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Effect of heat stress on physiological, haemato-biochemical and hormonal parameters of Vechur cattle in the tropical highlands of Kerala

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Abstract
Vechur, an indigenous cattle breed from Kerala is well known for its adaptability to local environmental conditions, including notable heat tolerance. The present study was done to see the effect of heat stress on blood parameters in Vechur cattle of similar age groups (12-14 months of age) and physiological status. Blood samples were collected and analyzed based on the temperature-humidity index (THI). Blood samples were collected and analyzed for haemoglobin, volume of packed red cells, red blood cell count, white blood cell count and blood pH. A significant rise (p<0.01) in physiological parameters such as respiration rate and surface body temperature was observed. Biochemical parameters such as total protein, cholesterol, AST and ALT and hormonal parameters such as T_3, T_4 and cortisol showed significant differences (p<0.01) during heat stress. From the present study, it can be concluded that THI is a sensitive indicator of heat stress.

Keywords: Vechur, heat stress, physiological parameters, haemato-biochemical parameters, hormonal parameters, temperature humidity index

Introduction
Climate change and seasonal fluctuations are critical factors which influence the welfare of livestock in extensive production systems, potentially reducing production and reproductive efficiency among grazing animals (Ali and Hayder, 2008) [3]. Animals with high productivity raised in tropical regions are negatively affected by climatic factors, particularly high temperatures and relative humidity, identified as significant factors compromising their production (Martello *et al*., 2010) [21]. Ambient temperature (AT), relative humidity (RH), geographic location, species, breeds, genetic potential, life stage, gender, and nutritional status contribute to the severity of heat stress. Heat stress also has adverse effects on their forage intake, feed conversion efficiency, milk production, reproductive efficiency, and susceptibility to diseases (Silanikove and Koluman, 2015) [27]. Hot dry weather increases the evaporative cooling whereas hot humid weather reduces the air's ability to absorb moisture which in turn increased the level of heat stress in animals (Kaliber *et al*., 2016) [19]. During higher temperature conditions, growth variables such as growth rate, average daily gain (ADG), solids daily gain, live body weight (BW) and dry body weight gets compromised in livestock (Pragna *et al*., 2018) [24]. Heat stress-induces homeorhetic mechanisms that prioritizes thermoregulation over other physiological processes, resulting in reduced milk production and increased susceptibility to metabolic disorders in cattle during the transition period (Baumgard and Rhoads, 2013) [4]. The reduction in milk yield by up to 50% in dairy animals resulted from a combination of factors: decreased feed intake and metabolic adaptations to heat stress. These metabolic responses included alterations in post-absorptive carbohydrate, protein and lipid metabolism, contributing further to the decline in milk production.

Temperature humidity index (THI), a critical tool in assessing the heat stress, integrates the effects of air temperature and humidity. The THI values below 72 denotes favorable environmental conditions without heat stress. For THI values ranging from 75 to 78, despite experiencing heat stress, the cows' thermoregulatory mechanisms effectively managed the conditions.

However, THI values surpassing 78 indicate severe stress, compromising the cows' ability to maintain normal body temperature and regulate their physiological functions effectively (Dimov *et al*., 2020) [10]. Prasad *et al*. (2017) [25] classified different agro-ecological zones of Kerala based on the meteorological data collected from thirteen different weather stations. It was found that Wayanad, a tropical highland in Kerala having a maximum THI of 84.17 and an average THI of 78.79, falls under the category of moderately comfortable zone with differing zones of comfort at different altitudes.

