. The yearly average of MnT(ºC) was 24.35±0.55, and the variation between seasons was significant (P<0.05), with the highest (26.30 ± 0.66) and lowest (22.09±1.19) mean values during MAM and DJF, respectively.

There was no significant association of THI with MxT and MxRH (Table II) attributable to the inverse relationship between these two variables. Elevation of MxT suppresses RH through alteration of air density and chances of evaporation [28], [29] unless intervened by rain. The highest daily MxRH was during JJA, coinciding with heavy rainfall, which also act to lower the AT. Even though the highest quarterly mean value of MxT was during MAM, the variation between quarters was not significant (Table III), showing the prevalence of high AT throughout the year. Correspondingly, the THI was very high during all the seasons (more than 78), exposing the animals to TS irrespective of the seasons in the locality [5], [26].

Even though the variation of MxRH between seasons was highly significant (P<0.001), the lack of a significant correlation of the same with THI is further indicative of the lower contribution of RH towards THI. However, despite no significant variation of MxT between quarters, the THI showed significant (P<0.05) seasonal variation contributed by remarkable elevation of MxRH as the result of increased rainfall in JJA [20], [30].

### C. Range of Daily Variation

AT is the primary determinant of THI and the extent of daily variation between MxT and MnT appears crucial in deciding the severity of TS [27], [31]. Strikingly, the differences between MnT and MxT as well as MxRH and MxRH had similar seasonal patterns with the lowest and highest variations during JJA and DJF, respectively (Fig. 1). During JJA with the highest mean RH, the range of variation was less, indicating persistence of elevated RH and more chances of TS [15]. At the same DJF was the period of lowest mean values for AT and RH. However, the range of variation was more comprehensive, contributing more TS even during that period [20].

Similar to MxT, the differences between MnT and MxT were not significant between seasons, indicating the persistence of high AT throughout the year. However, the extent of daily variation of AT and RH showed an inverse seasonal pattern with day length.

During the period of longer days, the extent of daily variations of AT and RH was minimum and vice versa.
TABLE IV: AMBIENT TEMPERATURE, RELATIVE HUMIDITY, AND THI OF HALF YEARS, ARRIVED BASED ON DAY LENGTH AND RAINING PATTERN

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Period (Months)</th>
<th>AT (°C)</th>
<th>RH (%)</th>
<th>THI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short days</td>
<td>(Sep to Feb)</td>
<td>28.54</td>
<td>72.56</td>
<td>79.54</td>
</tr>
<tr>
<td>Long days</td>
<td>(Mar to Aug)</td>
<td>29.45</td>
<td>78.24</td>
<td>81.78</td>
</tr>
<tr>
<td>Rainy season</td>
<td>(Jun to Nov)</td>
<td>28.23</td>
<td>82.10e</td>
<td>80.37</td>
</tr>
<tr>
<td>Rain pattern</td>
<td>Non-rainy</td>
<td>(Dec to May)</td>
<td>29.77</td>
<td>68.70</td>
</tr>
</tbody>
</table>

Values with superscripts vary significantly (P<0.01) between the half-years.

Instead of an absolute rise in AT alone or simultaneous with RH during the long-day periods (March to August), an increased range of variation was the primary reason for TS [27, 31] during other periods of medium and shorter day length (September to February). Thus the animals were exposed to TS throughout the year [5].

In most other parts of the sub-tropics, there occur distinct periods of high and low AT corresponding to the periods of long and short day lengths [15], [26]. However, the state of Kerala has a distinct climate regulated by the long period of rainfall [20], [25]. Accordingly, a comparison of the weather parameters across the two halves of a calendar year defined by day length and the prevalence of rainfall is shown in Table IV. RH showed significant variation (P<0.01) between rainy and non-rainy half-years. In contrast, AT and THI were almost similar across these two half-years. Even though AT gets elevated in the northern latitude during the long days, heavy rains in Kerala during the period JJA [23], [25], lowers the temperature. Accordingly, the mean AT of the long-day half-year becomes almost similar to that of the short-day half-year period, as evidenced in the study.

