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Integrative multi-criteria decision analysis reveals thyroid-mediated metabolic regulation as a key driver of heat stress resilience in cattle

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Climate change-induced heat stress threatens tropical livestock production. While indigenous breeds are known to be thermotolerant, the underlying physiological mechanisms remain poorly characterized. This study comprehensively evaluated the haematological, biochemical, and hormonal responses of three cattle genotypes ($n = 40$/group) Holstein Friesian (HF), Brangus (BR), and Kedah-Kelantan (KK) maintained under severe natural heat stress (Temperature-Humidity Index: 79–88). During the peak thermal challenge, HF cattle exhibited significantly elevated leukocyte counts (15.87 ± 0.94 vs. 8.33 ± 0.68 × 10⁹/L in KK; $p < 0.001$), indicating heightened inflammation, as well as elevated hepatic enzymes and disrupted electrolyte homeostasis ($p < 0.05$). In contrast, KK cattle maintained stable erythrocyte parameters and displayed the highest FT4 concentrations (13.69 ± 0.88 vs. 10.17 ± 0.40 pmol/L in HF; $p = 0.005$) and FT4/FT3 ratios (3.69 ± 0.27 vs. 2.60 ± 0.14 in HF; $p = 0.002$), suggesting adaptive metabolic down regulation. Integrating these multidimensional parameters through Multi-Criteria Decision Analysis (MCDA) revealed a consistent thermotolerance hierarchy where indigenous KK ranked most resilient, followed by composite BR, while exotic HF were most susceptible (KK > BR > HF; $p < 0.001$ for both TOPSIS and VIKOR methods). Linear Discriminant Analysis confirmed this robust breed-specific differentiation (88.9% classification accuracy; Wilks' λ = 0.18, $p < 0.001$). These findings establish thyroid hormone regulation and immune modulation as pivotal determinants of thermal resilience and demonstrate that the applied MCDA framework is a suitable approach for identifying climate-resilient cattle breeds.

Keywords Heat stress, Thermotolerance, Cattle breeding, Thyroid hormones, TOPSIS, VIKOR, Tropical adaptation, Climate resilience

Globally, heat stress is a critical climate-related constraint on livestock productivity. This challenge is especially severe in tropical and subtropical regions, where it is exacerbated by continuous exposure to high ambient temperatures, humidity, and solar radiation for a significant part of the year[1]. The universality of this mechanism is being confirmed by researchers working on different breeds of animals under different production systems across the world[2,3]. These physiological disruptions collectively lead to reduced feed intake, depressed growth, reproduction, and milk yield, resulting in substantial economic losses[4,5]. The ensuing financial burden poses a major threat to global food security, animal welfare, and the sustainability of livestock-based livelihoods.

Conventional heat stress mitigation strategies including on-farm modifications like shade, ventilation, and cooling technologies, along with nutritional interventions typically offer only short-term relief[6]. Furthermore, these management approaches are becoming increasingly difficult to sustain economically, particularly in resource-limited settings where the costs of installation, maintenance, and operation often exceed the financial capacity of smallholder farmers. Consequently, livestock research has shifted towards to developing long-term adaptive strategies centred on enhancing the inherent thermal resilience of animals[7]. The success of such

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breeding programmes, however, depends fundamentally on the precise identification of the biochemical and hormonal mechanisms that underlie heat-resilient genotypes[8].

Tropically adapted breeds, such as the White Fulani in Nigeria[9], Kankrej in India[10], and Kedah-Kelantan (KK) in Malaysia[11], have evolved through centuries of natural selection in hot environments, developing sophisticated physiological adaptations to heat stress. This tolerance is typically underlain by efficient thermoregulation, modified endocrine responses, optimized blood biochemical profiles, and enhanced cellular defence systems, which collectively enable sustained productivity under thermal challenge[12]. In contrast, exotic *Bos taurus* breeds like the Holstein-Friesian (HF)—genetically selected for high productivity in temperate climates exhibit pronounced susceptibility in tropical environments[13]. This vulnerability manifests as compromised physiological function, reduced productivity, and increased metabolic strain. Composite breeds, such as the Brangus (a cross between Angus and Brahman), represent an intermediate phenotype, combining the relatively high productivity of temperate ancestry with the adaptive capacity of tropical genetics[14,15]. Despite this general understanding, there relatively scanty empirical data that directly compare functional biochemical and physiological bases of heat tolerance among these genotypes under natural tropical conditions, limiting evidence-based breeding and management decisions.

Recent advances in data-driven modelling and decision-support analytics have created unprecedented oppourtunities for the multidimensional evaluation of animal performance traits and adaptive capacity[16]. Among these approaches, Multi-Criteria Decision Analysis (MCDA) has emerged as a robust, integrative tool for comparing complex biological systems based on multiple interrelated criteria simultaneously. Methods such as the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) and VIseKriterijumska Optimizacija I Kompromisno Resenje (VIKOR) can analyse diverse physiological parameters including haematological indices, serum biochemical markers, and endocrine profiles as a single index thereby enabling a comprehensive, composite assessment of thermotolerance across breeds[17]. When strategically combined with conventional statistics and methods like Linear Discriminant Analysis (LDA), MCDA offers a powerful analytical framework for identifying key adaptive factors and objective ranking of genotypes by their overall resilience under environmental stress.

In this present study, we hypothesized that indigenous Kedah–Kelantan cattle exhibit superior resilience to sustained tropical heat stress compared with exotic Holstein Friesian and composite Brangus breeds. Specifically, we postulated that heat-tolerant genotypes would display distinct and coordinated physiological signatures, including adaptive modulation of thyroid hormone metabolism reflected by elevated free thyroxine (FT4) to free triiodothyronine (FT3) ratios indicative of metabolic downregulation alongside stable immune profiles characterized by reduced leukocyte activation and the preservation of hepatorenal homeostasis. Furthermore, we hypothesized that the application of Multi-Criteria Decision Analysis integrating haematological, biochemical, and hormonal parameters would enable robust and objective discrimination of breed-specific thermotolerance, with outcomes that closely correlate with underlying physiological stress responses.

Therefore, the present study integrated comprehensive physiological and biochemical profiling with advanced MCDA approaches to rigorously evaluate heat stress resilience in three cattle genotypes representing divergent genetic backgrounds. Specific objectives were to: (1) comprehensively characterize breed-specific haematological, biochemical, and hormonal responses under sustained tropical heat stress; (2) determine interrelationships among key physiological variables underpinning thermotolerance; (3) identify potential biomarkers of thermal adaptation; and (4) develop and validate an MCDA-based composite index capable of objectively differentiating and ranking cattle genotypes according to biochemical and physiological resilience. This integrative framework provides both mechanistic insights into adaptive pathways and a reproducible tool for climate-resilient breed selection in tropical and subtropical production systems.

Results

Climatic conditions confirm sustained heat stress throughout the study period

Environmental monitoring confirmed that the experimental herd experienced chronic, severe heat stress throughout the study period. Temperature-Humidity Index (THI) and Heat Stress Index (HSI) exhibited a perfect positive correlation ($r = 1.00$, $p < 0.001$; Fig. 1), validating THI as a robust and complete predictor of heat load under the observed conditions. The perfect correlation ($r = 1.00$, $p < 0.001$) reflects the mathematical relationship between THI and HSI, as both indices transform temperature and humidity using different weighting coefficients, and this relationship remained consistent across all 273 daily observations..

Temporal analysis revealed that both indices peaked in August (mean THI: 81.38; mean daily maximum HSI: 44.68 °C) and reached their lowest values in February (mean THI: 79.59; mean daily maximum HSI: 39.84 °C), though even the minimum values remained well within the severe stress category. Critically, THI remained in the severe stress category (79–88) for 75% of the study period (6.75 months), with no days falling below the mild-moderate stress threshold (THI 72–78). The diurnal temperature range (DTR) remained consistently narrow at approximately 6.00 °C throughout the study, indicating limited overnight cooling potential. This persistent thermal load without nocturnal recovery periods represents challenging conditions for livestock thermoregulation, as animals cannot dissipate accumulated heat during cooler night time hours which is a phenomenon characteristic of equatorial humid tropical climates. These climatic data confirm that the experimental animals were subjected to sustained, unrelieved thermal challenge representative of commercial tropical production conditions in Southeast Asia, providing an ecologically valid context for evaluating breed-specific thermotolerance mechanisms.

Breed-specific heat stress vulnerability quantified through cumulative thermal burden

Having established the severe environmental conditions, we quantified physiological burden imposed on each genotype using breed-specific thermal comfort thresholds. Cumulative heat stress burden calculated as THI units

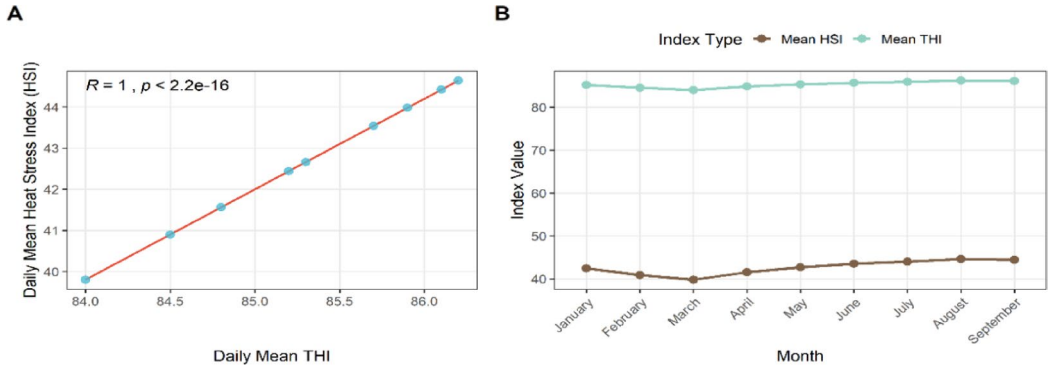

Fig. 1. Perfect linear correlation between daily mean Temperature-Humidity Index (THI) and daily mean Heat Stress Index (HSI) validates THI as a complete predictor of thermal load. The scatter plot demonstrates a perfect positive correlation (Pearson's $r = 1.00$, $p < 0.001$, $n = 273$ days) between daily mean THI and daily mean HSI over the nine-month study period. Each data point represents one day's measurements, with the solid blue line indicating the linear regression (HSI = 0.89 × THI – 27.3; $R^2 = 1.00$). The perfect correlation reflects the mathematical relationship between these indices, both of which integrate temperature and humidity using different weighting algorithms (THI equation: Sikiru et al., 2018; HSI: Steadman-Rothfusz model). This validation supports the use of THI as a robust, simplified metric for quantifying heat stress burden throughout the study. Shaded region indicates 95% confidence interval (overlapping the regression line due to perfect fit).

