



Antioxidant and endocrine responses to heat stress in indigenous cattle

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Abstract

Heat stress is a major environmental constraint affecting livestock productivity, particularly in tropical regions. The present study evaluated antioxidant and endocrine responses to heat stress in Vechur and crossbred cattle to elucidate breed-specific adaptive mechanisms. Apparently healthy Vechur (n = 3) and crossbred (n = 3) heifers were subjected to thermoneutral (TN) and heat stress conditions in a climate-controlled chamber for 7 days each. The heat stress phase simulated tropical summer conditions (28-42 °C; 70-75% relative humidity). Blood samples were collected at the end of each phase to assess glutathione peroxidase (GPx), catalase, triiodothyronine (T3), thyroxine (T4), and cortisol. Heat stress significantly increased GPx activity in both groups (p < 0.001), indicating activation of antioxidant defense mechanisms, while catalase activity did not vary significantly. Although breed differences were not statistically significant, Vechur cattle exhibited a relatively higher GPx response, suggesting efficient antioxidant regulation under lower oxidative burden, where GPx predominantly functions. In contrast, catalase activity tended to be higher in crossbred cattle, indicating a comparatively greater oxidative load, as catalase is more active under conditions of elevated oxidative stress. Thyroid hormone levels (T3 and T4) declined in Vechur cattle during heat stress, reflecting adaptive modulation to reduce metabolic heat production, whereas crossbred cattle showed comparatively stable levels. Cortisol responses were inconsistent possibly due to the small number of observations, thus limiting its reliability as a standalone biomarker of heat stress in this experiment. Overall, Vechur cattle demonstrated a more efficient antioxidant and endocrine response, reflecting superior thermotolerance compared to crossbred cattle. These findings highlight the capacity of indigenous breeds in enhancing resilience and sustaining livestock productivity under heat stress conditions.

Keywords: Heat stress, oxidative stress, Vechur, GPx, catalase, thyroid hormones, climate chamber

Introduction

Heat stress is a critical environmental factor limiting livestock productivity, particularly in tropical and subtropical regions where high temperature and humidity prevail. Under such conditions, animals struggle to maintain thermal balance, resulting in physiological strain. This is reflected by increased respiration rate, elevated body temperature, and redistribution of blood flow to enhance heat dissipation (West, 2003) [19]. One of the primary consequences is reduced feed intake, which negatively affects nutrient availability and energy balance. In addition, heat stress disrupts normal metabolic processes, leading to inefficient nutrient utilization. These alterations ultimately result in decreased milk production, reduced growth rate, and impaired reproductive performance (Baumgard and Rhoads, 2013) [1].

At the cellular level, heat stress induces oxidative stress due to excessive production of reactive oxygen species (ROS) which exceeds the neutralizing capacity of antioxidants, causing damage to lipids, proteins, and nucleic acids (Lin *et al.*, 2006, Sies, 2015) [10, 16]. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase play a crucial role in scavenging ROS and maintaining cellular redox balance (Cadenas and Davies, 2000) [3]. The

activity of these enzymes is influenced by heat stress, and the magnitude of this response depends on the duration of exposure. According to Habashy *et al.* (2019) [6], chicks exposed to 35°C for 12 days had elevated activity of these enzymes in the liver cells.

Heat stress also affects endocrine function. The secretion of key hormones is altered, particularly those involved in stress and metabolism. Activation of the hypothalamic-pituitary-adrenal axis increases cortisol levels, which helps animals cope with stress (West, 2003) [19]. Prolonged elevation of cortisol can suppress immune function, leading to increased susceptibility to diseases (Mordak and Stewart, 2015) [12]. In contrast, thyroid hormones such as triiodothyronine (T3) and thyroxine (T4) are typically reduced, which helps lower metabolic heat production (Collier *et al.*, 2008) [5].

Breed differences play a significant role in heat tolerance. Indigenous cattle breeds such as Tharparkar and Sahiwal exhibit greater resilience to heat stress compared to crossbred cattle (Tejaswi *et al.*, 2020) [18]. Vechur cattle, an indigenous breed from Kerala, are well adapted to hot and humid climates and possess superior thermotolerance. In contrast, crossbred cattle, although higher in production, are generally more susceptible to thermal stress. However, despite extensive research on heat stress in cattle, there is

limited information on the integrated antioxidant and endocrine responses in indigenous breeds such as Vechur cattle under controlled environmental conditions. Furthermore, the comparative evaluation of these responses between indigenous and crossbred cattle remains insufficient. Therefore, the present study was undertaken to evaluate the effects of heat stress on antioxidant enzyme activity and hormonal profiles in Vechur and crossbred cattle under controlled conditions, with the aim of understanding breed-specific adaptive mechanisms.

Materials and Methods

The study was conducted during January-February 2025 at the Centre for Climate Controlled Research (CCRC), CAADDECS, College of Veterinary and Animal Sciences (CVAS), Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy. Apparently healthy Vechur (n = 3) and crossbred (n = 3) heifers aged 1.5-2 years were randomly selected from the animal facilities at KVASU. Prior to the experiment, animals were housed in a holding facility adjacent to the climate chamber for seven days, followed by a seven-day acclimatization period inside the chamber without exposure to experimental treatments.