In most tropical regions, high THI causing moderate to severe TS and low THI within the comfort level of animals [32], [33] occur in separate half years. However, consequent to the early monsoon rainfall in Kerala, high RH and low AT cause reduced THI during JJA despite the day length increasing more than that of MAM [5]. Even though RH becomes slightly less during the other seasons, AT remains elevated, maintaining moderately high THI throughout the year [12]. DJF forms the winter season in the sub-tropics and temperate regions of the Northern Hemisphere [27]. In contrast, AT in Kerala does not fall into the cooling range even during DJF to designate the period as winter and is attributable to the latitude proximity to the equator. These features make the Kerala climate unique [20] and very adverse for high-producing animals, since the THI remains high at a level to cause mild to moderate stress throughout the year.

D. Biological Indicators of Thermal Stress

The biological response of animals exposed to TS-prone climate was assessed based on a comparison of HSP 70 and cortisol levels in serum with weather parameters. Values of HSP 70 in 544 samples ranged from 0.17 to 31.99 ng/mL, with a mean value of 3.54±0.16 ng/mL. Monthly, quarterly, and half-yearly averages of HSP 70 showed (Table V) highly significant variation (P<0.001) across these periods. A marked increase in HSP level was noticed among the seasons during summer (6.24 ng/mL), to almost three folds, compared to other seasons, which had more or less similar values.

The overall mean value of serum cortisol in 445 samples was 8.27±0.20 ng/mL falling within the normal range, even though the level was higher than the expected mean value of 4.5 to 5.1 ng/mL [34]. Comparison of mean cortisol values between months, quarters, and half years is shown in Table VI. The highest mean value of MAM (9.44±0.25 ng/mL) did not vary significantly from SON. Similarly, the lowest mean value was during DJF (6.84±0.61 ng/mL) and showed non-significant variation from the mean cortisol level during JJA.

Thus, even though the highest levels of HSP 70 and cortisol were in MAM, the pattern of variation was different during other seasons (Fig. 2), and the correlation between HSP 70 and cortisol levels across the seasons was non-significant.

The correlation of HSP 70 and cortisol levels with weather parameters is shown in Table VII to assess the biological influence of TS. Both HSP 70 and cortisol varied significantly between months, with the highest mean values coinciding with the period of high AT and THI. Also, there was a highly significant positive correlation of HSP 70 with AT (P<0.01) and THI (P<0.01), indicating the involvement of these factors in the causation of TS in the study animals, concurring with the report of [35]. However, despite some similarities with the variation pattern of RH, cortisol levels did not show a significant correlation with any of the weather parameters. This finding is in agreement with the earlier reports [18], [36], [37], indicating the involvement of some factors other than TS in regulating serum cortisol.

TABLE V: MEAN ± SE OF SERUM HSP 70 (NG/ML) ACROSS MONTHS, QUARTERS, AND HALF YEARS CATEGORIZED BASED ON DAY LENGTH AND RAINFALL PATTERN

<table>
<thead>
<tr>
<th>Month</th>
<th>Sample size</th>
<th>Mean ± SE of serum HSP 70 levels for the Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep</td>
<td>26</td>
<td>1.59±0.30</td>
</tr>
<tr>
<td>Oct</td>
<td>50</td>
<td>2.40±0.46</td>
</tr>
<tr>
<td>Nov</td>
<td>48</td>
<td>2.05±0.11</td>
</tr>
<tr>
<td>Dec</td>
<td>45</td>
<td>2.48±0.12</td>
</tr>
<tr>
<td>Jan</td>
<td>63</td>
<td>2.65±0.11</td>
</tr>
<tr>
<td>Feb</td>
<td>50</td>
<td>2.79±0.14</td>
</tr>
<tr>
<td>Mar</td>
<td>34</td>
<td>5.53±0.81</td>
</tr>
<tr>
<td>Apr</td>
<td>47</td>
<td>7.04±1.05</td>
</tr>
<tr>
<td>May</td>
<td>61</td>
<td>6.02±0.75</td>
</tr>
<tr>
<td>Jun</td>
<td>44</td>
<td>3.40±0.27</td>
</tr>
<tr>
<td>Jul</td>
<td>41</td>
<td>2.69±0.18</td>
</tr>
<tr>
<td>Aug</td>
<td>50</td>
<td>2.85±0.19</td>
</tr>
<tr>
<td>Overall</td>
<td>544</td>
<td>3.54±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DOI: http://dx.doi.org/10.24018/ejvetmed.2023.3.6.109
and cortisol (Fig. 3), further indicating the difference in their