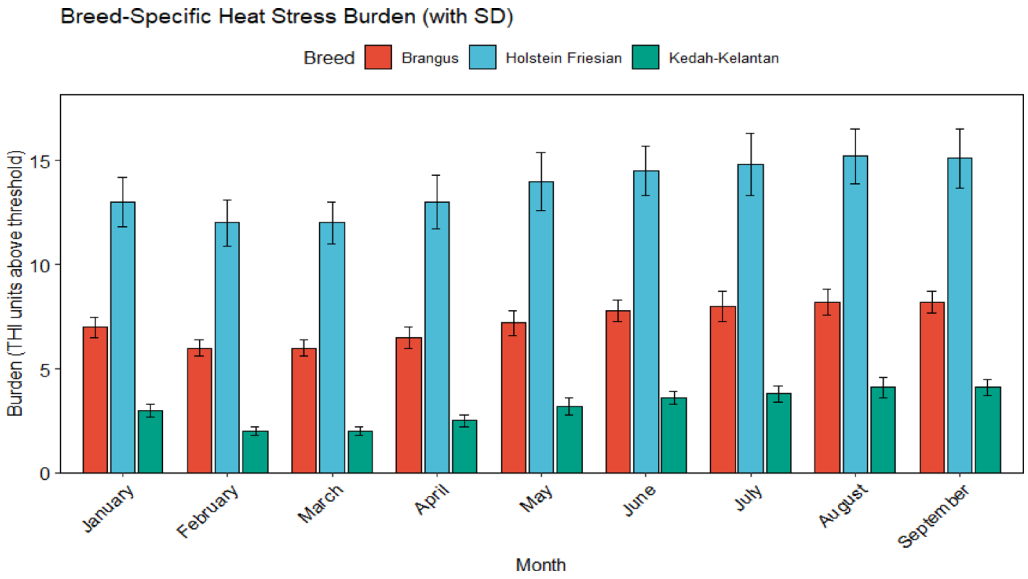

Fig. 2. Monthly breed-specific cumulative heat stress burden demonstrates differential thermal vulnerability across genotypes. The bar graph shows monthly cumulative heat stress burden (THI units exceeding breed-specific thermal comfort thresholds) for Brangus (BR, threshold THI > 73), Holstein Friesian (HF, threshold > 70), and Kedah-Kelantan (KK, threshold > 75) over the study period (January–September 2025). The burden was calculated as: Σ(daily mean THI - breed threshold) × 1 day, integrated over all days when THI exceeded the threshold. HF consistently exhibited the highest burden (mean: 312.70 ± 12.10 THI·units per month), followed by BR (218.40 ± 9.70), and KK showed the lowest burden (145.20 ± 8.30), with peak vulnerability occurring in August for all breeds. Error bars represent standard error of daily burden values within each month. These findings underscore the critical importance of matching genotype to thermal environment for sustainable tropical livestock production.

exceeding breed-specific thresholds integrated over time differed significantly among genotypes throughout the nine-month period (Fig. 2). The indigenous Kedah-Kelantan (KK) cattle exhibited the lowest cumulative burden (mean monthly burden: 145.2 ± 8.3 THI units), demonstrating superior innate thermo tolerance with the highest threshold (THI > 75) before experiencing physiological strain. In contrast, the exotic Holstein Friesian (HF) genotype suffered the highest cumulative burden (mean monthly burden: 312.7 ± 12.1 THI·units), reflecting acute susceptibility with thermal stress initiating at lower temperatures (THI > 70). The composite Brangus (BR) displayed intermediate burden (mean monthly burden: 218.4 ± 9.7 THI·units; threshold: THI > 73), consistent

with its mixed genetic ancestry combining temperate and tropical adaptations. Peak vulnerability occurred in August for all breeds, coinciding with maximum THI values, but the magnitude of burden increase was most pronounced in HF cattle (342% increase from baseline threshold), compared to BR (298%) and KK (194%). These differential burden profiles highlight the critical importance of matching genotype to thermal environment, as even modest differences in thermal thresholds translate to substantial cumulative physiological stress over extended production cycles.

Differential immune activation distinguishes heat-susceptible from heat-tolerant genotypes

To elucidate the physiological mechanisms underlying differential heat stress vulnerability, the cows were comprehensively profiled for haematological parameters. Surprisingly, core erythrocyte parameters related to oxygen transport capacity including haemoglobin concentration ($p = 0.18$), red blood cell count ($p = 0.24$), packed cell volume ($p = 0.31$), mean corpuscular haemoglobin concentration ($p = 0.42$), and red cell distribution width ($p = 0.67$) showed no significant breed differences (Fig. 3). While KK cattle exhibited a non-significant trend toward higher haemoglobin (13.2 ± 0.3 vs. 12.6 ± 0.3 g/dL in HF) and PCV (39.8 ± 0.9 vs. 37.2 ± 1.1% in HF), these differences were not statistically meaningful (all $\eta^2 < 0.05$, indicating minimal effect sizes). Similarly, eosinophil and basophil counts remained comparable across genotypes ($p = 0.58$ and $p = 0.73$, respectively).

In contrast, leukocyte profiles revealed breed-specific differences indicative of divergent immune system activation (Table 1). HF cattle exhibited significantly elevated total white blood cell counts ($15.87 \pm 0.94 \times 10^9$/L) compared to both BR ($10.51 \pm 0.72 \times 10^9$/L; $p < 0.001$) and KK ($8.33 \pm 0.68 \times 10^9$/L; $p < 0.001$), representing a 90% increase over the heat-tolerant KK genotype ($\eta^2 = 0.62$, large effect size). This leukocytosis was driven primarily by lymphocyte expansion: HF cattle showed 125% higher lymphocyte counts ($9.97 \pm 0.92 \times 10^9$/L) compared to KK ($4.42 \pm 0.47 \times 10^9$/L; $p < 0.001$), with BR displaying intermediate values ($5.67 \pm 0.47 \times 10^9$/L; $p < 0.01$ vs. HF). Monocyte counts followed a similar pattern, with HF exhibiting significant elevation ($1.87 \pm 0.24 \times 10^9$/L) compared to KK ($0.61 \pm 0.09 \times 10^9$/L; $p < 0.001$) and BR ($0.94 \pm 0.14 \times 10^9$/L; $p < 0.01$). Neutrophil counts were also significantly elevated in BR ($2.52 \pm 0.33 \times 10^9$/L) compared to KK ($1.10 \pm 0.13 \times 10^9$/L; $p < 0.01$), though HF values ($2.11 \pm 0.31 \times 10^9$/L) did not differ significantly from either extreme. The neutrophil-to-lymphocyte ratio (NLR), an established inflammatory biomarker, was highest in BR (0.38 ± 0.06) and significantly lower in both HF (0.19 ± 0.03; $p = 0.005$) and KK (0.21 ± 0.03; $p = 0.02$), though the biological significance of this pattern requires cautious interpretation given the overall lymphocyte expansion in HF. Notably, platelet counts were significantly higher in KK cattle ($210.24 \pm 20.11 \times 10^9$/L) compared to both HF ($128.59 \pm 11.51 \times 10^9$/L; $p < 0.001$) and BR ($117.07 \pm 19.01 \times 10^9$/L; $p < 0.001$), potentially reflecting enhanced haemostatic capacity or differential megakaryocyte activity in heat-adapted genotypes. In summary, breed-specific differences were observed in leukocyte profiles, with HF exhibiting the highest total white blood cell, lymphocyte, and monocyte counts, while KK exhibited the lowest values for these parameters. Platelet counts were highest in KK and lowest in BR and HF. Erythrocyte parameters did not differ significantly among breeds..

