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Breed-specific seasonal adaptation of Gir and Rathi cattle under tropical climate

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ABSTRACT

Heat stress is a major limitation to dairy production in tropical regions, influencing metabolic balance, oxidative stability, and overall performance of cattle. Although indigenous breeds are considered tolerant to high temperatures, comparative information on their thermotolerance at regional levels remains limited. The present study evaluated seasonal adaptability of two important Indian breeds, Gir and Rathi, by assessing their biochemical profile, mineral status, oxidative markers, antioxidant activity, and heat shock protein 70 (HSP70) concentration during autumn and hot-dry summer season. Six healthy lactating cows of each breed were examined in both seasons. Summer conditions were associated with a decline in serum glucose and triglycerides, while cholesterol, creatinine, and liver enzyme activities remained unchanged in both breeds. Calcium and magnesium concentrations decreased, and phosphorus levels increased in summer, with Gir showing a more evident reduction in calcium. Lipid hydroperoxides did not vary between seasons, whereas malondialdehyde levels were lower in summer, suggesting reduced oxidative damage. Antioxidant status improved under heat stress, indicated by elevated total antioxidant capacity, higher glutathione levels, and increased activities of major antioxidant enzymes, along with reduced superoxide dismutase activity. Notably, Rathi cattle exhibited stronger antioxidant responses and significantly higher HSP70 levels during summer, indicating comparatively superior thermotolerance under tropical conditions.

Keywords: Antioxidant enzymes, Heat tolerance, Indigenous cattle, Lipid peroxidation, Thermotolerance, Total antioxidant capacity

Climate change has intensified several environmental stressors such as high ambient temperature, solar radiation, relative humidity, and wind speed, all of which directly influence the performance, health, and welfare of domestic animals (Nardone *et al.* 2010; Das *et al.* 2016). To cope with these challenges, cattle activate a range of cellular, metabolic, physiological, and systemic mechanisms that help maintain homeostasis under stressful conditions (Bernabucci *et al.* 2010; Collier *et al.* 2019). However, these adaptive adjustments often divert energy away from productive functions, resulting in reduced growth, reproduction, and milk yield, thereby affecting the economic sustainability of dairy production systems (Rhoads *et al.* 2009; Bernabucci *et al.* 2010).

Although indigenous cattle generally produce lower milk yields as compared to exotic and crossbred cattle, their productivity remains relatively stable under harsh climatic conditions because of long-term physiological, morphological, and genetic adaptations to the local environment (Hoffmann, 2013; FAO, 2015; Das *et al.* 2016). Breeds that have evolved in regions characterized by high temperatures, water scarcity, and feed limitations possess superior heat tolerance and greater resilience to environmental stressors (Hoffmann, 2013; Prathap *et al.* 2017). Furthermore, the agro-climatic contrast between a breed's native tract and its current production environment influences its adaptive response to heat stress (Prathap *et al.* 2017). Therefore, identifying cattle breeds with superior thermo- and drought-tolerance under specific agro-climatic conditions is essential for developing breeding and management strategies that support climate-resilient dairy production (Hoffmann, 2013; FAO, 2015).

Gir is one of the principal dairy-type Bos indicus breeds of India, originating from the western plains of Gujarat, and is renowned for its superior milk production, inherent heat tolerance, and disease resistance (Patbandha *et al.* 2020). Rathi is an important indigenous dairy breed native to the arid districts of Rajasthan, particularly Bikaner, and is well adapted to hot, dry climatic conditions due to its resilience to heat stress and feed scarcity (NBAGR, 2021). However, despite the recognized thermotolerance of indigenous cattle breeds, comparative information on the biochemical, mineral, oxidative stress, antioxidant, and HSP70 responses of Gir and Rathi cattle maintained under identical environmental and management conditions outside their native breeding tracts remains limited. Therefore, the

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present study was undertaken to evaluate and compare the adaptive responses of these two indigenous breeds under tropical seasonal conditions.

MATERIALS AND METHODS

Ethics statement: This experimental protocol approved by the Institutional Animal Ethics Committee under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, (No.281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/028/Biochemistry/2022).