**.Significant (P<0.05), **.Highly significant (P<0.01)

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Fig. 3. Comparison of HSP 70 and Cortisol values in the serum.

The periods of lowest mean values were different for HSP and cortisol (Fig. 3), further indicating the difference in their regulatory factors [18]. Thus, it could be inferred that cortisol forms an indicator of composite stress and is not specific for TS, in agreement with Binsiya et al. [38]. Whereas HSP 70 has a highly significant correlation with AT and THI, to be considered a specific indicator of TS, in agreement with the earlier reports [35], [39].

Elevation of MnT usually occurs during morning hours, involves a narrow range of variation, and contributes to TS along with other variables. However, the negative association of MnT with HSP 70 (P<0.05) can be due to the lesser intensity of TS contributed solely by the elevation of MnT. HSPs are considered the potential mediators of animal adaptation to TS [40], [41]. Hence a response in the form of HSP elevation can be expected only at higher AT beyond the level of animal adaptation. However, the elevation of MnT falls within the range of adaptation that a response in the form of HSP elevation cannot be expected. At the same time, the negative association of MxRH with HSP 70 can be due to the association with rainfall, which also lowers the AT, minimizing the chances of TS.

Across the periods of comparison, such as months, quarters, and two-half years based on day length and rainfall pattern, there was a highly significant variation (P<0.001) of serum HSP 70 level. Such variation indicates that the increased response of the animals to elevated AT is by producing HSP 70 in the system, concurring with earlier reports [34], [35], [42]. Further, a significant correlation of HSP 70 with all the major climatic determinants of TS, such as MnT, MxRH, daily mean AT, daily mean RH, and THI, indicates the possibility of using HSP 70 as a biological marker for early detection of TS. Min et al. [43] also recommended HSP 70 as a potential biomarker to supplement THI and evaluate moderate TS in dairy cows.

Considerable elevation of HSP 70 was observed only during the season of the highest TS. During the other three seasons with moderately high AT and THI exceeding 78, the

** Highly significant, Means with different superscripts varied significantly between quarters.

TABLE VI: MONTHLY, QUARTERLY, AND HALF YEARLY MEAN VALUES OF SERUM CORTISOL (NG/ML) IN CROSSBRED DAIRY COWS

<table>
<thead>
<tr>
<th>Month</th>
<th>Sample size</th>
<th>Mean ± SE of serum cortisol across the periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Months</td>
<td>Quarters</td>
</tr>
<tr>
<td>Sep</td>
<td>23</td>
<td>8.07±0.60</td>
</tr>
<tr>
<td>Oct</td>
<td>38</td>
<td>9.14±0.52</td>
</tr>
<tr>
<td>Nov</td>
<td>33</td>
<td>9.03±0.46</td>
</tr>
<tr>
<td>Dec</td>
<td>36</td>
<td>5.37±0.34</td>
</tr>
<tr>
<td>Jan</td>
<td>40</td>
<td>8.76±1.53</td>
</tr>
<tr>
<td>Feb</td>
<td>29</td>
<td>6.05±0.33</td>
</tr>
<tr>
<td>Mar</td>
<td>33</td>
<td>11.49±0.58</td>
</tr>
<tr>
<td>Apr</td>
<td>46</td>
<td>9.53±0.38</td>
</tr>
<tr>
<td>May</td>
<td>50</td>
<td>0.08±0.28</td>
</tr>
<tr>
<td>Jun</td>
<td>37</td>
<td>9.64±0.47</td>
</tr>
<tr>
<td>Jul</td>
<td>38</td>
<td>9.31±0.40</td>
</tr>
<tr>
<td>Aug</td>
<td>42</td>
<td>4.81±0.47</td>
</tr>
<tr>
<td>Total</td>
<td>445</td>
<td>8.27±0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Highly significant, Means with different superscripts varied significantly between quarters.