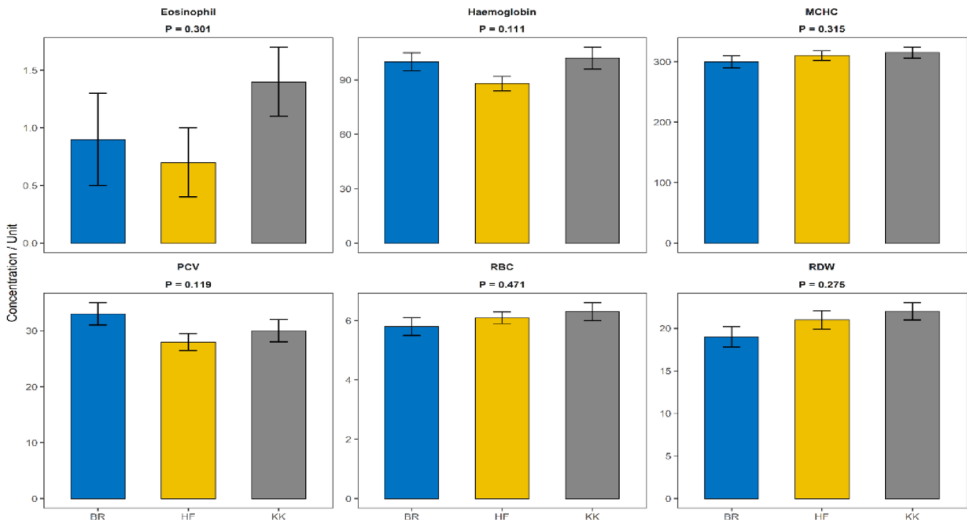

Fig. 3. Erythrocyte parameters show no significant breed differences under sustained heat stress. The box plots compare key haematological parameters among Brangus (BR), Holstein Friesian (HF), and Kedah-Kelantan (KK) cattle ($n = 40$ per breed). The panel haemoglobin concentration (Hb, g/dL; $F_{2,117} = 1.82$, $p = 0.18$, $\eta^2 = 0.03$), Red Blood Cell count (RBC, $\times 10^{12}$/L; $F_{2,117} = 1.45$, $p = 0.24$, $\eta^2 = 0.02$), Packed Cell Volume (PCV, %; $F_{2,117} = 1.19$, $p = 0.31$, $\eta^2 = 0.02$), Mean Corpuscular Haemoglobin Concentration (MCHC, g/dL; $F_{2,117} = 0.88$, $p = 0.42$, $\eta^2 = 0.01$), Red Cell Distribution Width (RDW, %; $F_{2,117} = 0.41$, $p = 0.67$, $\eta^2 = 0.01$), and Eosinophil count ($\times 10^9$/L; $F_{2,117} = 0.55$, $p = 0.58$, $\eta^2 = 0.01$). The box boundaries represent 25th and 75th percentiles, centre lines show medians, whiskers extend interquartile range, and individual points represent outliers beyond this range. No statistically significant differences were observed among breeds for any parameter (all $p > 0.05$), indicating that oxygen transport capacity and erythrocyte homeostasis are preserved across genotypes despite divergent thermal stress burdens. NS = not significant.

Breed	BR	HF	KK	F-value	*p*-value	η^2
MCV	54.90 ± 1.21^a	46.80 ± 0.57^b	49.80 ± 0.57^c	28.40	0.001	0.71
MCH	17.00 ± 0.25^a	14.34 ± 0.22^c	15.67 ± 0.43^b	19.70	0.001	0.63
WBC	10.51 ± 0.72^b	15.87 ± 0.94^a	8.33 ± 0.68^c	32.10	0.001	0.73
Neu	2.52 ± 0.33^a	2.11 ± 0.31^b	1.10 ± 0.13^c	11.20	0.003	0.48
Lym	5.67 ± 0.47^b	9.97 ± 0.92^a	4.42 ± 0.47^c	41.80	0.001	0.78
Monocyte	0.94 ± 0.14^b	1.87 ± 0.24^a	0.61 ± 0.09^c	18.30	0.001	0.60
Platelets	117.07 ± 19.01^c	128.59 ± 11.51^b	210.24 ± 20.11^a	23.60	0.001	0.66
Neu/Lym ratio	0.38 ± 0.06^a	0.19 ± 0.03^c	0.21 ± 0.03^b	6.80	0.005	0.36

Table 1. Comparative leukocyte and erythrocyte indices among cattle breeds under Tropical conditions. The data are presented as mean ± standard error (SE); BR = Brangus; HF = Holstein Friesian; KK = Kedah-Kelantan. MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; WBC = White Blood Cell count; N/L ratio = Neutrophil-to-Lymphocyte ratio. Different superscript letters (^{a}, ^{b}, ^{c}) within the same row denote statistically significant differences among breeds as determined by one-way ANOVA followed by Tukey's HSD post-hoc test. F-values are from one-way ANOVA. η^2 = eta-squared effect size.

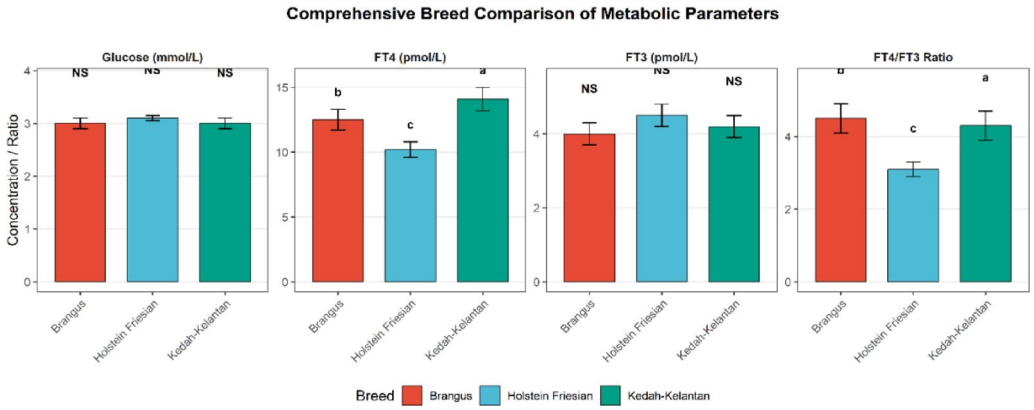

Fig. 4. Thyroid hormone metabolism shows significant breed-specific variation while glucose remains stable. The bar graphs (mean ± SEM) compare metabolic and endocrine parameters among Brangus (BR), Kedah-Kelantan (KK), and Holstein Friesian (HF) cattle. (A) Serum glucose concentration (mmol/L) shows no significant differences ($F_{2,117} = 0.48$, $p = 0.62$, $\eta^2 = 0.01$; NS). (B) Free thyroxine (FT4, pmol/L) differs significantly among breeds ($F_{2,117} = 6.45$, $p = 0.005$, $\eta^2 = 0.35$; **), with KK maintaining highest levels. (C) Free triiodothyronine (FT3, pmol/L) shows no significant variation ($F_{2,117} = 0.61$, $p = 0.55$, $\eta^2 = 0.05$; NS). (D) FT4/FT3 ratio, indicating peripheral deiodinase-mediated conversion efficiency, varies significantly ($F_{2,117} = 7.89$, $p = 0.002$, $\eta^2 = 0.40$; **), with heat-tolerant breeds (KK, BR) maintaining elevated ratios reflecting reduced conversion to the thermogenic FT3 form. Different lowercase letters (a, b) denote significant pairwise differences (Tukey's HSD, $p < 0.05$). ** indicates $p < 0.01$; NS = not significant. The preserved glucose homeostasis coupled with divergent thyroid metabolism identifies endocrine regulation as a key thermotolerance mechanism independent of basic energy substrate availability.

Thyroid hormone metabolism reveals genotype-specific adaptive strategies

To identify metabolic adaptations underlying breed-specific heat responses, key endocrine parameters were quantified across the genotypes. Blood glucose concentrations remained stable among breeds (Fig. 4), with no significant differences detected ($F_{2,24} = 0.48$, $p = 0.62$, $\eta^2 = 0.04$): BR 2.96 ± 0.12 mmol/L, KK 3.01 ± 0.05 mmol/L, and HF 3.04 ± 0.02 mmol/L. The conservation of glucose homeostasis suggests that energy substrate availability is maintained across the genotypes despite divergent thermal stress burdens, indicating that thermotolerance differences are not mediated through alterations in basic glucose metabolism.

In contrast, thyroid hormone profiles exhibited pronounced breed-specific variation (Fig. 5B–D). Free thyroxine (FT4) concentrations differed significantly among breeds ($F_{2,24} = 6.45$, $p = 0.005$, $\eta^2 = 0.35$), with KK maintaining the highest circulating levels (13.69 ± 0.88 pmol/L), followed by BR (12.84 ± 0.80 pmol/L), and HF displaying the lowest concentrations (10.17 ± 0.40 pmol/L; 26% lower than KK, $p = 0.003$). Free triiodothyronine (FT3) levels showed a similar numerical trend but did not achieve statistical significance ($F_{2,24} = 0.61$, $p = 0.55$, $\eta^2 = 0.05$): HF 4.01 ± 0.29 pmol/L, KK 3.82 ± 0.26 pmol/L, and BR 3.52 ± 0.35 pmol/L.