Animals and experimental design: The study was carried out at National Kamdhenu Breeding Centre (NKBC), Chintaladeevi, SPSR Nellore District, Andhra Pradesh, located at 14.9764°N latitude and 79.6467°E longitude. Six apparently healthy lactating cows each of Gir and Rathi breeds (total 12 animals) maintained under uniform managemental conditions were selected for the experiment. All animals were maintained under loose housing with adequate natural ventilation and free access to clean drinking water under uniform management practices throughout the experimental period.

Sample collection: Blood samples were collected from the jugular vein of all selected animals during early morning hours (6:00–8:00 AM) during autumn (October–November) and the hot-dry summer season (March–May). Samples were drawn into K_2-EDTA tubes and clot activator tubes. Serum was obtained by centrifuging clot activator samples at 2500 rpm for 5 minutes and used for biochemical and mineral analysis. Blood collected in EDTA tubes was used for assessing oxidative stress and antioxidant status.

Meteorological data: Maximum temperature and relative humidity were recorded using a digital thermo hygrometer for one month prior to sampling. Wind speed was recorded only to describe the prevailing environmental conditions and was not included in the THI calculation. (https://www.timeanddate.com/weather). The temperature–humidity index (THI) was calculated using the formula described by (Davis *et al.* 2003)

$$\text{THI}=\left(0.8\times \text{max. temp}\right)+\left[\frac{\%\text{RH}}{100}\times\left(\text{mean temperature-14.4}\right)\right]+46.4$$

where T is ambient temperature (°C) and RH is relative humidity (%).

The Davis *et al.* (2003) equation was selected because it is one of the most widely validated and commonly used temperature–humidity index equations for evaluating heat stress in cattle, allowing comparison with previous studies.

Serum biochemical profile: Serum biochemical parameters were analysed using a Biosystems A15 Automated Biochemical Analyzer (Biosystems, Spain). Commercial reagent kits compatible with the analyser were used according to the manufacturer's instructions, and the analytical principles were based on the cited standard reference methods. Estimation methods included: total protein by biuret method (Gornall *et al.* 1949), glucose by glucose oxidase–peroxidase method (Trinder, 1969), triglycerides by glycerol oxidase–peroxidase method (Fossati and Prencipe, 1982), total cholesterol by cholesterol oxidase–peroxidase method (Allain *et al.* 1974), creatinine by Jaffe enzymatic method (Peake and Whiting, 2006), alanine transaminase (ALT) by IFCC method (Schumann *et al.* 2002a), aspartate transaminase (AST) by IFCC method (Schumann *et al.* 2002b), calcium by arsenazo III method (Michaylova and Ilkova, 1971), magnesium by xylidyl blue method (Burtis *et al.* 2005), and phosphorus by phosphomolybdate method (Gamst and Try, 1980). Serum HSP70 concentration was estimated using a bovine HSP70 ELISA kit (Catalogue No. E0333Bo; Bioassay Technology Laboratory, Shanghai, China) with a detection range of 0.05–30 ng/mL and a sensitivity of 0.024 ng/mL.

Oxidative stress and antioxidative status: Whole blood collected in EDTA tubes was centrifuged at 3000 rpm for 15 minutes. Plasma was removed and the erythrocyte pellet was washed thrice with 0.9% NaCl solution, followed by lysis using chilled distilled water (Lakhani *et al.* 2018). Lipid peroxidation was assessed by malondialdehyde (MDA) estimation (Niehaus and Samuelsson, 1968) and lipid hydroperoxides by the method of (Jiang *et al.* 1992). Hemolysate protein was measured using the Lowry method (Lowry *et al.* 1951). Total antioxidant capacity (Prieto *et al.* 1999) and glutathione (Ellman, 1959) were estimated. Antioxidant enzyme activities were measured using standard methods for superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (Beers and Sizer, 1952), glutathione S-transferases (GST) (Habig *et al.* 1974), and glutathione peroxidase (GPx) (Rotruck *et al.* 1973).