TABLE VII: CORRELATION OF HSP 70 AND CORTISOL WITH WEATHER PARAMETERS

<table>
<thead>
<tr>
<th>Stress indicators</th>
<th>THI</th>
<th>AT</th>
<th>RH</th>
<th>MxT</th>
<th>MnT</th>
<th>MxRH</th>
<th>MnRH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(p-value)</td>
<td>(p-value)</td>
<td>(p-value)</td>
<td>(p-value)</td>
<td>(p-value)</td>
<td>(p-value)</td>
<td>(p-value)</td>
</tr>
<tr>
<td>HSP 70</td>
<td>0.701**</td>
<td>0.752**</td>
<td>-0.256</td>
<td>0.494</td>
<td>-0.619*</td>
<td>-0.619*</td>
<td>-0.187</td>
</tr>
<tr>
<td>(p-value)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.422)</td>
<td>(0.102)</td>
<td>(0.032)</td>
<td>(0.032)</td>
<td>(0.562)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.314</td>
<td>0.358</td>
<td>-0.159</td>
<td>0.313</td>
<td>-0.186</td>
<td>-0.186</td>
<td>-0.147</td>
</tr>
<tr>
<td>(p-value)</td>
<td>(0.320)</td>
<td>(0.254)</td>
<td>(0.623)</td>
<td>(0.323)</td>
<td>(0.562)</td>
<td>(0.562)</td>
<td>(0.648)</td>
</tr>
</tbody>
</table>

* Significant (P<0.05), ** Highly significant (P<0.01).
elevation of HSP 70 was not very high, and the levels were more or less similar. Even though there was no distinct association of HSP 70 levels with mean values of THI, daily mean AT, and RH during the three seasons, there was a positive relationship between monthly mean values of HSP 70 with AT (P=0.005) and THI (P=0.011), indicating the usefulness of HSP 70 for early detection of TS, concurring the report of Archana et al. [44]. However, for a better inference, there is a need to compare the HSP 70 levels during a period having no TS at all, since there was not a single day with THI less than 72 during the study period.

IV. CONCLUSION

AT, RH, and THI of the study locality were compared between seasons to understand the combined influence of various climatic factors contributing to TS in animals. Across seasons, THI was more than 78, indicating exposure of the animals to TS throughout the year. Prevalence of such high THI was mainly contributed by moderately high AT, evidenced by the persistence of elevated MxT throughout the year (mean value 33.64 °C) with non-significant variation across seasons. Even though MxT varied significantly between seasons (lowest mean 22 °C), the daily mean AT did not fall below 27 °C in any of the seasons. In addition, there was moderate to high RH (66 to 85%) contributed by the extended rainy season in the area, and even in those seasons with slightly lower AT, THI was high enough so that the animals were suffering moderate to severe TS throughout the year.

Primary determinants of TS such as THI and AT showed significant association with HSP 70 to be considered a biological indicator of TS in animals. Further, the HSP 70 level varied significantly between months, quarters, and half years with a quantum hike (6.24 ng/mL) during the period of maximum TS (MAM), indicating the role of HSP in the process of adapting to extreme TS. During the other three seasons of moderate THI, the elevation of HSP 70 was more petite, which might be due to the lower levels of TS, to which the animal has already adapted. Cortisol levels also varied significantly between seasons, but there was no significant correlation with the determinants of TS studied, including THI. Thus, even though cortisol is an indicator of general physiological stress, HSP 70 appears to be more specific as an indicator of TS. However, its usefulness for early detection of TS necessitates further studies.

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DECLARATION OF ANIMAL RIGHTS

The study was performed with due consideration for animal rights.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES


