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Reassessment of temperature-humidity index for measuring heat stress in crossbred dairy cattle of a sub-tropical region

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ABSTRACT

The temperature-humidity index (THI) has been extensively applied for assessing heat stress in moderate to hot conditions in dairy cattle. However, there exist wide variation between researchers in defining an appropriate range of THI values for denoting different levels of stress. The present study was aimed to reassess previously described heat stress indicators of dairy cattle of sub-tropical region of India. From comparative evaluation of meteorological data over previous four years (2014–2017) the period of year when high THI prevailed in the region was determined. Accordingly, the time period of sample collection and observation on animals was decided, so that a THI range of 68–86 could be covered. After analyzing physiological, biochemical parameters and expression profile of heat shock response (HSR) genes of animals in response to different THI, it was evident from the study that animal undergoes few or little changes at THI 72, but major physiological changes occurred after THI reached 74. At THI range 74–79, no drastic change in these parameters occurred suggesting animals undergo transient acclimatization in this range to maintain homeostasis. Once THI reached and crossed 80, this homeostasis was perturbed and animals experienced major physiological changes again. Overall, the study suggests that THI values indicating level of heat stress are dependent on the geographic location, as well as type of animal and therefore, existing THI should be recalibrated for different climatic region for accurate assessment of the heat stress.

1. Introduction

Climate is one of the limiting factors in dairy bovine production. Livestock being a biological entity is inherently sensitive to climate variability that alter homeostasis and perpetrate stress upon animal. Cattle as homeotherms are subjected to stress with sudden and abnormal rise or fall in environmental temperatures. The bovine thermal comfort zone is with a body temperature between 36.7 °C and 39.1 °C (Cunningham, 2002). Heat stress in livestock can be defined as an animal's inability to dissipate sufficient heat to maintain homeothermy, which is caused primarily due to high air temperature, but intensified by high humidity, thermal radiation, low air movement, and by metabolic heat. High ambient temperature accompanied by high air humidity, causes discomfort and enhances the stress level, which in turn results in depression of the physiological and metabolic activities of the animals (Ganaie et al., 2013). With unprecedented rates of Earth's climate change raising global temperature (IPCC, 2007), negative effects of heat stress are expected to accentuate further and are likely to be one of the main challenges for animal production.

A variety of indices were used to estimate heat stress in dairy cattle. The temperature-humidity index (THI), proposed by Thom (1959) has been extensively applied for assessing heat stress in moderate to hot conditions. The index uses dry bulb (T_{db}) and wet bulb (T_{wb}) temperatures to estimate the magnitude of heat stress but excludes the influence of solar radiation and wind speed. Even with recognized limitations related to airspeed and radiation heat loads (NOAA, 1976), original THI and several variations of it have been used extensively to estimate heat stress level of dairy cattle (Mader et al., 2006; Bohmanova et al., 2007). However, there exist variations among researchers in defining appropriate values or range of THI for denoting different levels of stress. According to Du Preez et al. (1990), at THI range between 35 and 72, milk production is not affected by heat stress but a range of 72–78 could seriously affect milk production and the range 78–82 could be dangerous affecting the performance of animals severely (Brown-Brandl et al., 2003; Broucek et al., 2009). Armstrong (1994) described THI < 71 as a thermal comfort zone, 72–79 as mild heat stress, 80–90

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as moderate heat stress, and > 90 as severe heat stress. Considering THI value 71–72 as critical point for onset of heat stress has been challenged by subsequent studies. Recently, De Rensis et al. (2015) defined THI < 68 as safe and outside the thermal danger zone for cows. THI range of 68–74 has been denoted as zone of mild stress and THI ≥75 as zone of severe stress causing drastic decreases in production performance (De Rensis et al., 2015). There exist breed differences in thermotolerance as Zebu (*Bos indicus*) cattle have been shown to have higher thermoregulatory ability compared to *Bos taurus* cattle (Pereira et al., 2014). Smaller size with low metabolic rates resulting from reduced growth rates and milk yields of zebu breeds has been attributed as a major contributing factor to thermotolerance of zebu cattle (Hansen, 2004). It appears that THI as indicator of heat stress is dependent on the geographic location, as well as cow breed and physical size (Polsky and von Keyserlingk, 2017). Depending upon environmental conditions of a region and type of animals, the correct range of THI has to be calibrated for defining different levels of heat stress (Mader et al., 2006). The present study was aimed at reassessment and validation of erstwhile temperature-humidity index (THI) for crossbred cattle of a sub-tropical region of India.

2. Materials and methods

2.1. Animals and sampling

A pool of 33 animals, maintained at experimental herd of the institute and farm owned by farmers, were selected for the experiment. The animals were crossbred of *Bos taurus* (Holstein Friesian) × *Bos indicus* (Hariana/Tharparkar) in their third to fifth parity and early to mid-lactation stages. The animals were monitored continuously, but recording of physiological parameters and blood sample collection were done only on test days. The selection of a test day was based on web based weather forecasting services (https://www.accuweather.com; https://www.timeanddate.com; https://www.weather.com) and actual THI at time of sample collection was calculated from real time meteorological data of weather data logger installed in the sheds. . For any test day, six animals were selected randomly from the pool and data were collected on environment variables, physiological, and biochemical parameters of these animals. The collection of samples were repeated at least thrice (three test days) for each THI interval. All the animal experiments were approved by Institutional Animal Ethics Committee (IEAC).

2.2. Calculation of THI

The environmental parameters during the experimental period were monitored through Weather Data Loggers and wireless data monitoring system (Testo- Saveris, Testo SE & Co. KGaA, Germany). Temperature Humidity Index (THI) was calculated from environmental variables using the following equations: (NRC, 1971). (Johnson et al., 1963),

$$THI_1 = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$$

$$THI_2 = (T_{db} + T_{wb}) \times 0.72 + 40.\ 6$$

where.

T_{db} = Dry bulb temperature (°C); RH = Relative humidity (%); T_{wb} = Wet bulb temperature (°C).

Both THI_1 and THI_2 were employed to calculate THI of previous years from data on environmental variables recorded at Agrometeorology section of the institute. THI during experiment were calculated using THI_1 from data recorded by weather data loggers installed at experimental animal sheds.

2.3. Infrared thermography

The skin temperatures of different regions of animals were analysed through a thermal imager (Testo 875-1i Thermal Imager; Testo SE & Co. KGaA, Germany). The thermal imager used operated in the 8- to 14-μm spectral range and was calibrated to measure temperature from −30 °C to 100 °C. Capturing images of animals and image processing were done as per previously described guidelines (Cardoso et al., 2015; Byrne et al., 2017). Animals were photographed in shed maintaining 1.5 m distance between animal and camera. Animals were imaged from frontal, lateral and dorsal planes using the laser point guide of the imager. The images were analysed by IR-Soft software (Testo SE & Co. KGaA, Germany). The thermal images were corrected and adjusted for corresponding ambient temperature and relative humidity as recorded in weather data logger during time of photography. The thermographs were adjusted to an emittance coefficient equal to 0.97 (Poikalainen et al., 2012). Different regions of the animal body were marked with the polygonal selection tool of the software. Average temperature, temperature profile histogram and hot-/cold-spots (highest and lowest temperature, respectively) of the corresponding area were then analysed with the software.

2.4. Measurement of haematological and biochemical parameters

For measuring haematological and biochemical parameters, about 10 ml of blood was collected in two separate vacutainers containing either EDTA or sodium fluoride (for estimation of blood glucose) as an anticoagulant. The samples were transported to the laboratory under refrigeration and were stored at 4 °C until further analysis.

Haemoglobin (Hb%), Packed Cell Volume (PCV%), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Blood glucose and Haematocrit values of whole blood were measured in a semi-automated blood analyser (Shenzhen Mind Ray Biomedical Electronics Ltd). For estimation of different biochemical parameters, 5 ml blood samples were centrifuged at 1500 g for 15 min. The plasma was aspirated in sterile vials and stored at −20 °C until further analysis. Estimation of oxidative markers (SOD and GPX) were performed within 24 h or collection. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in erythrocyte lysate was determined by the previously described methods (Marklund and Marklund, 1974; Hafeman et al., 1974). Estimation of sodium, potassium and chloride concentrations were performed in a semi-automated chemistry analyser (Medsource Ozone Biomedicals Pvt Ltd., HR, India) using commercially available kits (Beacon Diagnostic Pvt Ltd., India).

2.5. RNA isolation

Total RNA from peripheral blood mononuclear cells (PBMCs) was isolated by a modified Trizol method. The PBMCs from blood were isolated by density gradient centrifugation. Briefly, the buffy coat from whole blood was isolated by centrifugation (300 g for 8 min) and diluted with Dulbecco phosphate buffer saline (DPBS; Sigma-Aldrich, MO, USA). About 3 ml of the lymphocyte-enriched diluted buffy coat was layered over Histopaque-1077 (Sigma-Aldrich, MO, USA) and centrifuged (1500 g for 30 min) in a swing rotor centrifuge. The PBMC enriched interphase fraction was aspirated and washed twice with DPBS. The pellet was resuspended in residual DPBS and 500 μl trizol was added to this cell suspension and stored at −20 °C until further processing.

To the 500 μl trizol containing lysed PBMCs, 200 μl chloroform was added and vortexed briefly for uniform mixing. The suspension was centrifuged (10000 g for 10 min). The aqueous phase at the top layer was carefully aspirated and transferred to a microcentrifuge tube. Chilled isopropanol (Sigma-Aldrich, MO, USA; 0.8 vol) was added to this and the mixture was transferred to a RNA binding column (Zymo Research, CA, USA). The column with collection tube was centrifuged at 5000 g for 2 min. The silica pad of the column was washed twice with chilled ethanol (80%) and dried by centrifugation at 12,000*g* for 5 min.

Table 1
Details of the primers used for real time PCR (qRT-PCR) of Hsp70 and Hsf genes.

Gene Name	Primer name	Primer sequence (5′-3′)	Primer Size (bp)	T_m
Rps18	RPS18RTFW	TGCGAGTACTCAACACCAACATCGATGG	28	61
	RPS18RTRV	GGATTCTGCATAATGGTGATCACACGTTCC	29	62
Hsf1	HSF1RTFW	CCGCTCATCTGCTGGAGCCCGA	23	63
	HSF1RTRV	CATACATGTTGAGCTGCCGCACGAAGC	27	63
Hsf4	HSF4RTFW	CCAGTGCCTGCCTTCCTCGGCAAG	24	64
	HSF4RTRV	GCTGTGCTTGAAGTATTGGGGCAGCAC	27	63
HspA1A	HSPA1ARTFW	GCTGATCGGCCGCAAGTTCGG	21	60
	HSPA1ARTRV	GCTCACCTGCACCTTAGGCTTGT	23	59
HspA1L	HSPA1LRTFW	CGCGCCGTCTGATAGGCAGAAAATTTAA	28	60
	HSPA1LRTRV	CGCTTTTCCCCTTTGTAGGACACCAT	26	60
HspA6	HSPA6RTFW	CTCAGACACCGAGCGGCTGG	20	60
	HSPA6RTRV	CCCCGCGGTAGGACACGCG	19	62
HspA8	HSPA8RTFW	CGCGCGACTTATTGGACGAAGATTTGAT	28	60
	HSPA8RTRV	TGGATGACACCTCCTCTGGGTAAAAAC	27	60
HspA2	HSPA2RTFW	GGCTGATTGGTCGGAAGTTCGAG	23	59
	HSPA2RTRV	GAGATCTCCTCGGGGAAGAAGGT	23	59
SDHA	SDHRTFW	GCTGGGGAAGAATCTGTCATGAATCTTGACAA	32	62
	SDHRTRV	TTCTGCAGCTCCAAGGGTCTCCACCA	25	63

The RNA bound to silica pad was eluted by adding 20 µl of DEPC treated water, incubation for 2 min followed by centrifugation (12,000 g for 2 min). The quality of the RNA was checked by agarose gel electrophoresis (1.5%). The RNA was stored at −20 °C until further analysis.

2.6. End point and real-time PCR

Primers for real time PCR of Hsp70 (*HspA1A, HspA1L, HspA6, HspA8, HspA2*) and HSF (*HSF1* and *HSF4)* genes were designed from available ESTs and nucleotide sequence at NCBI (Table 1). For end point and Real-Time PCR, isolated RNA was converted to cDNA using a commercially available kit (RevertAid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific Inc., MA, USA). End point PCR was performed to check the specificity of primers and to check the quality of cDNA. All end-point PCR amplifications were performed in 25 µl reaction volume. Each reaction contained 2.5 µl 10 × buffer, 200 µM of dNTPs, 0.5 µl of each primer (10 pM/µl), 0.5 U of Taq DNA polymerase and nuclease free water to bring the total volume to 25 µl. Around 100 ng of cDNA was used as a template. The PCR products were resolved on a 2.0% agarose gel.

All Real-Time PCR (qRT-PCR) reactions were performed on a CFX Connect Real-Time PCR machine (Bio-Rad Laboratories, Inc., CA, USA). Each reaction consisted of 2 µl cDNA template, 5 µl of 2 × SYBR Green PCR Master Mix, 0.25 µl each of forward and reverse primers (10 pmol/µl) and nuclease free water for a final volume of 10 µl. Each sample was run in duplicate. Two housekeeping genes, Succinate dehydrogenase (*SDHA)* and ribosomal protein (*Rps18)*, were used for normalization of data. Analysis of real-time PCR (qRT-PCR) was performed by delta-delta-Ct ($^{\Delta\Delta}$Ct) method (Livak and Schmittgen, 2001).

2.7. Statistical analysis

Statistical analyses were carried out in SYSTAT v12.02 software (SYSTAT Software Inc.) One way analysis of variance (ANOVA) was used on different physiological and haemato-biochemical parameters to test the effect of different THI group intervals using following model.

$$Y_{ij} = \mu + THI_i + e_{ij}$$

where.

Y_{ij} = jth observation of physiological/haemato-biochemical parameter at ith THI.

µ = Overall mean.

THI_i = effect of ith THI (i = 69, 72, 74, … …. .84).

e_{ij} = random error associated with each observation.

When significance was found, pairwise comparison among different THI group intervals on different physiological and haemato-biochemical parameters was performed with Fischer's restricted least significant differences (LSD) criterion.

3. Result

The major goals of this study were to reassess and revalidate previously described heat stress indicators of dairy cattle for a subtropical region. To know the trend of climatic variables and likely period of heat stress for the region, a comparative evaluation of THI over last four years (2014–2017) was analysed (Supplementary File, Fig. S1). Based on this analysis, actual data recording and sample collection from experimental animals were made during Feb–Jul 2018 to cover a THI range of 68–86. During this period, data on animals' physiological and biochemical parameters were recorded at different THI. The result of THI mediated change in animals' physiological, biochemical parameters and expression profile of heat shock responsive (HSR) genes (*HspA1A, HspA1L, HspA6, HspA8, HspA2* from Hsp70 family and *Hsf1, Hsf4* from Hsf family*)* have been presented in the following sections.

3.1. Effect of THI on rectal temperature (RT) and respiration rate (RR) and skin temperature

Dairy animals exposed to higher THI had a pronounced effect on the RT and RR (Fig. 1). First significant ($p < 0.05$) increase in RT and RR

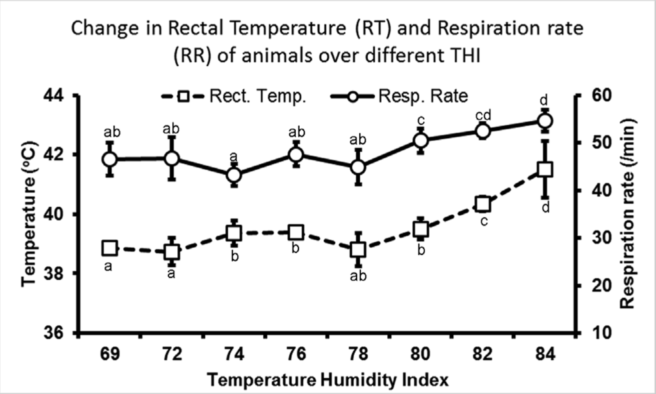

Fig. 1. Effect of different THI on Rectal Temperature (RT) and Respiration Rate (RR) (Mean ± SE) of animals. Markers with different super- and sub-scripts within a parameter differ significantly ($p < 0.05$) among themselves.

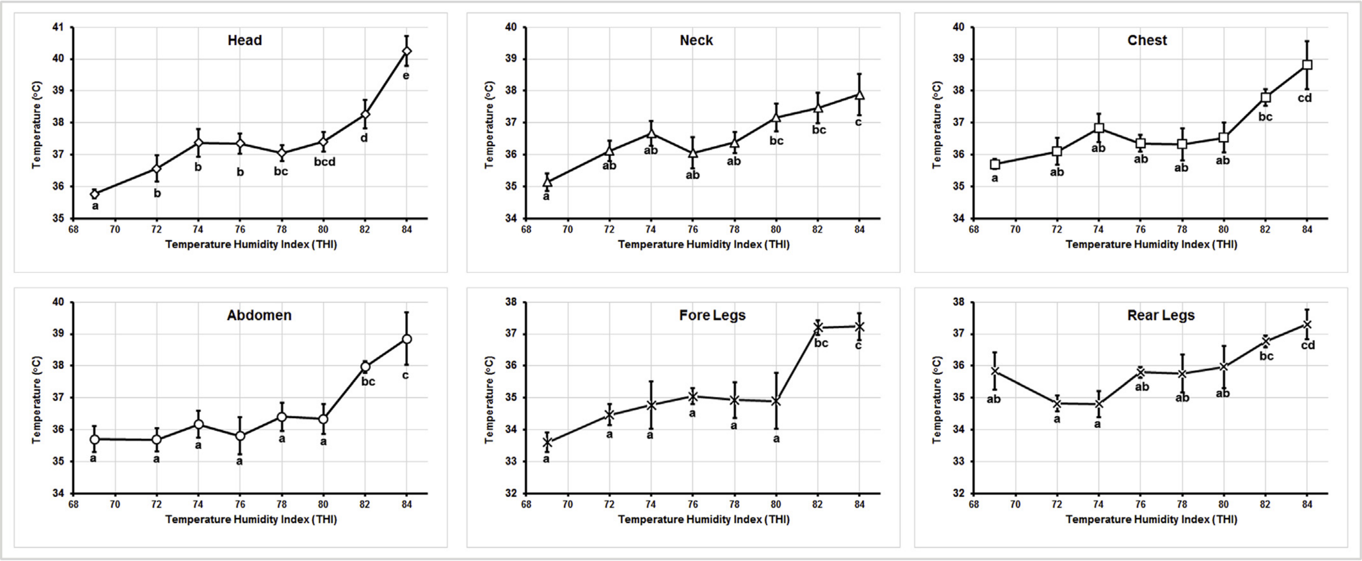

Fig. 2. Effect of different THI on surface temperature (Mean ± SE) hotspot of different body regions. Markers with different subscripts differ significantly ($p < 0.05$) among themselves.

was observed at THI 74. The RT thereafter, was steady until THI 80, after which another significant ($p < 0.05$) increase in RT was observed at THI 82. Also, the RT at THI 84 was highest and the value was significantly ($p < 0.05$) higher than values observed at lower THIs. The RR followed a pattern alike RT with higher but non-significant ($p > 0.05$) increase at THI 76. There was significant ($p < 0.05$) increase of RR observed at THI 80 and THI 82. The RR, alike RT was highest at THI 84, although there was no significant difference of RR between THI 82 and THI 84. Overall, the result suggested that both RT and RR increased with the increase in THI, however, this increase did not maintain a linear relationship with increase in THI.

The temperature hotspot of different body regions increased with increase in THI (Fig. 2). Almost all the region analysed showed increase in skin temperature with increased THI. The analysis revealed a non-linear relationship between THI and skin temperatures of body regions. Among different region analysed, the surface temperature of head region showed an interesting and perhaps more direct relationship with THI. The temperature hotspot of head region followed a similar pattern of increase to that of RT. The first significant ($p < 0.05$) increase was observed at THI 74, after which a steady profile up to THI 80 was maintained, followed by another significant ($p < 0.05$) increase at THI 82 and then at THI 84. The changes in temperature profile of neck, chest and abdomen were different and significant ($p < 0.05$) deviations were observed only after THI reached 80 or higher. The variations in temperature profile of fore and rear legs were minimal in the THI range of 72–80, with marginal but non-significant increase in skin temperature with increased THI. Significant ($p < 0.05$) increase in skin temperature of fore and rear legs were observed only after THI reached 82 or high values. THI mediated change in skin temperature of different regions particularly of head, neck, chest and abdomen regions were also obvious in respective temperature histograms (Supplementary File, Fig. S2). For majority of the animals, the poll in the head region was the point where, maximum temperature was recorded.

3.2. Effect of different THI on haematogram of animals

Heat stress had significant effect on the haematological parameters of the animals (Fig. 3). The change in mean WBC and RBC counts showed an inverse pattern and a non-linear relationship with different THI. At THI 76, both RBC and WBC counts were significantly ($p < 0.05$) different from that were observed at THI 72–74. However, at THI 76, the WBC count increased while RBC counts showed a decline. At THI 84, WBC count showed significantly ($p < 0.05$) higher and maximum value, while RBC count showed a significant ($p < 0.05$) decline at this THI. The overall trend indicated an increase in THI is associated with increase in WBC counts but decline in RBC counts. Increase in THI was not significantly ($p > 0.05$) associated with Hb%. The PCV concentration showed a marked increase only at THI 78 but no definite association of PCV and THI was observed. The lymphocyte and monocyte counts over different THI followed a similar pattern and there was an non-significant increase in the lymphocyte and monocyte percentage at THI 74 and 82. The MCH and MCHC levels had marked and significant ($p < 0.05$) increase at THI 76 and 84, although there existed animal-to-animal variations.

3.3. Effect of different THI on levels of oxidative stress markers

The activity of superoxide dismutase and glutathione was significantly ($p < 0.05$) associated with increase in THI, however, no linear relationship between the THI and level of either SOD or GPX was observed in the study (Fig. 4). The GPX level showed two marked and significant ($p < 0.05$) increase at THI 74 and THI 82 and maintained basal or near basal levels otherwise. Level of SOD increased significantly ($p < 0.05$) at THI 74, but no significant increase was observed at other THI.

Change in blood glucose level was significantly ($p < 0.05$) associated with a change in THI. The glucose level decreased with an increase in THI with major and significant ($p < 0.05$) decline was observed at THI 74 and thereafter at THI 80–82. Blood sodium level significantly ($p < 0.05$) increased only at THI 76 and thereafter returned to basal level. Blood potassium level showed a flip-flop pattern where the levels significantly ($p < 0.05$) increased at THI 76 and THI 80 with an intermediary decline at THI 78. Blood chloride level showed non-significant increase with the increase in THI.

3.4. Effect of THI on expression levels of HSR genes

The expression pattern of different HSR genes in response to increased THI have been presented in Fig. 5. At lower THI (≤74), the expression of *Hsf4*, a negative regulator of Hsp70 genes, was prominent but its expression ceased at THI 74. From THI 74 onwards, the expression of *HspA8* started and gradually increased as THI increased. Thus, expression of *HspA8* followed a direct and linear relationship with increased THI. No significant expression levels of other Hsp70 genes