Statistical analysis: Data were expressed as mean ± standard error (SE). Seasonal and breed-wise comparisons were performed using the *t*-test at a significance level of $P \leq 0.05$ (Snedecor and Cochran, 1994). Analysis was done using SPSS Statistics Software (Version 22; SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Thermo-Humidity Index (*THI*): The THI values recorded during the study period (Table 1) indicated that both seasons imposed measurable thermal stress on cattle (Helal *et al.* 2010). Summer conditions, however, represented a more intense heat load than autumn, confirming the greater environmental challenge during this season. THI reflects the combined effects of ambient temperature and relative humidity; although wind speed can contribute to evaporative cooling, it is not typically included in THI

Table 1. Meteorological parameters recorded during the experimental period

	Autumn	Summer
Mean Temperature (°C)	27.92 ± 0.43	32.22 ± 0.44
Mean Humidity (%)	80.51 ± 2.01	72.53 ± 1.89
Mean THI	79.43 ± 0.54	84.90 ± 0.46
Mean wind speed (Km/hr)	5.55 ± 0.28	6.53 ± 0.33

Table 2. Serum biochemical profile of Gir and Rathi cattle during autumn and summer seasons

	Breed	Autumn	Summer
Total protein (g/dl)	Gir	$8.28^{aX} \pm 0.50$	$8.52^{aX} \pm 0.36$
	Rathi	$7.38^{aX} \pm 0.42$	$7.98^{aX} \pm 0.18$
Glucose (mg/dl)	Gir	$53.00^{bX} \pm 3.49$	$40.67^{aY} \pm 2.59$
	Rathi	$50.17^{bX} \pm 2.30$	$40.00^{aY} \pm 2.08$
Triglycerides (mg/dl)	Gir	$47.67^{aX} \pm 2.39$	$36.67^{aY} \pm 3.25$
	Rathi	$48.67^{aX} \pm 1.77$	$33.17^{aY} \pm 4.27$
Total cholesterol (mg/dl)	Gir	$144.17^{aX} \pm 11.00$	$136.17^{aX} \pm 4.83$
	Rathi	$122.50^{aX} \pm 7.18$	$130.00^{aX} \pm 6.67$
Creatinine (mg/dl)	Gir	$2.47^{aX} \pm 0.11$	$2.25^{aX} \pm 0.13$
	Rathi	$2.21^{aX} \pm 0.10$	$2.10^{aX} \pm 0.11$
Alanine Transaminase (U/L)	Gir	$35.80^{aX} \pm 1.17$	$38.25^{aX} \pm 1.83$
	Rathi	$34.67^{aX} \pm 3.10$	$38.77^{aX} \pm 2.38$
Aspartate Transaminase (U/L)	Gir	$65.92^{aX} \pm 3.79$	$62.93^{aX} \pm 3.33$
	Rathi	$63.62^{aX} \pm 3.51$	$67.63^{aX} \pm 3.34$

$^{a, b\ Means}$ bearing different superscripts differ significantly between the breeds within a season ($P \leq 0.05$).

$^{X, Y}$ Means bearing different superscripts differ significantly between the seasons within a breed ($P \leq 0.05$).

calculations (Grewal *et al.* 2021; Vasantha *et al.* 2021). Thus, the conventional THI model was retained for the present study. Recent reports have also emphasized THI as a reliable early indicator for identifying thermotolerant breeds under climate change conditions (Cartwright *et al.* 2023).

Serum biochemical profile: All measured biochemical parameters remained within normal physiological limits (Table 2), but clear seasonal shifts were observed. Serum glucose and triglyceride concentrations declined significantly ($P \leq 0.05$) during summer in both breeds. Similar heat stress-associated hypoglycaemia has been attributed to reduced feed intake, altered insulin sensitivity, increased glucose utilization, and impaired gluconeogenesis, leading to adaptive changes in energy metabolism during thermal stress (Chandrashekhar *et al.* 2017, Nikhil *et al.* 2018, Sammad *et al.* 2020, Cartwright *et al.* 2023, Altyeb *et al.* 2025). The decline in triglycerides might be reflective of enhanced peripheral utilization or increased lipoprotein lipase activity under heat stress (Dar *et al.* 2019).