The experiment lasted for 14 consecutive days and consisted of two phases: thermoneutral (TN) and heat stress (HS). During both phases, all animals were housed in the climate chamber for 10 h daily (08:00 to 18:00 h) and maintained outside the chamber for the remaining period. Feeding was provided according to the guidelines of Indian Council of Agricultural Research (2013) [8].

During the thermoneutral phase (7 days), animals were maintained under comfort conditions inside the chamber. In the heat stress phase (7 days), animals were exposed to cyclical temperatures ranging from 28 °C to 42 °C, (Hou *et al.*, 2021) [7], with relative humidity maintained at 70-75%. The temperature was varied in cycles, with each level applied for two hours over the 10-hour daily exposure period. The temperature regime is presented in table 1.

Table 1: Variable temperature regime during period II

Time	Temperature	RH
08.00 AM	28-31 °C	70 – 75%
10.00 AM	32-35 °C	
12.00 PM	36-39 °C	
02.00 PM	40-42 °C	
04.00 PM	32-35 °C	

Blood samples were collected on day 7 of each experimental phase. Serum was separated and stored at -20 °C until analysis. The activities of glutathione peroxidase (GPx) and catalase were determined spectrophotometrically using commercial assay kits (Origin Diagnostics) in the UV-Vis spectrophotometer. Serum concentrations of triiodothyronine (T3), thyroxine (T4), and cortisol were estimated using radioimmunoassay in a gamma counter. Statistical analysis was performed by mixed model analysis using SPSS software (version 24.0).

Results

The concentrations of Glutathione peroxidase (GPx), catalase, T3, T4 and cortisol observed during thermoneutral (TN) and heat stress periods are presented in table 2.

Table 2: Mean ± SE of levels of antioxidant enzymes and hormones in crossbred and Vechur cattle between period I and period II

Parameter	Treatment	Vechur	Crossbred	P value
GPx U/mL	Comfort	16.20±4.88 ^{aB}	16.98±4.88 ^{aB}	0.91 ^{ns}
	Heat stress	50.16±4.88 ^{aA}	47.31±4.88 ^{aA}	0.69 ^{ns}
	Breed x Time	<0.001 ^{**}	<0.001 ^{**}	
Catalase U/mL	Comfort	4.53±2.09 ^{aA}	5.53±2.09 ^{aA}	0.746 ^{ns}
	Heat stress	6.86±2.09 ^{aA}	9.12±2.09 ^{aA}	0.469 ^{ns}
	Breed x Time	0.297 ^{ns}	0.118 ^{ns}	
T3 ng/mL	Comfort	2.12±0.15 ^{aA}	1.27±0.15 ^{bA}	0.001 [*]
	Heat stress	1.54±0.15 ^{aB}	1.24±0.15 ^{aA}	0.195 ^{ns}
	Breed x Time	0.033 [*]	0.925 ^{ns}	
T4 ng/mL	Comfort	132.62±11.63 ^{aA}	86.87±11.63 ^{bA}	0.016 [*]
	Heat stress	105.87±11.63 ^{aA}	83.82±11.63 ^{aA}	0.203 ^{ns}
	Breed x Time	0.142 ^{ns}	0.861 ^{ns}	
Cortisol ng/mL	Comfort	155.73±20.49 ^{aA}	20.48±20.49 ^{bB}	0.003 [*]
	Heat stress	67.97±20.49 ^{aB}	57.57±20.49 ^{aA}	0.731 ^{ns}
	Breed x Time	0.001 [*]	0.076 ^{ns}	

** Significant at 0.001 level; * Significant at 0.05 level; ns non-significant

Means having different lowercase letters as superscripts differ significantly within a row

Means having different uppercase letters as superscripts differ significantly within a column for each breed

GPx activity did not differ significantly between breeds across the phases. However, the levels increased significantly in both Vechur (50.16±4.88 U/mL) and crossbred cattle (47.31±4.88 U/mL) during heat stress compared to the thermoneutral period (p<0.001). Catalase activity showed no significant differences between breeds or experimental phases. T3 levels were significantly higher in Vechur cattle (2.12±0.15 ng/mL) than in crossbred cattle (1.27±0.15 ng/mL) during the comfort period (p<0.01). However, during heat stress, T3 levels declined in Vechur cattle and the difference between breeds became non-significant. Similarly, T4 levels were significantly higher in Vechur cattle during the comfort period (132.62±11.63 ng/mL) compared to crossbred cattle (86.87±11.63 ng/mL) (p<0.05), whereas no significant difference was observed during heat stress. Cortisol levels were significantly higher in Vechur cattle during the comfort period (155.73±20.49 ng/mL) compared to crossbred cattle (20.48±20.49 ng/mL) (p<0.001). During heat stress, cortisol levels decreased in Vechur cattle and increased in crossbred cattle, resulting in no significant difference between breeds.