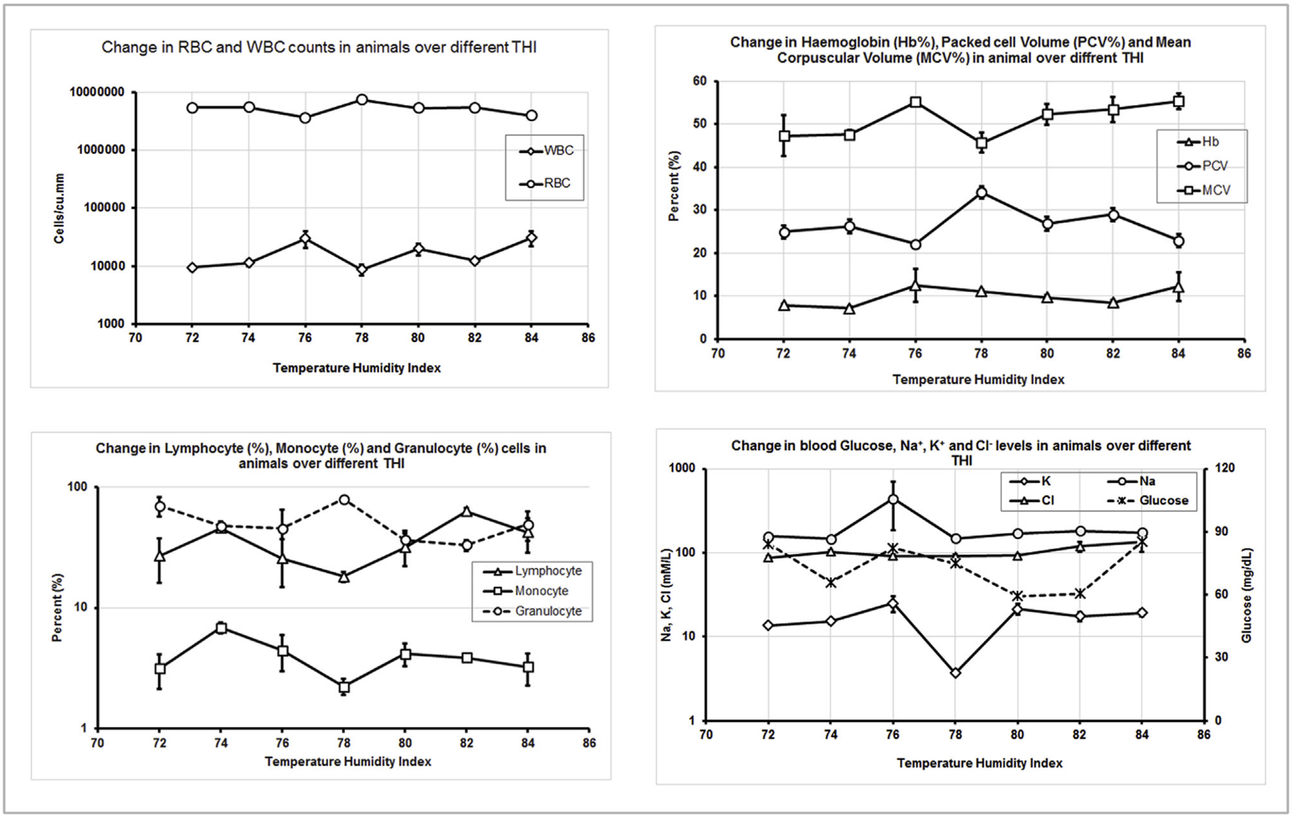

Fig. 3. Effect of different THI on haematological parameters (Mean ± SE) of animals.

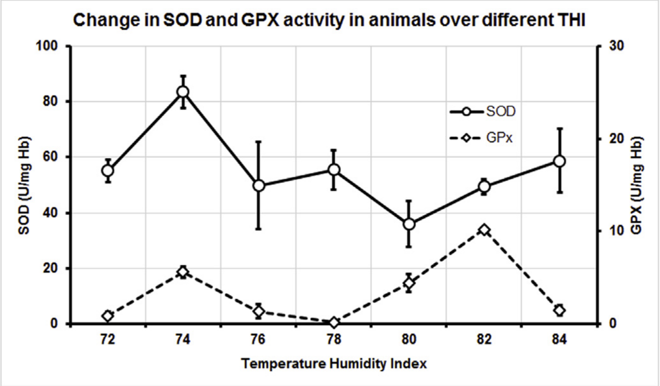

Fig. 4. Effect of different THI on SOD and GPX activity (Mean ± SE) of animals.

(*HspA1A, HspA1L, HspA2* and *HspA6*) were observed in this study. Being a positive regulator of Hsp70 genes, it was expected that expression of *Hsf1* would follow a pattern similar to that of *Hsp70* genes. However, significantly ($p < 0.05$) higher expression of *Hsf1* was only observed at THI 84, suggesting that the gene is expressed in a temporal fashion.

4. Discussion

Several studies have documented the deleterious effects of heat stress on the production and welfare of dairy animals. For tropical and subtropical regions, the challenge is even greater, as most of these geographical areas experience prolonged summer and there is a constant presence of radiant solar energy and high humidity, resulting in minimal relief from the heat. To begin with the corrective measures well in advance and before the negative impacts of heat stress on an animal could set on, it is extremely imperative that the occurrence of

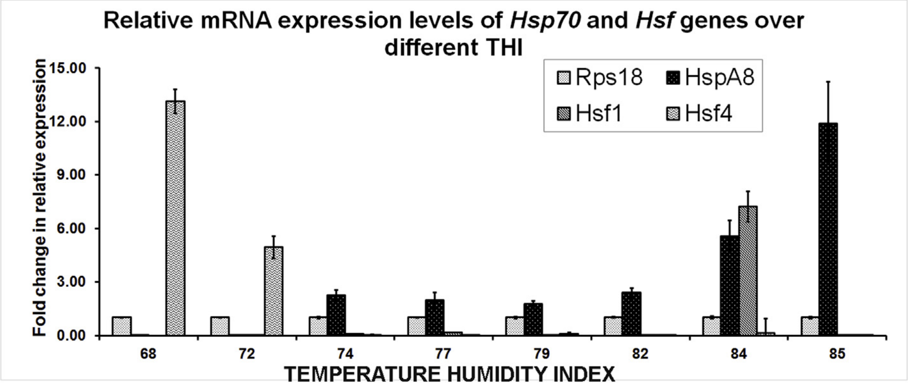

Fig. 5. Relative mRNA expression levels (Mean Fold change ± SE) of heat shock responsive (HSR) genes at different THI.

heat stress is detected and diagnosed at proper time. Temperature-humidity index (THI), a unitless index that incorporates the effects of environmental temperature with relative humidity has been widely adapted to indicate heat stress in dairy cattle (De Rensis et al., 2015). However, the threshold levels of THI to indicate the level of heat stress vary between researchers and climatic conditions (Armstrong, 1994; De Rensis et al., 2015). It appears that THI values are dependent on the geographic location, as well as cow breed and physical size (Polsky and von Keyserlingk, 2017). This necessitates that the THI should be revalidated and calibrated for different geographical locations and types of animals. The present study aimed at addressing this specific concern on recalibration of THI for a subtropical region that experience diverse climatic conditions with environmental temperature reaching extreme during summer and winter months.

Concurring to earlier findings (Joshi and Tripathi, 1991; Kumar, 2005; Padilla et al., 2006), rectal temperature and respiration rate in the present study increased with the increase in THI. The changes in RT and RR with increased THI were non-linear and in phases with first significant increase in RT and RR at THI 74. Both respiration rate and rectal temperature showed a steady phase at THI 74–80, after which it increased again. This finding suggests that THI had direct influence on RT or RR but the relationship is not linear. There existed intermediary phases, where animal tried to cope up with the conditions and maintained homeostasis. When THI exceeded a certain threshold, this homeostasis failed and again animals responded with higher RT or RR. In the present study, these thresholds of THI were < 74, 74–80 and > 80.