Total cholesterol did not differ significantly between seasons, aligning with Alberghina *et al.* (2013) but contrasting with earlier studies which have reported reduced cholesterol under heat stress (Nikhil *et al.* 2018; Dar *et al.* 2019). Such inconsistencies might have arisen from differences in THI, breed physiology, metabolic efficiency, or production stage.

Serum total protein, creatinine, ALT, and AST values showed no significant seasonal variation, indicating stable hepatic and renal function across THI ranges (Garcia *et al.* 2015; Nikhil *et al.* 2018; Vasantha *et al.* 2021).

Overall, the biochemical responses of both breeds remained within physiological limits; however, Rathi cattle exhibited comparatively greater metabolic stability during summer, suggesting superior adaptation to heat stress.

Serum mineral profile: Significant seasonal shifts were recorded in all major minerals (Table 3). Calcium and magnesium levels were reduced during summer, whereas phosphorus levels increased in both breeds. Similar reports of low calcium and magnesium during hot periods have been attributed to intracellular calcium accumulation and altered neuromuscular responses (Ermak and Davies, 2002; Sharma *et al.* 2018). Higher phosphorus levels may be indicative of stress-induced mobilization from intracellular reserves (Shah *et al.* 2017).

Gir cattle exhibited a significantly greater reduction ($P \leq 0.05$) in serum calcium concentration during summer than Rathi cattle, indicating comparatively poorer maintenance of mineral homeostasis under heat stress. Conversely, the significantly smaller decline in serum calcium observed in Rathi cattle suggested more effective maintenance of mineral homeostasis and superior physiological adaptation to elevated environmental temperatures.

Recent studies also emphasized the role of mineral balance in the physiological resilience of heat-stressed dairy cows, linking mineral shifts to broader metabolic and reproductive challenges (Altyeb *et al.* 2025).

Oxidative stress indicators: Despite higher THI during summer, lipid hydroperoxide (LPO) levels did not differ significantly between seasons (Table 4). Malondialdehyde (MDA), a key marker of lipid peroxidation, decreased significantly ($P < 0.05$) in summer. Lipid hydroperoxides (LPO) and malondialdehyde (MDA) represent different stages of lipid peroxidation. Lipid hydroperoxides are primary oxidation products formed during the initiation phase, whereas MDA is a secondary end-product generated following the decomposition of lipid hydroperoxides (Jiang *et al.* 1992; Ayala *et al.* 2014). Therefore, these biomarkers do not necessarily exhibit parallel changes (Ayala *et al.* 2014). In the present study, the unchanged LPO levels together with reduced MDA suggested that although lipid

Table 3. Serum mineral profile of Gir and Rathi cattle during autumn and summer seasons

	Breed	Autumn	Summer
Calcium (mg/dl)	Gir	$10.04^{aX} \pm 0.08$	$8.62^{aY} \pm 0.17$
	Rathi	$10.89^{aX} \pm 0.2$	$9.40^{bY} \pm 0.28$
Magnesium (mg/dl)	Gir	$3.63^{aX} \pm 0.10$	$2.52^{aY} \pm 0.06$
	Rathi	$4.47^{bX} \pm 0.11$	$2.61^{aY} \pm 0.09$
Phosphorus (mg/dl)	Gir	$4.48^{aX} \pm 0.48$	$5.98^{aY} \pm 0.28$
	Rathi	$3.94^{aX} \pm 0.41$	$5.26^{aY} \pm 0.24$

$^{a, b\ Means}$ bearing different superscripts differ significantly between the breeds within a season ($P \leq 0.05$).

$^{X, Y}$ Means bearing different superscripts differ significantly between the seasons within a breed ($P \leq 0.05$).

Table 4. Status of oxidative stress and antioxidants in Gir and Rathi cattle during autumn and summer seasons