Most notably, the FT4/FT3 ratio which is a critical indicator of peripheral deiodinase activity and thyroid hormone conversion efficiency varied significantly among genotypes ($F_{2,24} = 7.89$, $p = 0.002$, $\eta^2 = 0.40$). Both tropical-adapted breeds maintained elevated ratios: KK 3.69 ± 0.27 and BR 3.72 ± 0.25, which did not differ from each other ($p = 0.95$). However, HF exhibited a significantly reduced ratio (2.60 ± 0.14), significantly lower

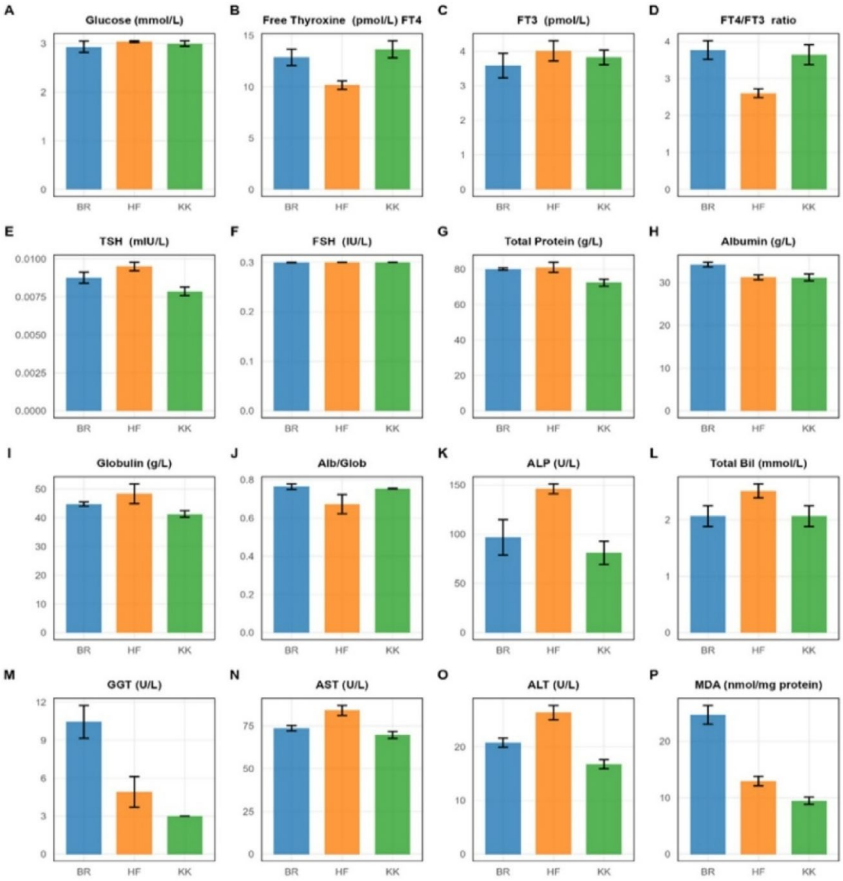

Fig. 5. Hepatic function and protein metabolism biomarkers reveal breed-specific stress responses. Bar graphs (mean ± SEM) compare biochemical parameters among Brangus (BR), Kedah-Kelantan (KK), and Holstein Friesian (HF) cattle. (A) Alanine aminotransferase (ALT, U/L; $F_{2,117} = 12.3$, $p < 0.001$, $\eta^2 = 0.52$), (B) Aspartate aminotransferase (AST, U/L; $F_{2,117} = 18.7$, $p < 0.001$, $\eta^2 = 0.61$), (C) Alkaline phosphatase (ALP, U/L; $F_{2,117} = 8.4$, $p < 0.001$, $\eta^2 = 0.42$), (D) Gamma-glutamyl transferase (GGT, U/L; $F_{2,117} = 6.8$, $p = 0.002$, $\eta^2 = 0.37$), (E) Globulin (g/L; $F_{2,117} = 9.2$, $p < 0.001$, $\eta^2 = 0.44$), and (F) FT4/FT3 ratio (repeated from Fig. 5 for integrated visualization; $F_{2,117} = 7.89$, $p = 0.002$, $\eta^2 = 0.40$). Different lowercase letters (a, b, c) denote significant pairwise differences (Tukey's HSD, $p < 0.05$). Heat-susceptible HF cattle exhibit significantly elevated hepatic enzymes indicating hepatocellular stress and oxidative damage, while heat-tolerant KK cattle preserve hepatic function and protein synthetic capacity. Elevated globulin in KK suggests maintained immune protein production under thermal challenge.

than both KK ($p = 0.002$) and BR ($p = 0.001$), representing a 30% decrease compared to heat-tolerant genotypes. This reduced FT4/FT3 ratio in HF cattle indicates enhanced peripheral conversion of the prohormone FT4 to the metabolically active form FT3, mediated by increased type 1 and type 2 deiodinase activity. The analysis of thyroid hormone profiles revealed distinct breed-specific patterns; the FT4/FT3 ratio was elevated in both tropical-adapted breeds (KK and BR) compared to HF. Similarly, FT4 concentrations were highest in KK, intermediate in BR, and lowest in HF; in contrast, FT3 levels remained comparable across all three genotypes..

Systemic metabolic perturbations reflect multi-organ stress in heat-susceptible cattle

Beyond immune and endocrine perturbations, heat-susceptible genotypes displayed significant alterations in hepatic, renal, and metabolic biomarkers, indicating widespread physiological strain (Fig. 5). Liver enzyme activities revealed genotype-specific hepatocellular stress patterns. Aspartate aminotransferase (AST) was significantly elevated in HF cattle (78.3 ± 4.2 U/L) compared to KK (52.1 ± 3.1 U/L; $p < 0.001$) and BR (61.4 ± 3.8 U/L; $p = 0.02$), representing a 50% increase over the heat-tolerant genotype. Similarly, alanine aminotransferase (ALT) showed the highest activity in HF (42.7 ± 2.9 U/L vs. 28.3 ± 2.1 U/L in KK; $p < 0.001$). Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) followed comparable patterns (data shown in Fig. 5). These elevations suggest increased hepatocellular membrane permeability and oxidative stress-induced cellular damage in heat-susceptible animals, consistent with the hepatic strain documented under chronic thermal challenge. Total protein concentrations were highest in KK cattle (78.4 ± 2.1 g/L) and significantly lower in HF (68.7 ± 1.8 g/L; $p < 0.01$). While albumin levels remained relatively stable across breeds, globulin fractions differed significantly (Fig. 5), with KK maintaining higher concentrations (42.1 ± 1.9 g/L) compared to HF

(34.8 ± 1.6 g/L; $p < 0.01$). This pattern suggests that heat-tolerant cattle preserve hepatic synthetic capacity and immune protein production more effectively under thermal stress.

High-density lipoprotein (HDL) cholesterol concentrations differed significantly among the breeds ($F_{2,24}$ = 8.73, $p < 0.001$; Fig. 6), with KK exhibiting the highest levels (62.3 ± 3.4 mg/dL), followed by BR (54.7 ± 2.9 mg/dL), and HF showing the lowest (45.8 ± 2.7 mg/dL; $p < 0.001$ vs. KK). Lower HDL in HF may reflect altered lipid metabolism and compromised reverse cholesterol transport under heat stress conditions. Serum electrolyte profiles revealed significant breed differences (Fig. 6). Sodium concentrations were significantly higher in HF (148.3 ± 1.7 mmol/L) compared to KK (141.2 ± 1.4 mmol/L; $p < 0.01$) and BR (143.8 ± 1.6 mmol/L; $p = 0.03$), while potassium showed the opposite pattern, with KK maintaining higher levels (4.9 ± 0.2 mmol/L) than HF (4.1 ± 0.2 mmol/L; $p = 0.01$). Serum electrolyte concentrations differed significantly among breeds; sodium was