Above 35 ℃ of surface body temperature, all the heat exchange mechanisms get activated in the animal's body (Collier *et al*., 2003) [8]. The animal starts to store heat which leads to cutaneous heat loss and an increase in respiratory rate and rectal temperature when the surface body temperature rises above 35℃ (Pollard *et al*., 2004) [23]. Brown-Brandl *et al*. (2005) [6] observed that respiration rate was responsive to atmospheric temperature with a small percent of total variation and concluded that it was an easy and direct indicator of heat stress to be measured without any equipment. Heat stress alters the levels of total proteins and cholesterol in cattle indicating metabolic disturbances and also alters the AST and ALT levels indicating liver dysfunction and cellular damage that occurs due to extreme heat exposure (Herbut *et al*., 2019) [15]. Heat stress exerts a profound influence on the endocrine system of cattle, particularly by modulating the levels of cortisol, triiodothyronine (T_3), and thyroxine (T_4). The elevation of cortisol, a hallmark of the activation of the hypothalamic-pituitary-adrenal axis in response to environmental stressors, signifies an intensified stress response (Aggarwal and Upadhyay, 2013) [2]. Simultaneously, a notable alteration in T_3 and T_4 levels occurs, a physiological adaptation aimed at reducing metabolic heat production, which is crucial for maintaining homeostasis during periods of thermal stress (Kahl *et al*., 2015; Blond *et al*., 2024) [18, 5].

Within the spectrum of Indigenous cattle breeds, Vechur cattle, the only native breed from Kerala, are distinguished by their adaptability to local environmental conditions, including notable heat tolerance, higher disease resistance and less maintenance (Lali and Bindu, 2011) [20]. Their compact stature is considered an adaptation to heat, particularly in tropical climates, as it enhances heat tolerance by reducing the amount of metabolic heat produced and facilitating heat dissipation. Smaller animals have a higher surface area-to-body mass ratio, which allows them to more efficiently release excess heat into the environment. This trait is especially useful in cattle breeds like the Vechur, which have evolved smaller body sizes as a genetic adaptation to better cope with heat stress (Elayadeth-Meethal *et al.*, 2023) [11].

This study was done to investigate changes due to heat stress in the physiological, haemato-biochemical and hormonal parameters of Vechur heifers reared in tropical highlands of Kerala during thermo-neutral (TN) and heat stress (HS) periods.

Materials and methods

The experiments were conducted at the cattle farm maintained at the Instructional Livestock Farm Complex (ILFC), Pookode, located in Wayanad district of Kerala. Six heifers of Holstein Vechur cattle at 12-14 months of age maintained at Cattle farm, ILFC, Pookode, Wayanad were selected for the study. The experiment was conducted during low THI period (November-January) of 2022 and high THI period of (March-May) of 2023 (Jyothi *et al*., 2017) [17]. The study duration was two months in each period (December and January in low THI period, March and April in high THI period). The macroclimatic parameters such as environmental temperature (°C) and relative humidity (RH) (%) were recorded from the automatic weather station at Department of Livestock Production Management, College of Veterinary and Animal Sciences, Pookode. The temperature humidity index (THI) was calculated using the formula given below (Ravagnolo *et al*., 2000) [26].

$$THI= (1.8 X AT + 32)-[(0.55-0.0055 X RH) X (1.8 X AT-26)]$$

Physiological parameters such as respiration rate (RR) and surface body temperature were recorded daily before (7 a.m.) and after grazing (1 p.m.) for two months in thermoneutral and heat stress periods to analyse the animal responses to the heat stress. The RR (bpm) was observed by counting the inward and outward abdominal movements. One outward movement was counted as one respiration. The surface body temperature (℃) was calculated by averaging the temperature recorded from four points, head (at the middle of the line joining the two lateral canthus of the eye), foreleg (lateral side of elbow joint), hindleg (lateral side of stifle joint) and thigh (middle gluteal muscle region) using infrared thermometer (HTC^{TM} – MTX-2) (Yadav *et al*., 2016) [28].