	Breed	Autumn	Summer
Malondialdehyde (MDA) (µmol/ml hemolysate)	Gir	$42.30^{bx} \pm 4.06$	$7.42^{ay} \pm 1.22$
	Rathi	$23.14^{ax} \pm 2.48$	$9.44^{ay} \pm 1.46$
Lipid hydroperoxides (LPO) (x10^{3}mmol/ml hemolysate)	Gir	$2.55^{ax} \pm 0.12$	$2.47^{ax} \pm 0.24$
	Rathi	$2.15^{ax} \pm 0.10$	$2.53^{ax} \pm 0.25$
Total antioxidant capacity (TAC) (µg of ascorbic acid equivalents/ml hemolysate)	Gir	$3.97^{ax} \pm 0.25$	$4.78^{ay} \pm 0.26$
	Rathi	$4.35^{ax} \pm 0.12$	$5.44^{ay} \pm 0.24$
Reduced glutathione (GSH) (µg/ ml hemolysate)	Gir	$44.40^{ax} \pm 2.72$	$180.40^{by} \pm 20.64$
	Rathi	$51.01^{ax} \pm 3.40$	$120.20^{ay} \pm 9.10$

[a, b] Means bearing different superscripts differ significantly between the breeds within a season ($P \leq 0.05$). [X, Y] Means bearing different superscripts differ significantly between the seasons within a breed ($P \leq 0.05$).

peroxidation may have been initiated, enhanced antioxidant defence mechanisms, as evidenced by increased TAC, GSH, catalase, GPx and GST activities, might have limited the propagation of oxidative damage and the formation of secondary lipid peroxidation products (Li *et al.* 2020; Cartwright *et al.* 2023; Altyeb *et al.* 2025).

A similar decline in oxidative stress markers with increasing THI had been reported in dairy cattle (Li *et al.* 2020). Increased HSP70 levels has also been linked to reduced oxidative injury in other species (Oksala *et al.* 2014), which was consistent with the patterns observed here. The loose-housing management system and adequate ventilation maintained at the experimental farm might have also contributed to minimizing excessive oxidative damage despite high ambient temperatures.

The comparatively stronger antioxidant response observed in Rathi cattle, together with enhanced antioxidant enzyme activities, indicated a more efficient cellular defence mechanism against heat-induced oxidative stress than in Gir cattle.

Both breeds exhibited enhanced antioxidant responses during summer (Table 4). Total antioxidant capacity (TAC) and glutathione (GSH) levels increased significantly ($P \leq 0.05$), which was consistent with earlier findings (Li *et al.* 2020). Elevated GSH might be due to increased glutathione synthesis driven by heat-induced oxidative signalling (Habeeb *et al.* 2018).

Although Gir cattle exhibited higher glutathione concentrations during summer, the greater coordinated enhancement of GPx and GST activities together with higher HSP70 expression in Rathi cattle suggested a more efficient overall antioxidant defence mechanism against heat-induced oxidative stress.

Catalase, glutathione peroxidase (GPx), and glutathione-S-transferase (GST) activities increased significantly during summer in both breeds, indicating activation of enzymatic antioxidant defence mechanisms (Lakhani *et al.* 2018; Nikhil *et al.* 2018; Sihag, 2021).

Superoxide dismutase (SOD) activity decreased significantly during summer, a finding consistent with previous observations in cattle and buffaloes (Yeotikar *et al.* 2019; Li *et al.* 2020). Reduced SOD activity under chronic heat exposure might be indicative regulatory down-modulation as other antioxidant pathways become more active.

Table 5. Activity of antioxidant enzymes in Gir and Rathi cattle during autumn and summer seasons

	Breed	Autumn	Summer
Superoxide dismutase (SOD) (U/mg protein)	Gir	$0.9^{ay} \pm 0.08$	$0.59^{ay} \pm 0.07$
	Rathi	$0.92^{ay} \pm 0.16$	$0.48^{ax} \pm 0.07$
Catalase (U/mg protein)	Gir	$0.2^{ax} \pm 0.02$	$0.67^{by} \pm 0.09$
	Rathi	$0.16^{ax} \pm 0.01$	$0.24^{ay} \pm 0.08$
Glutathione peroxidase (GPx) (U/mg protein)	Gir	$101.35^{bx} \pm 2.01$	$122.16^{by} \pm 3.31$
	Rathi	$22.74^{ax} \pm 2.1$	$103.1^{ay} \pm 8.7$
Glutathione-S-transferase (GST) (U/mg protein)	Gir	$0.18^{bx} \pm 0.01$	$0.74^{ay} \pm 0.12$
	Rathi	$0.08^{ax} \pm 0.02$	$0.73^{ay} \pm 0.17$

[a, b] Means bearing different superscripts differ significantly between the breeds within a season ($P \leq 0.05$).