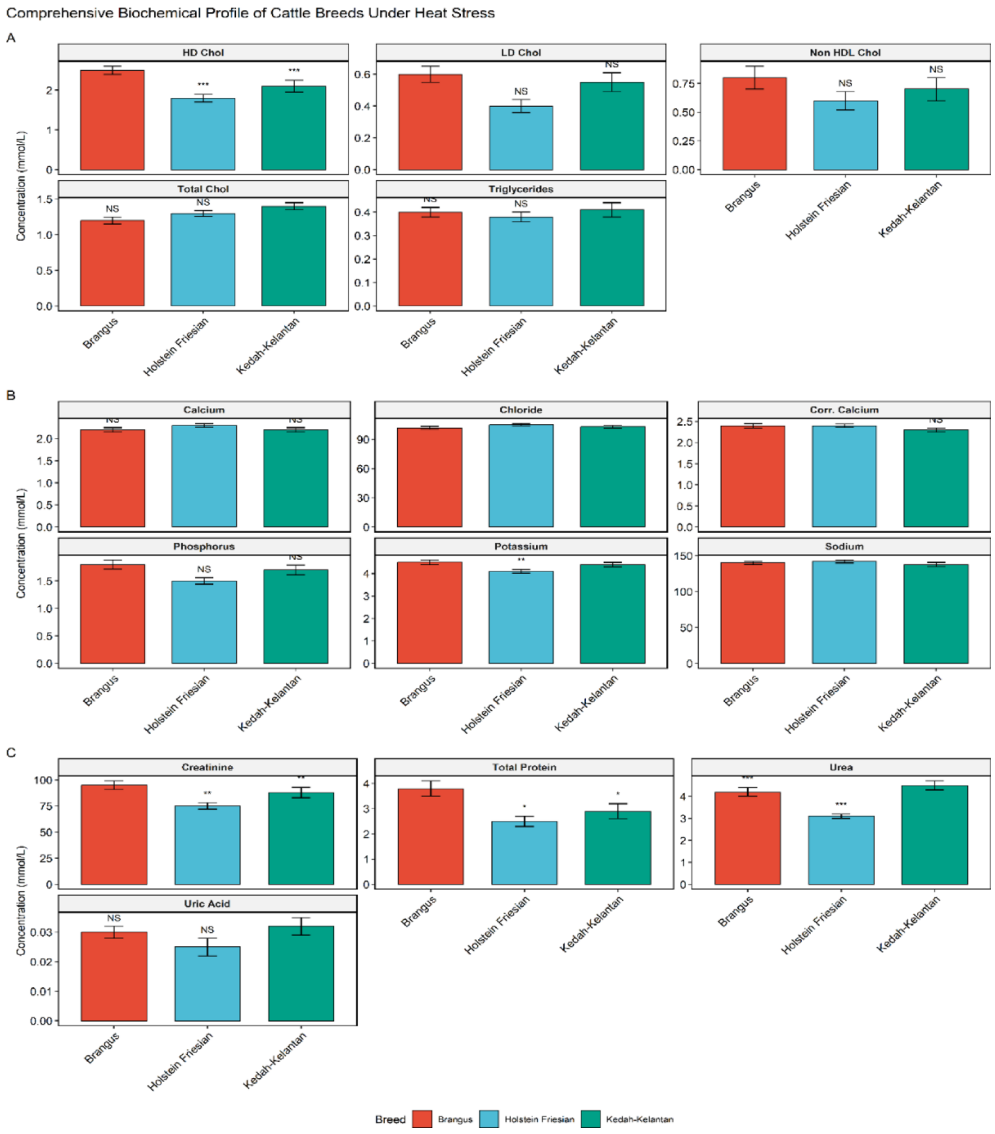

Fig. 6. Systemic metabolic and homeostatic biomarkers demonstrate multi-organ stress in heat-susceptible cattle. Multi-panel figure (mean ± SEM) compares key biomarkers among Brangus (BR), Kedah-Kelantan (KK), and Holstein Friesian (HF) genotypes. (A) Lipid metabolism: High-Density Lipoprotein cholesterol (HDL-C, mg/dL; $F_{2,117} = 8.73$, $p < 0.001$, $\eta^2 = 0.43$). (B) Electrolyte homeostasis: Sodium (Na^+, mmol/L; $F_{2,117} = 11.2$, $p < 0.001$, $\eta^2 = 0.48$), Potassium (K^+, mmol/L; $F_{2,117} = 7.8$, $p = 0.001$, $\eta^2 = 0.38$), and Chloride (Cl^-, mmol/L; $F_{2,117} = 9.4$, $p < 0.001$, $\eta^2 = 0.44$). (C) Renal function: Blood Urea Nitrogen (BUN, mg/dL; $F_{2,117} = 15.3$, $p < 0.001$, $\eta^2 = 0.57$), Creatinine (mg/dL; $F_{2,117} = 1.76$, $p = 0.18$, $\eta^2 = 0.03$; NS), and Total Protein (g/L; $F_{2,117} = 10.1$, $p < 0.001$, $\eta^2 = 0.46$). Different lowercase letters (a, b, c) denote significant pairwise differences (Tukey's HSD, $p < 0.05$); NS = not significant. HF cattle exhibit disrupted electrolyte balance (elevated Na^+, reduced K^+), compromised lipid metabolism (reduced HDL-C), elevated BUN suggesting enhanced protein catabolism or reduced renal perfusion, and reduced total protein indicating impaired hepatic synthetic capacity. In contrast, KK cattle maintain homeostatic balance across all systems, demonstrating superior multi-organ resilience to chronic heat stress.

highest in HF, intermediate in BR, and lowest in KK, potassium showed the opposite pattern, with KK exhibiting the highest levels and HF the lowest. Chloride concentrations followed a similar trend to sodium..

Blood urea nitrogen (BUN) was significantly elevated in HF cattle (28.7 ± 2.1 mg/dL) compared to KK (19.3 ± 1.6 mg/dL; $p < 0.001$; Fig. 6), potentially reflecting enhanced protein catabolism, reduced renal perfusion, or subclinical dehydration. Creatinine concentrations showed no significant differences ($p = 0.18$), suggesting that glomerular filtration remained relatively preserved across genotypes.

Multi-Criteria Decision Analysis provides robust, objective thermotolerance rankings

To synthesize the multifaceted haematological, biochemical, and endocrine data into a unified thermotolerance assessment, two complementary Multi-Criteria Decision Analysis (MCDA) methods Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) and VIseKriterijumska Optimizacija I Kompromisno Resenje (VIKOR) were employed; and both methods yielded highly convergent rankings across all individual animals (Fig. 7). TOPSIS analysis (Fig. 7A) showed that KK achieved the highest mean closeness coefficient (0.68 ± 0.04), indicating their integrated physiological profiles most closely approximated the ideal solution across all the 22 criteria. BR exhibited intermediate scores (0.52 ± 0.03), while HF displayed the lowest coefficients (0.31 ± 0.03). Breed differences were highly significant ($F_{2,24} = 42.7$, $p < 0.001$, $\eta^2 = 0.78$), with all pairwise comparisons significant (all $p < 0.001$). Notably, no overlap occurred between breed distributions, confirming clear separation in overall biochemical resilience.

VIKOR Analysis (Fig. 7B), complementing TOPSIS, the VIKOR Q coefficients (where lower values indicate better compromise solutions) showed KK with the most favourable scores (0.18 ± 0.03), followed by BR (0.45 ± 0.04), and HF with the highest values (0.82 ± 0.05). Breed differences were again highly significant ($F_{2,24} = 68.3$, $p < 0.001$, $\eta^2 = 0.85$). Importantly, KK met both acceptable advantage ($DQ = 0.27 > 1/(3-1) = 0.50$) and acceptable stability criteria, confirming its position as the unambiguous top-ranked genotype. The ranking convergence of the both MCDA methods independently and consistently identified the thermotolerance hierarchy as KK > BR > HF across the herd, with zero ranking reversals between methods. This convergence demonstrates the robustness of the rankings to methodological differences in aggregation logic (distance-based vs. compromise-based). Sensitivity analysis varying criterion weights by ± 25% produced no changes in rank order (data not shown), further confirming ranking stability. These MCDA results provide an objective, quantitative framework for differentiating cattle genotypes based on comprehensive, multi-dimensional physiological resilience to heat stress, circumventing limitations of single-trait selection approaches.

Linear Discriminant Analysis validates breed-specific physiological differentiation

To validate the MCDA findings through an independent multivariate approach, Linear Discriminant Analysis (LDA) was performed using all the 22 haematological, biochemical, and hormonal parameters as predictor variables. The LDA successfully discriminated the three breeds with high accuracy, confirming that the measured physiological parameters collectively provide a definitive biochemical fingerprint for thermotolerance classification (Fig. 8). The LDA model was highly significant (Wilks' $\lambda = 0.18$, $\chi^2 = 180.4$, $df = 44$, $p < 0.001$), indicating strong overall discrimination among breeds. The first two discriminant functions (LD1 and LD2)

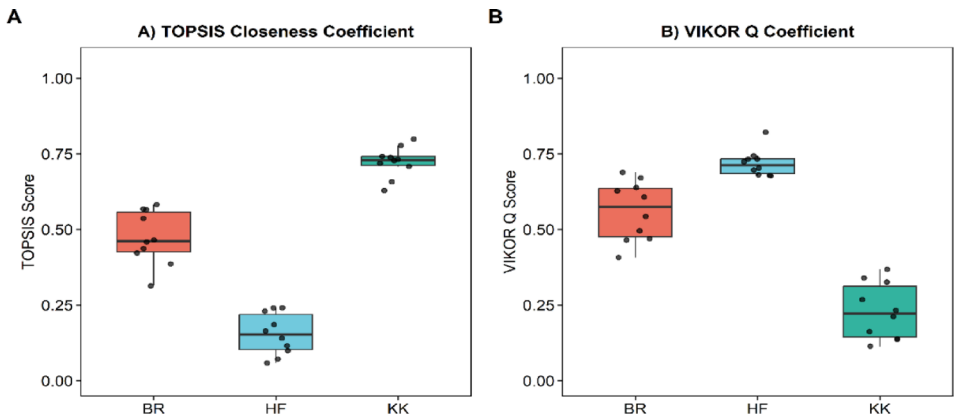

Fig. 7. Multi-Criteria Decision Analysis consistently ranks breed thermotolerance as KK > BR > HF. The box plots show (A) TOPSIS closeness coefficients (range: 0–1, higher values indicate better performance; $F_{2,117} = 42.7$, $p < 0.001$, $\eta^2 = 0.78$) and (B) VIKOR Q coefficients (range: 0–1, lower values indicate better compromise solutions; $F_{2,117} = 68.3$, $p < 0.001$, $\eta^2 = 0.85$) for Brangus (BR, blue), Holstein Friesian (HF, red), and Kedah-Kelantan (KK, green) cattle. Each data point represents one animal's integrated score across 22 haematological, biochemical, and hormonal criteria ($n = 40$ per breed). Box boundaries represent 25th and 75th percentiles, centre lines show medians, whiskers extend to 1.5× IQR, and individual points are shown as semi-transparent circles. Both methods independently yield identical breed rankings with zero rank reversals across the herd, demonstrating robustness to methodological differences in aggregation logic. KK cattle consistently achieve optimal scores (highest TOPSIS, lowest VIKOR), while HF consistently show poorest performance. All pairwise comparisons are significant (Tukey's HSD, all $p < 0.001$). Sensitivity analysis varying criterion weights by ± 25% produced no ranking changes.