Blood samples were collected from the Vechur heifers during the thermo-neutral and heat stress periods after estimating the THI during peak hours of the day (1pm) based on the macro- climatic variables and if a lower THI between the range of 66-72 during the thermo-neutral period and a higher THI (≥ 78) during the heat stress period was observed consecutively for three days, then on the third day the blood was collected after sterilizing the area of blood collection using 70 per cent ethanol. Haematological parameters such as haemoglobin, volume of packed red cells, red blood cell count, white blood cell count were estimated by auto haematology analyser (Mindray BC2800Vet). The blood pH was estimated using epoc Blood Analysis System (epoc® NXS host). The serum biochemical parameters were estimated using a semi-automated clinical chemistry analyser (Mispa VIVA, Agappe, ADL/BR/VIVA/R05-03/19). The diagnostic kits for estimation of Total protein (Cat. No. 51013002), cholesterol (Cat. No. 51403003), AST (Cat. No. 51408003) and ALT (Cat. No. 51409003) were procured from Agappe Diagnostics Limited, India. Cortisol was estimated in serum using Cortisol C. T. RIA kit (M/s Beckman Coulter, United States) supplied by Anand Brothers, Delhi, India, using a gamma counter (Perkin Elmer, model Wizard) and Triiodothyronine (T_3) and Thyroxine (T_4) were estimated in serum by using BRIA MAG 3 Radioimmunoassay (RIA) kit supplied by Board of Radiation and Isotope Technology, Mumbai, India, under gamma counter (Perkin Elmer, model Wizard).

For statistical analysis, Mixed analysis of variance (ANOVA) was applied.

Results

Macro-climatic variables

For calculating the THI, the macroclimatic variables such as ambient temperature and relative humidity were recorded on a half-hourly basis and their readings at the mid-day peak hours (from 12:00 P.M. to 1:00 P.M) were used. An average of THI was calculated from these three timings. The ambient temperature, relative humidity and the average THI for both the periods are depicted in Table 1. The heat stress period had a THI above 78 indicating that the animals were under severe stress (Dimov *et al*., 2020) [10].

Table 1: Macroclimatic data across the mid-day peak hours

Period	Ambient Temperature (℃)	Relative humidity (%)	Average THI
TN	24.99±0.34	60.11±1.78	71.42±0.34
HS	28.74±0.30	57.39±1.75	78.73±0.27

Physiological parameters

The respiration rate was found to be 26.61±0.08 bpm in the forenoon and 36.78±0.08 bpm in the afternoon during TN period, and 29.75±0.09 bpm in the forenoon and 61.84±0.23 bpm in the afternoon during HS period. The Vechur cattle also showed a surface body temperature of 31.03±0.01 ℃ in the forenoon and 32.87±0.01 ℃ in the afternoon during TN period, and 31.86±0.02 ℃ in the forenoon and 34.27±0.03 ℃ in the afternoon during HS period. The statistical analysis revealed a significant increase (p≤0.01) in RR and surface body temperature during the afternoon sessions in both TN and HS periods and a very high significant increase (p≤0.01) in both the physiological parameters during heat stress. The results are depicted in Table 2.

Table 2. Physiological parameters of Vechur cattle in thermo-neutral and heat stress periods

Parameters	Time	Thermo-neutral period		Heat stress period	
		Mean ± SE	p-value	Mean ± SE	p-value
Respiration rate (bpm)	7 am	26.61±0.08	0.00**	29.75±0.09	0.00**
	1 pm	36.78±0.08	0.00**	61.84±0.23	0.00**
Surface body temperature (℃)	7 am	31.03±0.01	0.00**	31.86±0.02	0.00**
	1 pm	32.87±0.01	0.00**	34.27±0.03	0.00**

** Significant at 0.01 level (*p*<0.01)

Haemato-biochemical parameters

The Vechur animals had Hb levels of 13.45±0.35 g/dL in TN and 13.47±0.37 g/dL in HS period. The VPRC was found to be 36.34±0.35 % in TN period and 40.83±2.05 % during HS. The RBC counts were 8.49±0.12 10^6 /µL in TN period and 9.15±0.25 10^6 /µL in HS and the WBC counts were 10.80±0.19 10^3/µL in TN period and 22.97±1.28 10^3/µL in HS. The mean blood pH for Vechur was 7.43±0.02 in TN period and 7.39±0.01 in HS. Hb and VPRC showed a non-significant (*p*>0.05) increase during HS but a significant increase (*p*<0.05) in RBC and a highly significant increase (p≤0.01) in WBC and a highly significant decrease in blood pH was also observed. The results are depicted in Table 3.