[X, Y] Means bearing different superscripts differ significantly between the seasons within a breed ($P \leq 0.05$).

Although antioxidant activity increased in both breeds during summer, Rathi cattle exhibited a greater coordinated enhancement of GPx and GST activities together with higher HSP70 expression, indicating a more effective adaptive response to heat stress.

HSP70 expression: HSP70 is widely recognized as a biomarker of cellular adaptation to heat stress in cattle (Bharathi *et al.* 2017). Its expression increases with elevated temperature and prolonged heat exposure (Osei-Amponsah *et al.* 2019). Several studies have associated higher HSP70 levels with improved thermotolerance in cattle and goats (Bhanuprakash *et al.* 2016; Mayengbam *et al.* 2016; Rout *et al.* 2016).

In the present study, Rathi cattle exhibited significantly higher serum HSP70 levels during summer compared with Gir (Fig. 1), along with a greater seasonal fold-change. This is suggestive a more vigorous cellular defence response and supports the breed's superior thermotolerance. Recent evidence has identified HSP70 as a reliable biomarker for

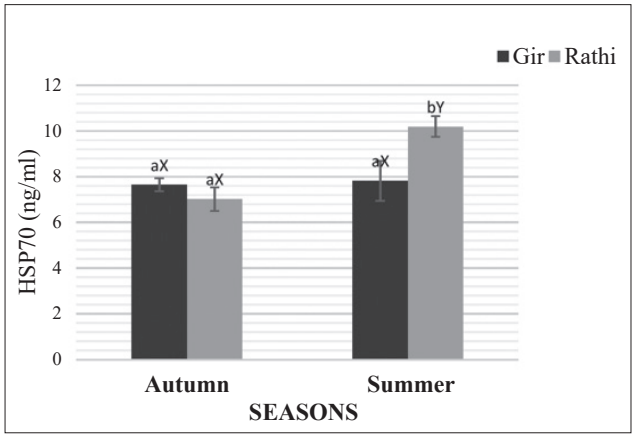


Fig. 1. Serum HSP70 concentration in Gir and Rathi cattle during autumn and summer seasons

[a, b] Means bearing different superscripts differ significantly between the breeds within a season ($P \leq 0.05$).

[X, Y] Means bearing different superscripts differ significantly between the seasons within a breed ($P \leq 0.05$).

assessing heat tolerance and selecting climate-resilient dairy cattle under warming environmental conditions (Rakib *et al.* 2026). Supporting this, Vaidya *et al.* (2026) reported breed-dependent variation in thermotolerance among indigenous milch cattle, with Kankrej cows exhibiting superior physiological and molecular adaptations, including enhanced heat shock protein expression, compared with Sahiwal during seasonal heat stress. Consistent with these findings, the higher HSP70 levels observed in Rathi cattle further supported its superior cellular adaptation to heat stress compared with Gir cattle.

When interpreted together with the observed biochemical, mineral and antioxidant responses, the significantly increased HSP70 expression in Rathi cattle provided additional evidence of superior cellular protection and adaptive resilience under tropical heat stress.

The present study demonstrated that both Gir and Rathi cattle activate coordinated metabolic, mineral, oxidative, and antioxidant adaptations to cope with seasonal heat stress. However, the magnitude and pattern of these responses indicated that Rathi cattle possess comparatively superior thermotolerance under tropical summer conditions. Rathi cattle exhibited more effective maintenance of mineral homeostasis, a more robust antioxidant defence system, and significantly higher HSP70 expression, reflecting enhanced cellular protection against heat-induced stress. Collectively, the biochemical, mineral, oxidative stress, antioxidant, and heat-shock protein responses observed in the present investigation provided a strong evidence for the greater adaptive resilience of Rathi cattle compared with Gir cattle. These findings suggested that Rathi cattle may be a more suitable indigenous breed for sustainable dairy production in heat-prone tropical regions. Although the study included all eligible animals available at the study site, validation in larger populations across multiple herds and diverse geographical regions would further strengthen the generalizability of the present findings.

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