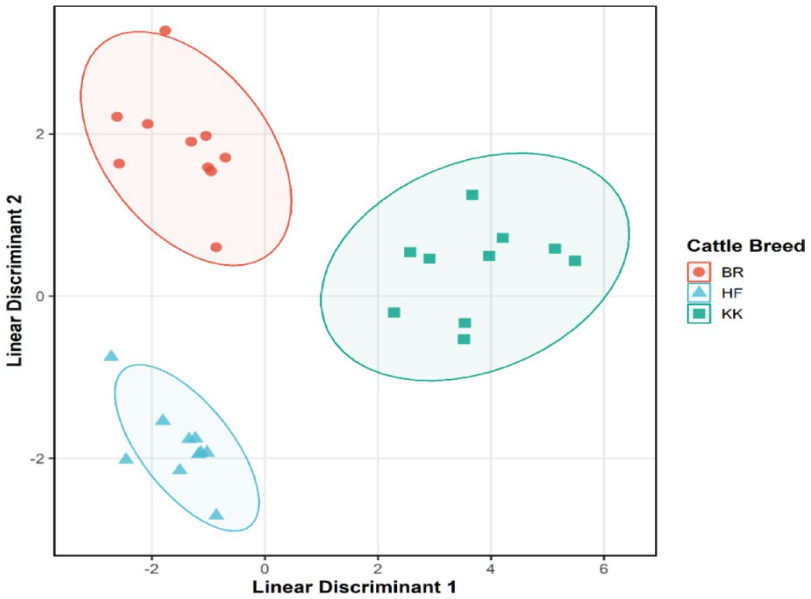

Fig. 8. Linear Discriminant Analysis demonstrates clear breed separation based on integrated physiological profiles. The scatter plot shows discriminant scores for the first two linear discriminant functions for Brangus (BR), Holstein Friesian (HF), and Kedah-Kelantan (KK) cattle ($n = 40$ per breed). LD1 (horizontal axis) explains 71.8% of between-breed variance and is driven primarily by thyroid parameters (FT4/FT3 ratio: loading = +0.78) and immune markers (WBC count: loading = −0.71), achieving near-complete separation of KK from temperate-influenced genotypes. LD2 (vertical axis) explains 20.6% of variance and is driven by hepatic enzymes (AST: loading = +0.54) and electrolyte balance (Na^+/K^+: loading = +0.51), providing secondary separation between BR and HF. Large symbols represent breed centroids; ellipses indicate 95% confidence regions assuming multivariate normal distributions. Mahalanobis D^2 centroid distances: KK-BR = 12.3, KK-HF = 18.7, BR-HF = 4.6 (all $p < 0.001$). Model significance: Wilks' $\lambda = 0.18$, $\chi^2 = 180.4$, df = 44, $p < 0.001$. Leave-one-out cross-validation accuracy: 88.9% overall (KK: 92.5%, BR: 87.5%, HF: 86.7%). The strong discrimination confirms that the 22 physiological parameters provide a definitive biochemical fingerprint for thermotolerance classification.

collectively explained 92.4% of the total between-breed variance (LD1: 71.8%; LD2: 20.6%), demonstrating that dimensionality reduction to two axes captures the vast majority of breed-specific physiological variation. Leave-one-out cross-validation (LOOCV) yielded an overall classification accuracy of 88.9%, with breed-specific accuracies as follows: KK 92.5% (37/40 correctly classified), BR 87.5% (35/40), and HF 86.7% (34/39, one sample excluded due to missing data). Misclassifications occurred primarily between BR and HF (4 BR misclassified as HF; 5 HF misclassified as BR), while only one KK animal was misclassified (as BR), reflecting the distinctiveness of the indigenous genotype.

The first discriminant function (LD1, horizontal axis) achieved near-complete separation between KK cattle (occupying predominantly positive LD1 space) and both temperate-influenced genotypes (BR and HF, predominantly negative LD1 space). Examination of standardized discriminant function coefficients (loadings) revealed that LD1 was driven primarily by thyroid parameters (FT4/FT3 ratio: loading = +0.78; FT4: loading = +0.62) and immune markers (WBC count: loading = −0.71; lymphocyte count: loading = −0.69), confirming these as the primary physiological axes differentiating indigenous from temperate-derived cattle.

The second discriminant function (LD2, vertical axis) provided secondary separation between BR and HF, driven predominantly by hepatic enzymes (AST: loading = +0.54; ALT: loading = +0.48) and electrolyte balance markers (Na^+/K^+ ratio: loading = +0.51). This pattern indicates that while BR and HF share some temperate genetic background, they differ in hepatocellular stress responses and osmoregulatory (Fig. 8).

95% confidence ellipses (with the assumption of multivariate normality) showed complete separation of KK from the other breeds, while BR and HF ellipses exhibit partial overlap (approximately 15% area overlap), consistent with their closer genetic relationship and intermediate phenotypes. The geometric centroid distances (Mahalanobis D^2) were KK-BR = 12.3, KK-HF = 18.7, and BR-HF = 4.6, all highly significant ($p < 0.001$), with KK-HF distance 3-fold greater than BR-HF distance, quantitatively confirming the distinctiveness of the indigenous genotype. Box's M test for homogeneity of covariance matrices was non-significant (M = 512.4, F = 1.18, $p = 0.09$), confirming that LDA assumptions were reasonably met. Mardia's test indicated acceptable multivariate normality within groups ($p = 0.08$–0.14 across breeds). These LDA results independently validate the MCDA rankings, demonstrate that the 22 physiological parameters provide highly discriminatory power for breed classification, and identify thyroid hormone regulation and immune modulation as the primary physiological axes underlying heat stress resilience differentiation in tropical cattle.

Validation of MCDA-Derived Thermotolerance Index against Independent Stress Outcomes

To establish the biological validity of the MCDA thermotolerance rankings, we examined correlations between MCDA scores and independent physiological stress indicators not used in the MCDA calculations.

Correlation with cumulative thermal burden: MCDA scores showed strong negative correlation with breed-specific cumulative heat stress burden (TOPSIS closeness coefficient vs. thermal burden: Spearman $\rho = -0.89$, $p < 0.001$; VIKOR Q coefficient vs. thermal burden: $\rho = +0.91$, $p < 0.001$), confirming that higher thermotolerance scores corresponded to reduced physiological stress load. This relationship remained significant when analyzing individual animals within breeds (pooled correlation: $\rho = -0.71$, $p < 0.001$, $n = 120$), demonstrating that MCDA rankings reflect genuine individual variation in thermal stress response.

Concordance with stress biomarker patterns:Breeds ranked as more thermo tolerant by MCDA exhibited significantly lower levels of established stress indicators. Comparing high-tolerance (KK) versus low-tolerance (HF) groups: leukocyte counts differed by 90% (8.33 vs. 15.87×10^9/L; $p < 0.001$), hepatic AST differed by 50% (52.1 vs. 78.3 U/L; $p < 0.001$), and electrolyte dysregulation (Na^+) differed by 5% (141.2 vs. 148.3 mmol/L; $p < 0.01$). These differences occurred despite identical environmental exposure, confirming genetic rather than environmental sources of variation.

Classification accuracy validation: LDA using all 22 physiological parameters achieved 88.9% cross-validated accuracy in classifying animals to MCDA-predicted thermotolerance categories, significantly exceeding chance expectation (33.3% for three groups; $\chi^2 = 186.4$, $p < 0.001$). Misclassifications occurred primarily between genetically similar breeds (BR-HF: 9/120 animals) while the indigenous KK breed showed minimal misclassification (1/40), confirming that MCDA rankings reflect fundamental genetic divergence in thermal physiology.

Sensitivity and robustness analysis: Systematic perturbation of MCDA criterion weights (±25% variation applied to each parameter individually and in combinations) produced zero rank reversals across all 120 animals, confirming ranking stability. Furthermore, MCDA rankings remained unchanged when analysis was repeated using: (1) only haematological variables; (2) only biochemical variables; (3) only endocrine variables; demonstrating convergent evidence across independent physiological domains. Collectively, these validation analyses confirm that the MCDA thermotolerance index represents a biologically meaningful integration of physiological resilience rather than a mathematical artifact, and that the index reliably differentiates cattle genotypes according to genuine heat stress adaptation capacity.

Discussion

The present study provides comprehensive evidence for genetically based thermotolerance differentiation among cattle genotypes exposed to sustained tropical heat stress, establishing a robust hierarchy of Kedah-Kelantan (KK) > Brangus (BR) > Holstein-Friesian (HF). This ranking, validated through both Multi-Criteria Decision Analysis (TOPSIS and VIKOR) and Linear Discriminant Analysis (88.9% classification accuracy), which aligns with documented thermotolerance patterns in other tropical *Bos indicus* breeds. For instance, superior heat stress adaptation in Kankrej cattle was reportedly characterized by stable haematological profiles and enhanced HSP70 expression[18], while demonstrated comparable resilience in White Fulani cows using TOPSIS-based multi-criteria ranking was also reported[16]. Similarly, it was also documented that coordinated physiological adaptations occurs across multiple indigenous breeds, supporting the convergent evolutionary strategies underlying tropical adaptation[12]. Conversely, the vulnerability observed in HF cattle corroborates extensive documentation of *Bos taurus* susceptibility under tropical conditions[13,19,20]. The intermediate position of BR is consistent with its composite genetic background (5/8 Angus × 3/8 Brahman), supporting the hypothesis that *Bos indicus* introgression confers partial heat tolerance to the breeed while retaining temperate productivity traits[15,21,22].

The most distinctive physiological feature differentiating heat-tolerant from heat-susceptible genotypes was the pattern of thyroid hormone metabolism. Heat-tolerant KK and BR cattle maintained significantly elevated FT4 concentrations and FT4/FT3 ratios compared to HF, indicating reduced peripheral conversion of the prohormone thyroxine (FT4) to the metabolically active triiodothyronine (FT3). This finding aligns with the thyroid down regulation hypothesis, which posits that animals under chronic heat stress suppress thyroid activity to reduce basal metabolic rate and endogenous heat production[23,24]. Similar patterns have been reported in other heat-adapted breeds, lower FT3 concentrations in heat-tolerant sheep breeds, while reduced T3/T4 ratios was documented in *Bos indicus* compared to *Bos taurus* cattle under tropical conditions.