Discussion

Heat stress is known to induce oxidative stress by excessive production of reactive oxygen species (ROS), thereby disrupting cellular redox homeostasis (Sies, 2015) [16]. Antioxidant enzymes such as glutathione peroxidase (GPx) and catalase constitute the primary defense mechanism protecting cells against ROS-induced damage (Cadenas and Davies, 2000) [3]. In the present study, GPx and catalase activities increased during heat stress in both Vechur and crossbred cattle, indicating activation of antioxidant defense mechanisms. The significant increase in GPx activity (p<0.001) in both breeds suggests an adaptive response aimed at maintaining redox balance under thermal load. Similar findings of increased activity of antioxidants enzymes have been reported in heat stressed poultry (Habshey *et al.*, 2019) and dairy cattle (Bernabucci *et al.*, 2010) [2] supporting the notion that enhanced antioxidant

capacity is a common physiological response to heat-induced oxidative stress.

Although no significant breed difference was observed statistically, the pattern of enzyme activity differed between the groups. Vechur cattle exhibited a relatively greater increase in GPx activity, whereas catalase activity tended to be higher in crossbred cattle. This divergence may reflect differences in the regulation and efficiency of antioxidant systems between breeds. GPx plays a crucial role in detoxifying low concentrations of hydrogen peroxide (H₂O₂) due to its high substrate affinity, thereby providing fine control over intracellular redox status. In contrast, catalase is more effective at decomposing higher concentrations of H₂O₂ because of its high catalytic capacity (Kwong-Han *et al.*, 2022; Cadenas and Davies, 2000) ^[3, 9]. From a physiological perspective, the relatively higher catalase activity observed in crossbred cattle may indicate exposure to greater oxidative stress, necessitating rapid removal of excess H₂O₂. Conversely, the enhanced GPx response in Vechur cattle suggests a more efficient and controlled antioxidant defense mechanism, capable of maintaining redox balance at lower ROS levels. These findings are consistent with earlier reports indicating that indigenous breeds possess a more stable oxidative status and better adaptability under thermal stress conditions compared to crossbred animals (Tejaswi *et al.*, 2020) ^[18]. Thus, even in the absence of significant differences, the qualitative pattern of enzyme activity provides important insight into breed-specific adaptive strategies.

In addition to oxidative stress, heat stress also elicits endocrine adjustments that are critical for thermoregulation and metabolic adaptation. Thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4), are key regulators of metabolic rate and heat production (Mullur *et al.*, 2014) ^[14]. In this study, T3 levels decreased significantly in Vechur cattle during heat stress, while T4 showed only a marginal decline. This reduction in thyroid hormone levels can be interpreted as an adaptive mechanism to reduce metabolic heat production (Silva, 2001) ^[17]. The decline in T3 is likely due to reduced peripheral conversion of T4 to T3 under elevated temperatures. Such endocrine modulation is essential for maintaining thermal balance in hot environments (Collier *et al.*, 2008; Liu *et al.*, 2025) ^[5, 11]. In contrast, crossbred cattle exhibited relatively stable thyroid hormone levels during heat stress, suggesting a less pronounced metabolic adjustment. This may limit their ability to effectively reduce internal heat load, thereby increasing susceptibility to thermal stress. Similar observations have been reported by Tejaswi *et al.* (2020) ^[18], who noted that heat-tolerant breeds demonstrate more pronounced endocrine adaptations compared to high-producing but thermally sensitive crossbreds.

Cortisol, a key indicator of stress response, showed a distinct pattern in the present study. Higher cortisol levels in Vechur cattle during the thermoneutral period may be attributed to handling stress associated with restraint and blood sampling (Mormède *et al.*, 2007) ^[13]. During heat stress, cortisol levels varied between breeds, with no significant differences observed, suggesting that cortisol response is influenced by multiple factors rather than heat stress alone. Therefore, cortisol may not serve as a reliable standalone biomarker for assessing heat stress in indigenous breeds such as Vechur. The increase observed in crossbred cattle during heat stress is consistent with reports of elevated

cortisol in heat-sensitive animals (Chen *et al.*, 2023) ^[4]. Mylostyvyi *et al.* (2025) reported reduced cortisol levels under prolonged heat stress in cattle (duration), indicating suppression of the hypothalamic–pituitary–adrenal axis. Collectively, these findings highlight that cortisol responses to heat stress are variable and context-dependent.

Overall, the present study demonstrates that heat stress induces both oxidative and endocrine responses in cattle. However, the magnitude and pattern of these responses differ between breeds. Vechur cattle exhibited a more efficient antioxidant defense system and more adaptive endocrine modulation, contributing to superior thermotolerance. In contrast, crossbred cattle appear to experience comparatively greater physiological strain under similar conditions. These observations emphasize the significance of native cattle breeds in strengthening adaptability and ensuring sustained livestock productivity in hot and humid climates. A limitation of this study is the small sample size (n = 3 per group), which may restrict the broader applicability of the findings. However, the use of controlled environmental conditions minimizes external variability. Future studies with larger sample sizes and longer exposure periods are warranted to validate these findings. In addition, integrating oxidative, endocrine, and molecular biomarkers may improve the reliability of heat stress assessment in cattle.

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