Vechur cattle had total protein values of 4.86±0.03 g% in TN period and 8.76±0.07 g% in HS and cholesterol levels of 129.92±2.13 mg/dL in TN period and 110.15±2.24 mg/dL in HS. The AST levels were 78.98±0.27 IU/L in TN period and 78.24±0.8 IU/L in HS and the ALT level was 31.05±1.34 IU/L during TN period and 60.29±0.47 IU/L during HS. The analysis found highly significant (p≤0.01) period effects on total protein, cholesterol, AST and ALT levels. The results are depicted in Table 3.

Hormonal parameters

The cortisol levels were 13.45±0.35 ng/mL in TN period and 60.61±6.71 ng/mL during HS in Vechur. The T_3 levels were 1.18±0.04 ng/mL in TN period and 1.38±0.05 ng/mL in HS and a T_4 levels of 36.99±1.06 ng/mL in TN period and 25.22±1.33 ng/mL in HS. The analysis indicated highly significant (*p*≤0.01) effects of period on cortisol, T_3 and T_4 levels. The results are depicted in Table 3.

Table 3: Haemato-biochemical and hormonal parameters of Vechur cattle in thermo-neutral and heat stress periods

Parameters	Period		p-value
	Thermo-neutral	Heat stress	
Haemoglobin (g/dL)	13.45±0.35	13.47±0.36	0.44^{ns}
Volume of packed red cells (%)	36.34±0.35	40.83±2.05	0.06^{ns}
RBC (10^6 /µL)	8.49±0.12	9.15±0.25	0.03*
WBC (10^3/µL)	10.80±0.19	22.97±1.28	0.00**
Blood pH	7.43±0.02	7.39±0.01	0.01**
AST (IU/L)	78.98±0.27	78.24±0.80	0.01**
ALT (IU/L)	31.05±1.34	60.29±0.47	0.00**
Cholesterol (mg/dL)	129.92±2.13	110.15±2.24	0.00**
Total protein (g %)	4.86±0.03	8.76±0.07	0.00**
T_3 (ng/mL)	1.18±0.04	1.38±0.05	0.00**
T_4 (ng/mL)	36.99±1.06	25.22±1.32	0.00**
Cortisol (ng/mL)	13.45±0.35	60.60±6.71	0.00**

** Significant at 0.01 level (*p*<0.01); *Significant at 0.05 level (*p*<0.05); ns non-significant (*p*>0.05).

Discussion

The increased respiration rate and surface body temperature observed in cattle during heat stress are a result of intensified evaporative cooling mechanisms, which compensate for the diminished sensible heat loss caused by elevated ambient temperatures (Collier *et al*., 2012) [9]. Heat stress reduces oxygen availability due to increased respiration, triggering erythropoiesis, where erythropoietin stimulates RBC production to ensure adequate oxygen delivery to tissues. Additionally, the body's thermoregulatory processes, such as sweating and panting, increase blood viscosity, prompting further RBC release. Heat stress also induces inflammation and activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of stress hormones like cortisol, which mobilizes WBCs from the spleen and bone marrow. This immune response increases WBC levels to counteract cellular damage and potential infections associated with heat-induced stress. The cumulative effect of these processes enhances both oxygen transport and immune protection in cattle during periods of elevated temperature (Cardoso *et al.*, 2015) [7]. Heat stress in cattle causes a decline in blood pH primarily through respiratory and metabolic disturbances. As cattle pant to cool down, they expel excessive carbon dioxide (CO_2), initially leading to respiratory alkalosis due to reduced carbonic acid levels. To compensate, the body excretes bicarbonate (HCO_3^-), reducing its buffering capacity, which, combined with increased lactic acid production from heightened metabolic demands, results in metabolic acidosis and a drop in blood pH. Additionally, dehydration from heat stress disrupts electrolyte balances, further contributing to acid-base imbalances and promoting a shift toward acidosis (Farooq *et al*., 2010) [12].