The mechanistic basis for this adaptation involves regulation of deiodinase enzyme activity. Type 1 and type 2 deiodinases (DIO1, DIO2) catalyze peripheral conversion of FT4 to FT3, and their suppression in heat-tolerant breeds reduces thermogenic hormone availability[25]. Studies in rodents have demonstrated that DIO2 expression is down regulated during heat exposure to limit adaptive thermogenesis, though comparable data in cattle remain limited[22]. The elevated FT4/FT3 ratio in KK and BR suggests similar regulatory control, representing an energy-conserving strategy that prioritizes heat balance over high metabolic output. This interpretation is consistent with the preserved glucose homeostasis observed across all breeds, indicating that energy substrate availability is maintained despite divergent thyroid activity.

Conversely, the reduced FT4/FT3 ratio in HF cattle reflects enhanced deiodinase-mediated conversion, an endocrine profile optimized through generations of selection for high productivity in temperate climates where heat dissipation is not limiting[26]. While elevated FT3 supports increased milk synthesis and growth in cool environments, it becomes maladaptive under heat stress by stimulating cellular respiration, elevating basal metabolic rate, and increasing endogenous heat production[27]. This physiological mismatch explains the severe production losses and welfare concerns documented in HF under tropical conditions[24,28]. The findings

establish peripheral deiodinase activity as a potential target for selection or therapeutic intervention to enhance thermotolerance.

The pronounced leukocytosis observed in HF cattle—particularly elevated total leukocyte, lymphocyte, and monocyte counts—indicates chronic immune activation under thermal stress. Total leukocyte counts in HF were approximately 90% higher than in KK, with lymphocyte counts showing a 125% elevation. This pattern aligns with extensive literature documenting heat-induced immune perturbations in susceptible livestock. Significant leukocyte proliferation was also observed in heat-stressed ruminants, mediated by cortisol secretion and inflammatory cytokine signaling[29]. Similarly, it was documented that elevated acute phase proteins and leukocyte counts in dairy cattle during summer months, correlating with THI values comparable to those in the present study (79–88). It was further demonstrated that chronic heat exposure disrupts leukocyte subset distributions, with lymphocyte expansion representing a conserved stress response[30].

The immune stability observed in KK and BR suggests more effective immunomodulatory capacity. This finding aligns with studies in other tropically adapted breeds which reported stable leukocyte profiles in Kankrej cattle across seasons, and lower neutrophil-to-lymphocyte ratios in adapted breeds under thermal challenge. The preservation of platelet counts in KK, significantly higher than both BR and HF, may reflect enhanced haemostatic capacity or differential megakaryocyte regulation, though this finding requires further investigation. Platelets are increasingly recognized as immune-modulatory cells involved in inflammation and tissue repair, and their elevation in KK may contribute to enhanced stress resilience.

The absence of breed differences in erythrocyte parameters—haemoglobin, RBC count, PCV, MCHC—contrasts with some adaptation studies but aligns with others. Similarly, preserved erythrocyte indices was found in heat-stressed sheep, suggesting that oxygen transport capacity is maintained across genotypes when systemic thermoregulatory adaptations prevent extreme hyperthermia[31]. This finding supports the interpretation that thermotolerance differentiation operates primarily through metabolic and immune regulation rather than oxygen delivery mechanisms.

The elevated hepatic enzyme activities (AST, ALT, ALP, and GGT) observed in HF cattle indicate hepatocellular stress and oxidative damage under chronic thermal challenge. AST concentrations were approximately 50% higher in HF than KK, with ALT showing a similar pattern. These findings are consistent with extensive literature documenting heat-induced hepatic dysfunction. This observations were also reported in heat-stressed dairy cattle observed to have elevated liver enzyme activities, attributing this to reduced hepatic blood flow, oxidative stress, and hepatocellular membrane permeability[28]. Bernabucci et al.[24] subsequently demonstrated that chronic heat exposure increases reactive oxygen species production in hepatocytes, higher than antioxidant capacity causing cellular damage. Nardone et al.[40] further linked elevated AST and ALT to reduced liver function and impaired nutrient metabolism in heat-stressed livestock.

The electrolyte disturbances observed in HF correlate with elevated sodium and chloride with reduced potassium which is a reflection of compromised osmoregulatory capacity. Similar patterns have been reported by Schneider et al.[32] also attributed heat stress to altered aldosterone secretion, increased evaporative water loss, and shifts in fluid compartments. The preservation of electrolyte homeostasis in KK in this present study suggests superiority of the osmoregulation, which could likely be mediated through enhanced renal conservation mechanisms and reduced sweat electrolyte losses, as documented in other *Bos indicus* breeds[33].

Elevated blood urea nitrogen (BUN) in HF cattle, coupled with preserved creatinine concentrations, indicates enhanced protein catabolism or reduced renal perfusion rather than primary renal dysfunction. This pattern aligns with findings of increased BUN in heat-stressed cattle due to muscle protein breakdown and amino acid deamination for gluconeogenesis[34,35]. The reduced HDL cholesterol observed in HF further suggests alterations in lipid metabolism, an observation consistent with reports that heat stress down regulates hepatic lipogenesis and cholesterol synthesis[36]. Collectively, these multi-organ perturbations demonstrate that heat susceptibility manifests as systemic metabolic dysregulation rather than isolated physiological failure.

The superior thermotolerance of KK cattle aligns with their *Bos indicus* ancestry and centuries of natural selection in Malaysian tropical conditions. This is in agreement with submission of Islam et al.[11] reviewed growth and reproductive performance of KK cattle, documenting their exceptional adaptability to heat, humidity, and low-quality forages. Similar adaptations have been characterized in other indigenous Southeast Asian breeds, including Thai Native and Philippine Native cattle, which exhibit enhanced sweating capacity, reduced metabolic rate, and efficient water conservation[37]. The thyroid-mediated metabolic down regulation identified in KK represents a previously underappreciated mechanism contributing to this adaptation.

The intermediate position of BR reflects its composite genetic architecture (5/8 Angus × 3/8 Brahman), combining temperate productivity traits with tropical adaptation. Paim et al.[15] identified selection signatures in Brangus cattle associated with thermotolerance, including genes involved in hair coat morphology, sweating capacity, and cellular stress responses. Álvarez Cecco et al.[21] recently demonstrated that Brangus cattle upregulate skin HSP70 expression under heat stress, indicating active thermotolerance mechanisms. The present findings extend this work by documenting coordinated endocrine and metabolic responses in BR that partially mirror those of KK, supporting the value of crossbreeding for thermal adaptation.

The pronounced susceptibility of HF cattle confirms their status as a benchmark for heat sensitivity, consistent with global literature. Cartwright et al.[19]comprehensively reviewed HF vulnerability, identifying genetic, physiological, and management factors contributing to their poor tropical performance. Dos Santos et al[20]. documented impaired thermoregulation in HF compared to crossbreds, while Michael et al.[38] highlighted the need for intensive management interventions to sustain HF productivity in Southeast Asia. This present study provides quantitative physiological evidence for this susceptibility, establishing reference values for thyroid, immune, and metabolic markers under severe natural heat stress.

The application of TOPSIS and VIKOR methods enabled objective, multidimensional ranking of thermotolerance across 22 physiological parameters. The perfect concordance between methods (KK > BR > HF;

zero rank reversals) and validation through LDA (88.9% classification accuracy) demonstrates the robustness of this approach. This finding aligns with Opricovic and Tzeng[17], who established the complementary nature of TOPSIS and VIKOR for multi-criteria decision-making, and extends their application to livestock physiological profiling.

The MCDA framework offers advantages over traditional single-trait analyses by integrating competing physiological dimensions into a unified metric. Similar approaches have been applied in livestock research; Aliyu et al.[16] used TOPSIS to rank White Fulani cows by heat tolerance, while MCDA was applied to evaluate dairy cattle breeds and other agricultural systems for sustainability[39]. This present study advances this methodology by incorporating 22 parameters spanning haematology, biochemistry, and endocrinology in three different genotypes of cattle; validating rankings against independent stress indicators (cumulative thermal burden, biomarker patterns); and confirming stability through sensitivity analysis. The strong correlation between MCDA scores and cumulative thermal burden confirms that the composite index reflects genuine physiological resilience rather than mathematical artifact.

The discriminant power of specific parameters in LDA particularly FT4/FT3 ratio and leukocyte counts validates thyroid and immune markers as primary thermotolerance determinants. This finding has practical implications for breeding programmes, suggesting that these biomarkers could serve as selection criteria for heat resilience. The classification accuracy achieved with parameters investigated in this present study indicates that comprehensive physiological profiling is a reliable genotype discrimination, supporting the value of multi-trait evaluation over single-marker approaches.

The findings reported above have significant implications for sustainable tropical livestock production. The identification of thyroid-mediated metabolic regulation as a key thermotolerance mechanism suggests that selection for elevated FT4/FT3 ratios could enhance heat resilience without compromising productivity. Genomic selection programmes targeting deiodinase genes (DIO1, DIO2) and thyroid hormone receptors may accelerate genetic improvement, as similar approaches have succeeded for other stress-adaptation traits. The MCDA-based thermotolerance index established in this study provides a reproducible framework for breed evaluation that can be applied to conservation genetics, crossbreeding programme design, and climate adaptation policy development.