The skin tissue is an important mediator of heat dissipation and could be an indirect indicator of core body temperature. However, there exist a few studies (Godyń et al., 2019; Taylor et al., 2014; Salles et al., 2016) describing effect of different levels of heat stress on skin temperature of animals. The results of present study were encouraging as it was observed that the temperature hotspot of different body regions increased with increase in THI. Almost all the region analysed, showed THI mediated change in skin temperature, although the analysis revealed a non-linear relationship between THI and skin temperatures of body regions, as was experienced for RT and RR. Among different region analysed, the surface temperature of head region showed more direct relationship with THI. The head region is less likely to be influenced by secondary factors (*e.g.* contact with ground during lying, soaking of water by body parts while lying *etc.*) that could impact temporal fluctuation in skin temperature. The initial physiological responses initiated increased heat loss which redirect blood to the periphery, by vasodilatation of skin blood vessels promoting enhanced heat loss from the skin through sweating. A lesser surface area of head region contributes less heat loss through sweating compared to other body regions. Considering both standing and lying postures of animal, heat loss by convection through direct contact with floor is less likely for head region. These factors could account for direct correlation of skin temperature of head region and RT. Infrared thermography, a non-invasive and practical method has been identified as a potential tool for both diagnostic and scientific research (McManus et al., 2016) including evaluation of thermal stress in animals (Salles et al., 2016) and detection of minimal variations in body temperature (Knížková et al., 2007). However, surface temperature acquired in thermograph is influenced by several factors including surrounding environment, the type and colour of the hair coat, thickness of the adipose tissue, blood perfusion volume and metabolic rate of tissues (Racewicz et al., 2018). This necessitates minimization of stimuli interfering with the measurements during thermal imaging and normalization of thermographic data against factors such as variable temperature, humidity and emissivity of skin of the animals. In animals, major means (40–60%) of heat loss is thermal radiation falling within the emission factor range of 0.97–0.98 (Poikalainen et al., 2012). The accuracy of thermal imaging is also influenced by light intensity and solar radiation (Church et al., 2014) and therefore, it has been recommended that animals should be thermographed in shed in low intensity light (Poikalainen et al., 2012). It has been suggested that a minimum 1.0–1.2 m distance between the camera and the animal should be maintained for accuracy of thermograph (Cook et al., 2016). Analysis of thermal images has shown that forehead temperatures were most closely related to rectal temperatures in Jersey cattle (Salles et al., 2016). In zebu cattle, thermography showed a high correlation between rectal and surface temperatures with eye and brain surface temperatures being most affected by environmental parameters (Cardoso et al., 2015). Together, the finding suggests skin temperature of head region could be a reliable, alternate of RT and non-invasive indicator for predicting amount of heat load or level of heat stress in dairy animals.

The reticuloendothelial system is the major body defence systems to protect and cope against environmental stressors. Primary indicators of stress response include white blood cells (WBCs), red blood cells (RBCs), haemoglobin (Hb), packed cell volume (PCV), glucose and electrolyte concentration in blood that get altered in thermal stress. Indeed, it was observed that with increased THI, WBC counts tend to increase while RBC count decreased. These results were in accordance with earlier reports where, WBC (leukocytes) count increased by 21–26% (Abdel-Samee, 1987) and RBC count decreased by 12–20% (Habeeb, 1987) in thermally stressed cattle due to thyromolymphatic involution or destruction of erythrocytes. Although no significant change in Hb%, MCV% in response to increased THI was observed in the study, the PCV% and plasma glucose showed a marked variation in response to increased THI. Sejian et al. (2013) reported a similar finding of highly significant variation of PCV and plasma glucose levels for the different temperature exposure in Malpura ewes. This higher PCV value is an adaptive mechanism to provide water necessary for evaporative cooling process (Al-Haidary, 2004). It has been argued that reduction in blood glucose in heat-stressed dairy cows could be a consequence of greater blood insulin (Rhoads et al., 2009; Baumgard and Rhoads, 2013) reduced energy intake compensating thermogenesis of animal, and negative effect of heat on gluconeogenesis (Abeni et al., 2007; Kim et al., 2018).

Heat stress on an animal leads to oxidative stress resulting in an increase in reactive oxygen species (ROS) in different cells and tissues of heat-stressed animals. The body has a defence mechanism to counter oxidative stress in the form of enzymatic superoxide dismutase (SOD), glutathione peroxidase (GSH), and catalase antioxidants, which increases as a result of heat stress (Bernabucci et al., 2002). A higher activity level of both SOD and GPX was observed in the study at THI 74 and 82. The results again follow a similar trend to other physiological parameters, where increased levels are associated with the certain threshold of THI, between which body attempts to maintain homeostasis. Significantly higher SOD levels have been observed in lactating cows during summer compared to winter (Lallawmkimi et al., 2009). Bernabucci et al. (2002) also observed that when cows were exposed to moderate heat stress (73.2 ± 2.5 THI), showed higher erythrocyte SOD, GSH-Px-E compared with spring cows. The increased production of SOD during heat stress resulted in increased activity of H_2O_2 along with a coordinated increase in GPX (Ganaie et al., 2013; Lallawmkimi et al., 2009).

It is now well established phenomenon that cellular heat shock response (HSR) to heat stress includes synthesis of a group of molecular chaperones called heat-shock proteins (HSP) and among different classes of HSPs, Hsp70 family play an important role in HSR (Verghese et al., 2012; Kim et al., 2013). Seasonal variations of Hsp70 genes (*HspA1A* and *HspA1B*) have been reported in cattle, buffalo and goats with higher expression during summer and winter compared to spring season (Mohanarao et al., 2014; Kumar et al., 2015; Nagayach et al., 2017). The expression profile of *Hsp70* found in this study was little different in the sense that expression of only *HspA8* was observed in the THI range 74–85. The fold change in *HspA8* was moderate in the THI range 74–82 but was higher at THI 84–85. The seasonal variability of *Hsp70* could not be applicable for this study since the present study did not span different seasons, but a few months to cover THI range 68–84 or more. Also, there exist breed to breed variation of *Hsp70* expression and higher expression of *Hsp72* had been reported to be associated with lower heat tolerance in Brown Swiss cows (Lacetera et al., 2006). The findings in this study were straightforward as *HspA8* expression increased with increased THI and its expression started only after expression of *Hsf4* ceased at THI 74. Expression of Hsp's is regulated by a transcription factor family known as the heat shock factor (HSF) (Akerfelt et al., 2007), implicated as important first responders during the onset of elevated cell temperature (Trinklein et al., 2004). Although mammals express HSF1, 2, and 4, HSF1 is primarily responsible for inducing Hsp gene expression during hyperthermia (Pirkkala et al., 2001). Intermolecular interaction between *Hsf1* and *Hsf4* determines the expression pattern of heat shock proteins and *Hsf4a*, a transcript variant of *Hsf4*, suppresses the expression of heat shock proteins when it is overexpressed (Nakai et al., 1997). One of the potential inhibitory mechanisms is by associating with and inhibiting *Hsf1's* transcription activity (Zhang et al., 2001). While increased expression of *HspA8* should have been associated with increased expression of *Hsf1,* no significant increase in *Hsf1* over different THI was found in the study. This suggests the gene is expressed in a temporal fashion to regulate Hsp expression. The beginning of the expression of *HspA8* gene, a member of *Hsp70* gene family at THI 74 suggests that this THI is critical as at this point other physiological parameters for example RR, RT *etc.* also showed significant deviation from the normal range.