When cattle are exposed to high temperatures their bodies undergo significant metabolic shifts to adapt to the stress.

One of the key changes is a pronounced reduction in plasma lipid concentrations, particularly cholesterol, which can drop to around 60% of normal levels (Noble *et al*., 1976) [22]. This decline is largely due to the diminished activity of lecithin-cholesterol-acyl transferase, an enzyme vital for cholesterol metabolism. As heat stress impairs the enzyme's function, cholesterol processing becomes less efficient, leading to lower plasma cholesterol levels.

Another important effect of heat stress is the alteration of aspartate aminotransferase (AST) levels, an enzyme involved in amino acid metabolism and liver function. AST levels typically decrease during heat stress, likely due to reduced muscle metabolism and changes in liver function. To cope with the heat, the body reallocates energy and resources to prioritize thermoregulation, shifting focus away from other metabolic processes, such as those in muscles and the liver. These physiological adjustments help the animal manage heat stress, but prolonged exposure can lead to adverse effects on overall health and productivity (Herbut *et al*., 2019) [15].

An increase in total protein and alanine aminotransferase (ALT) levels are primarily caused by dehydration, hormonal imbalances, and shifts in metabolism. As cattle lose water due to heat, dehydration results in haemo-concentration, meaning there is less fluid in the bloodstream, which leads to higher concentrations of erythrocytes and total proteins (Ferreira *et al*., 2006) [13]. Additionally, during heat stress, elevated cortisol levels—part of the stress response—alter how the body metabolizes proteins and impact liver function, contributing to higher ALT levels, a marker of liver stress or damage. The rise in body temperature and increased respiratory rate during heat stress further disrupt normal physiological processes, influencing both protein production and liver enzyme activities. Moreover, the stress response may boost protein synthesis as the body tries to cope, leading to elevated protein levels. Liver function, however, can become compromised, causing an increase in ALT as the liver struggles to eliminate waste and maintain balance (Hansen, 2013) [14].

Heat stress in cattle induces significant physiological changes, most notably characterized by elevated levels of T_3 and cortisol, which are essential for thermoregulation and stress adaptation. The increase in T_3 is largely driven by a rise in metabolic rate, where the body boosts energy expenditure and thermogenesis to counteract excess heat (Herbut *et al.*, 2019) [15]. This response is regulated by activating the hypothalamic-pituitary-thyroid axis, promoting the secretion of thyroid hormones to facilitate metabolic adjustments during heat exposure. In parallel, cortisol levels rise due to increased adrenocortical activity (Abilay *et al*., 1975) [1], directly linked to higher body and environmental temperatures (Idris *et al*., 2021) [16]. As a key stress marker, elevated cortisol is associated with increased respiratory rate and body temperature, reflecting the animal's effort to maintain homeostasis under thermal stress.

Heat stress also disrupts thyroid function, specifically affecting the production of thyroxine (T_4), a hormone crucial for regulating metabolic rate. The reduction in T_4 levels serves as an adaptive mechanism, allowing the body to slow down its metabolic rate, thereby minimizing heat production and conserving energy during thermal stress. By downregulating thyroid activity, the animal reduces unnecessary energy expenditure, which helps it maintain thermal equilibrium in hot conditions (Collier *et al*., 2012) [9].

Conclusion

The present study demonstrated variations in physiological, haemato-biochemical and hormonal parameters in response to changes in ambient temperature and temperature-humidity index, although these values remained within the physiological range for Vechur cattle. Thus, we can conclude that seasonal fluctuations can impact the physiological, haemato-biochemical and hormonal profiles in Vechur. These findings offer valuable insights into the physiological responses of dairy cattle to elevated temperatures, enabling a more accurate assessment of their capacity to adapt to and manage environmental stress.

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