5. Conclusions

After analyzing physiological, biochemical parameters and HSR gene expression profile of animals in response to different THI, the study revealed that animal undergoes few or little changes at THI 72 but major physiological responses happened after THI reaches 74. At THI range 74–79, no drastic change in these parameters occurred suggesting transient acclimatization in animals in this range to maintain homeostasis. Once THI reached and crossed 80, this homeostasis is disturbed and animals experienced major physiological changes again. Our experiment indicated that crossbred cattle in this particular subtropical region experienced mild or no stress at THI < 74, THI 74–79 induced moderate stress, and THI $\geq$ 80 induced severe stress in the animals. The study therefore, reports a shift in thresholds of THI as indicator of heat stress and suggests that thresholds of THI as indicator of heat stress could be dependent upon geographical location. However, our study included limited number of animals and outcome of this experiment should not be interpreted and generalized for overall cattle population of subtropical region. Further population study over a large number of animals from different locations is warranted before accepting this new THI ranges as indicator of heat stress for overall population of sub-tropical regions. The study also suggests skin temperature of head region as potential non-invasive indicator for monitoring heat stress in animals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.03.017.

References

Abdel-Samee, A.M., 1987. The Role of Cortisol in Improving Productivity of Heat-Stressed Farm Animals with Different Techniques. Zagazig, Egypt: Ph.D. thesis. Faculty of Agriculture, Zagazig University.

Abeni, F., Calamari, L., Stefanini, L., 2007. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. Int. J. Biometeorol. 52, 87–96.

Akerfelt, M.D., Trouillet, V., Mezger, Sistonen, L., 2007. Heat shock factors at a crossroad between stress and development. Ann. N. Y. Acad. Sci. 1391, 1–13.

Al-Haidary, A.A., 2004. Physiological responses of Naimey sheep to heat stress challenge under semi-arid environments. Int. J. Agric. Biol. 6 (2), 307–309.

Armstrong, D.V., 1994. Heat stress interactions with shade and cooling. J. Dairy Sci. 77, 2044–2050.

Baumgard, L.H., Rhoads, R.P., 2013. Effects of heat stress on post absorptive metabolism and energetics. Annual Rev. Anim. Biosci. 1, 311–337.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci. 85, 2173–2179.

Bohmanova, J., Misztal, I., Cole, J.B., 2007. Temperature humidity indices as indicators of milk production losses due to heat stress. J. Dairy Sci. 90 (4), 1947–1956.

Broucek, J., Novak, P., Vokralova, J., Soch, M., Kisac, P., Uhrinccat, M., 2009. Effect of high temperature on milk production of cows from free-stall housing with natural ventilation. Slovak J. Anim. Sci. 42, 167–173.

Brown-Brandl, T.M., Nienaber, J.A., Eigenberg, R.A., Freetly, H.C., Hahn, G.L., 2003. Thermoregulatory responses of feeder cattle. J. Therm. Biol. 28, 149–157.

Byrne, D., Berry, D., Esmonde, H., McHugh, N., 2017. Temporal, spatial, inter-, and intra-cow repeatability of thermal imaging. J. Anim. Sci. 95, 970–979.

Cardoso, C.C., Peripolli, V., Amador, S.A., Brandão, E.G., Esteves, G.I.F., Sousa, C.M.Z., França, M.F.M.S., Gonçalves, F.G., Barbosa, F.A., Montalvão, T.C., Martins, C.F., Fonseca Neto, A.M., McManus, C., 2015. Physiological and thermographic response to heat stress in zebu cattle. Livest. Sci. 182, 83–92.

Church, J.S., Hegadoren, P.R., Paetkau, M.J., Miller, C.C., Regev-Shoshani, G., Schaefer, A.L., Schwartzkopf-Genswein, K.S., 2014. Influence of environmental factors on infrared eye temperature measurements in cattle. Res. Vet. Sci. 96, 220–226.

Cook, N., Timsit, E., Colyn, J., Chabot, B., Liu, T., Wolfger, B., Schaefer, A., 2016. Assessing consistency in radiated thermal output of beef steers by infrared thermography. J. Imaging. 2, 21. https://doi.org/10.3390/jimaging2030021.

Cunningham, J.G., 2002. Text Book of Veterinary Physiology, third ed. W.B. Saunders, Philadelphia, PA.

De Rensis, F., Garcia-Ispierto, I., López-Gatius, F., 2015. Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows. Theriogenology 84, 659–666.

Du Preez, J.H., Giesecke, W.H., Hattingh, P.J., 1990. Heat stress in dairy cattle and other livestock under Southern African conditions. Temperature-humidity index mean values during the four main seasons. Onderstepoort J. Vet. Res. 57 (1), 77–86.

Ganaie, A.H., Shanker, G., Bumla, N.A., Ghasura, R.S., Mir, N.A., 2013. Biochemical and physiological changes during thermal stress in bovines. J. Vet. Sci. Technol. 4 (1), 1–6.

Godyń, D., Herbut, P., Angrecka, S., 2019. Measurements of peripheral and deep body temperature in cattle – a review. J. Therm. Biol. 79, 42–49.

Habeeb, A.A.M., 1987. The Role of Insulin in Improving Productivity of Heat Stressed Farm Animals with Different Techniques. Zagazig, Egypt: Ph.D. Thesis. Faculty of Agriculture, Zagazig University.

Hafeman, D.G., Sunde, R.A., Hoekstra, W.G., 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. 104, 580–587.

Hansen, P.J., 2004. Physiological and cellular adaptations of zebu cattle to thermal stress. Anim. Reprod. Sci. 82, 349–360.

IPCC, 2007. Intergovernmental panel on climate change) climate change 2007: synthesis report. In: Pachauri, R.K., Reisinger, A. (Eds.), Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change IPCC, Geneva, Switzerland, pp. 104.

Johnson, J.E., Stone, E.J., Frye Jr., J.B., 1963. Effects of Hot Weather on the Productive Function of Dairy Cows. Louisiana Agricultural Experiment Station Bulletin, Baton Rouge, LA, USA.

Joshi, B.C., Tripathi, K.C., 1991. Heat stress effect on weight gain and related physiological responses of buffalo calves. J. Vet. Physiol. Allied Sci. 9, 33–43.

Kim, Y.E., Hipp, M.S., Bracher, A., Hayer-Hartl, M., Hartl, F.U., 2013. Molecular chaperone functions in protein folding and proteostasis. Annu. Rev. Biochem. 82, 323–355.

Kim, W.S., Lee, J.S., Jeon, S.W., Peng, D.Q., Kim, Y.S., Bae, M.H., Jo, Y.H., Lee, H.G., 2018. Correlation between blood, physiological and behavioral parameters in beef calves under heat stress. Asian-Australas. J. Anim. Sci. 31, 919–925.

Knížková, I.K.P., Gürdil, G.A.K., Pinar, Y., Selvi, K.Ç., 2007. Applications of infrared thermography in animal production. O.M.U. J. Faculty Agril. 22, 329–336.

Kumar, A., 2005. Status of Oxidative Stress Markers in Erythrocytes of Heat Exposed Cattle and Buffaloes. MS Thesis. NDRI, Deemed University, Karnal, India.

Kumar, A., Ashraf, S., Sridhar Goud, T., Grewal, A., Singh, S.V., Yadav, B.R., Upadhyay, R.C., 2015. Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) under tropical climatic condition. J. Therm. Biol. 51, 55–64.

Lacetera, N., Bernabucci, U., Scalia, D., Basirico, L., Morera, P., Nardone, A., 2006. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. J. Dairy Sci. 89, 4606–4612.

Lallawmkimi, C.M., 2009. Impact of Thermal Stress and Vitamin-E Supplementation on

Heat Shock Protein 72 and Antioxidant Enzymes in Murrah Buffaloes. Ph D Thesis. NDRI, Deemed University, Karnal, India.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2_T^{-\Delta\Delta C}$ method. Methods 25, 402–406.

Mader, T.L., Davis, M.S., Brown-Brandl, T., 2006. Environmental factors influencing heat stress in feedlot cattle. J. Anim. Sci. 84, 712–719.

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.

McManus, C., Tanure, C.B., Peripolli, V., Seixas, L., Fischer, V., Gabbi, A.M., Menegassi, S.R.O., Stumpf, M.T., Kolling, G.J., Dias, E., Costa, J.B.G., 2016. Infrared thermography in animal production: an overview. Comput. Electron. Agric. 123, 10–16.

Mohanarao, G., Mukherjee, A., Banerjee, D., Gohain, M., Dass, G., Brahma, B., Datta, T.K., Upadhyay, R.C., De, S., 2014. HSP70 family genes and HSP27 expression in response to heat and cold stress in vitro in peripheral blood mononuclear cells of goat (*Capra hircus*). Small Rumin. Res. 116 (2–3), 94–99.

Nagayach, R., Gupta, U.D., Prakash, A., 2017. Expression profiling of hsp70 gene during different seasons in goats (*Capra hircus*) under sub-tropical humid climatic conditions. Small Rumin. Res. 147, 41–47.

Nakai, A., Tanabe, M., Kawazoe, M., Inazawa, J., Morimoto, R.I., Nagata, K., 1997. HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. Mol. Cell Biol. 17, 469–481.

NOAA, 1976. Livestock Hot Weather Stress. Operations Manual Letter C-31–76. NOAA, Kansas City, MO.

Padilla, L., Matsui, T., Kamiya, Y., Kamiya, M., Tanaka, M., Yano, H., 2006. Heat stress decreases plasma vitamin C concentration in lactating cows. Livest. Sci. 101, 300–304.

Pereira, A.M.F., Titto, E.L., Infante, P., Geraldo, A.M., Alves, A., Leme, T.M., Baccari Jr., F., Almeida, J.A., 2014. Evaporative heat loss in *Bos taurus*: do different cattle breeds cope with heat stress in the same way? J. Therm. Biol. 45, 87–95.

Pirkkala, L., Nykanen, P., Sistonen, L., 2001. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. International J. Current Microbiol. Applied Sci. 15, 1118–1131.

Poikalainen, V., Praks, J., Veerma, E.I., Kokin, E., 2012. Infrared temperature patterns of cow's body as an indicator for health control at precision cattle farming. Agron. Res. Biosystem Eng., Special Issue 1, 187–194.

Polsky, L., von Keyserlingk, M.A.G., 2017. Effects of heat stress on dairy cattle welfare. J. Dairy Sci. 100, 8645–8657.

Racewicz, P., Sobek, J., Majewski, M., Zawieja, J.R., 2018. The use of thermal imaging measurements in dairy cow herds. Sci. Annals Polish Soc. Anim. Prod. 14, 55–69.

Rhoads, M.L., Rhoads, R.P., Baale, M.J., Collier, R.J., Sanders, S.R., Weber, W.J., Croocker, B.A., Baumgard, L.H., 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92 (5), 1986–1997.

Salles, M.S., da Silva, S.C., Salles, F.A., Roma, L.C., El Faro Jr., L., Bustos Mac Lean, P.A., Lins de Oliveira, C.E., Martello, L.S., 2016. Mapping the body surface temperature of cattle by infrared thermography. J. Therm. Biol. 62 (Pt A), 63–69.

Sejian, V., Indu, S., Naqvi, S.M.K., 2013. Impact of short term exposure to different environmental temperature on the blood biochemical and endocrine responses of Malpura ewes under semi-arid tropical environment. Indian J. Anim. Sci. 83 (11), 1155–1160.

Taylor, N.A., Tipton, M.J., Kenny, G.P., 2014. Considerations for the measurement of core, skin and mean body temperatures. J. Therm. Biol. 46, 72–101.

Thom, E.C., 1959. The discomfort index. Weatherwise 12, 57–59.

NRC, 1971. A Guide to Environmental Research on Animals, vol. 12. National Academy of Science, Washington, DC, pp. 57–59 Thom, E. C. 1959. The discomfort index. Weather-wise.

Trinklein, N.D., Murray, J.I., Hartman, S.J., Botsein, D., Myers, R.M., 2004. The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response. Mol. Biol. Cell 15, 1254–1261.

Verghese, J., Abramsa, J., Wanga, Y., Kevin, A., 2012. Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. Microbiol. Mol. Biol. Rev. 76 (2), 115–158.

Zhang, Y., Frejtag, W., Dai, R., Mivechi, N.F., 2001. Heat shock factor-4 (HSF-4a) is a repressor of HSF-1 mediated transcription. J. Cell. Biochem. 82, 692–703.