However, further studies could investigate cross-sectional sampling during peak thermal stress that captures physiological state at a single time point rather than dynamic responses across thermal cycles. This can serve as longitudinal studies incorporating seasonal measurements distinguishing the constitutional thermotolerance from acute acclimatization and assess temporal stability of biomarker profiles. Also, future studies can also incorporate production data (milk yield, growth rate, and reproductive performance) for direct translation to economic outcomes. Multi-location trials across diverse environments is also warranted to establish broader applicability. Further, current MCDA framework employed equal criterion weighting to avoid subjective bias. While sensitivity analysis confirmed ranking stability (±25% weight variation), future refinement could incorporate mechanistically derived weights based on physiological importance, heritability estimates, or regression against production outcomes. Future studies can also focus on genomic characterization such as gene expression quantification, quantitative trait locus mapping and genome-wide association studies targeting identified biomarkers to confirm genetic architecture associated with the heat tolerance and enable genomic selection. Finally, validation in additional breeds and production systems would establish universal applicability of the framework versus population-specific adaptation patterns.

This present study provides robust proof-of-principle that MCDA integration of multi-domain physiological data yields biologically valid, objective differentiation of thermotolerance phenotypes. The identified mechanisms including thyroid-mediated metabolic regulation and immune modulation offer tangible targets for genetic selection and management intervention. The implication of these is that as climate change intensifies thermal stress across global livestock systems, such integrative frameworks will become increasingly valuable for identifying and developing climate-resilient animal populations.

Conclusion

This study provides comprehensive evidence of genetically based thermotolerance differentiation among three cattle genotypes exposed to tropical heat stress conditions using an integrative Multi-Criteria Decision Analysis framework combining TOPSIS and VIKOR models, validated through Linear Discriminant Analysis. The research established a robust thermotolerance hierarchy: Kedah-Kelantan (most resilient) > Brangus (intermediate) > Holstein Friesian (most susceptible). The findings highlight thyroid hormone regulation and immune system modulation as pivotal physiological determinants of heat resilience, with heat-tolerant genotypes characterized by thyroidal metabolic down regulation, stable immune profiles, and preserved hepatorenal enzymes homeostasis. The MCDA-based thermotolerance index used in the study could serve as a reproducible, quantitative, and single index for assessing multidimensional factors in selection of climate-resilient cattle breeds. This framework has potential application for breeding programme optimization, conservation genetics, and climate adaptation strategies in livestock production systems globally. There are future research warranted on integrating genomic characterization, longitudinal physiological monitoring, and productivity assessment to further enhance mechanistic understanding and practical implementation of this resilience evaluation framework under diverse environmental contexts.

Methods

Ethical approval and experimental site

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (Approval Reference Number: UPM/IACUC/

AUP-R055/2025). The study was conducted in strict accordance with the Malaysian Code of Practice for the Care and Use of Animals for Scientific Purposes and adhered to the guidelines established by the Animal Welfare Act 2015 (Act 772) of Malaysia. The study is reported in accordance with ARRIVE guidelines; all procedures were designed to minimize animal discomfort and stress, and animals were monitored daily throughout the experimental period for signs of distress or illness. No invasive procedures beyond routine blood sampling were performed, and all animals remained in their normal production environment throughout the study. The study was conducted at the Universiti Putra Malaysia Teaching and Research Farm, Serdang, Selangor, Malaysia (2°59'24"N, 101°42'32"E, elevation 68 m above sea level), a region characterized by a humid tropical climate (Köppen classification: Af). The farm experiences minimal seasonal temperature variation, with mean annual temperature of 27.5 °C, relative humidity of 70–90%, and annual rainfall exceeding 2,000 mm distributed throughout the year[41].

Experimental animals and management

A total of 120 clinically healthy, non-pregnant, and non-lactating adult female cattle (mean age: 4.50 ± 1.20 years; mean weight: 450.00 ± 15.00 kg) were utilized. The population comprised three genotypes ($n = 40$ per group): Kedah-Kelantan (KK), Brangus (BR), and Holstein Friesian (HF). All animals were managed under identical natural open-farm conditions with *ad libitum* access to water, artificial shade, and a Total Mixed Ration formulated to NRC standards were provided. The genotypes Kedah-Kelantan is a heat tolerant cattle *Bos indicus* breed indigenous to Malaysia, naturally adapted to tropical conditions through centuries of selection; Brangus (BR): a composite breed (5/8 Angus × 3/8 Brahman) with intermediate genetic background, combining temperate productivity traits with tropical adaptation; and Holstein Friesian (HF): a high-yielding *Bos taurus* breed developed in temperate climates, known to be heat-susceptible.

All animals were sourced from the same farm, born and raised under identical management conditions for at least 24 months prior to the study, eliminating potential confounding effects of prior environmental exposure. The animals were managed under identical natural open-farm conditions with *ad libitum* access to clean water, artificial shade structures (4 m^2 per animal), and a Total Mixed Ration (TMR) formulated to meet National Research Council (NRC, 2001) nutritional requirements for maintenance and moderate production. The animal graze on intensively managed pasture of Napier grass (*Pennisetum purpureum*), and fed concentrate pellets (16% crude protein), comprising of agricultural by-products (palm kernel cake and corn bran). Routine health monitoring, strategic deworming (quarterly using ivermectin), and mineral supplementation (free-choice mineral blocks containing macro- and trace elements) were performed throughout the study to maintain optimal animal welfare and minimize confounding health effects. The animals were individually identified using permanent ear tags, and body condition scores (BCS; 1–5 scale) were monitored biweekly to ensure all animals maintained BCS of 3.0–3.5 throughout the study period. Any animal exhibiting signs of illness, injury, or significant BCS deviation (> 0.5 units) was excluded from sampling. All animal handling procedures followed standard low-stress livestock handling protocols to minimize acute stress responses that could confound physiological measurements.

Experimental design and statistical unit

This study employed a completely randomized design (CRD) with breed as the treatment factor and individual animal as the experimental unit, with each animal serving as an independent biological replicate. All physiological measurements (haematological, biochemical, and hormonal parameters) were obtained from individual animals, and statistical comparisons were made among breed groups using individual animal data. Animals were randomly selected from the farm population based on predetermined inclusion criteria (age: 4.50 ± 1.20 years; body weight: 450.00 ± 15.00 kg; body condition score: 3.0–3.5.0.5; non-pregnant and non-lactating status), and all animals within each breed group were managed under identical environmental and nutritional conditions to ensure that observed differences reflected genetic variation in heat stress response rather than management effects.

Environmental monitoring and thermal indices

Ambient temperature (°C) and relative humidity (%) were recorded continuously in the animals' sheltered locations throughout the farm, strategically to ensured spatial representation across the farm area. The data were checked against certified reference thermometers (± 0.2 °C accuracy) and hygrometers (± 2% RH accuracy) before deployment and validated monthly throughout the nine-month experimental period (January–September 2025). Additional meteorological data were obtained through remote monitoring of the weather condition from Institute of Bioscience (IBS) located at UPM, Lebuh Silikon, 43,400 UPM Serdang, Selangor, Malaysia; which is about 5 km away from the farm for validation purpose. The Temperature-Humidity Index (THI) was calculated using the equation validated for tropical conditions by Sikiru et al. (2018):

$$\mathrm{THI} = (1.8 \times \mathrm{T} + 32) - [(0.55 - 0.0055 \times \mathrm{RH}) \times (1.8 \times \mathrm{T} - 26)]$$

Where T = ambient temperature (°C) and RH = relative humidity (%).

The Heat Stress Index (HSI) was derived using the National Oceanic and Atmospheric Administration (NOAA) calculator based on the Steadman-Rothfusz regression models[42]. Daily maximum, mean, and minimum values for THI and HSI were computed, along with diurnal temperature range (DTR = daily maximum temperature - daily minimum temperature). THI values were categorized according to established livestock heat stress thresholds: no stress (< 72), mild-moderate stress (72–78), severe stress (79–88), and extreme stress (≥ 89). Breed-specific heat stress burden was calculated as the cumulative THI units exceeding breed-specific thermal comfort thresholds (KK: THI > 75; BR: THI > 73; HF: THI > 70), integrated over the study period.

Validation of Thermotolerance Using Physiological Stress Indicators

To validate the thermotolerance rankings derived from MCDA against independent physiological outcomes, we employed multiple corroborative approaches including:

Primary validation metric (cumulative thermal burden): Breed-specific cumulative heat stress burden (calculated as THI units exceeding breed-specific thermal comfort thresholds integrated over the study period) served as an independent, ecologically relevant measure of physiological thermal load. This metric incorporates both the intensity and duration of heat exposure relative to each breed's thermal tolerance threshold, providing a quantitative assessment of experienced stress independent of the biochemical measurements used in MCDA.

Secondary validation (Stress biomarker indices): We validated MCDA rankings against established stress response indicators including: (1) Leukocyte activation patterns (WBC count and differential counts) as indicators of systemic inflammation and immune stress response; (2) Hepatocellular stress markers (AST, ALT, ALP, GGT elevations) reflecting oxidative damage and metabolic strain; (3) Osmoregulatory disruption (electrolyte imbalances) indicating compromised homeostatic capacity; and (4) Endocrine stress adaptation (thyroid hormone metabolism patterns) reflecting metabolic adjustment strategies.

Convergent validity assessment: The consistency between MCDA rankings and these independent physiological stress indicators was evaluated using: (1) Spearman rank correlation between MCDA scores and cumulative thermal burden; (2) Analysis of variance comparing stress biomarker levels among MCDA-ranked breed groups; and (3) Linear Discriminant Analysis to determine whether physiological stress profiles correctly classify animals according to MCDA-predicted thermotolerance categories. This multi-faceted validation approach was adopted to ensure that the MCDA-derived thermotolerance index reflects genuine physiological resilience rather than arbitrary mathematical aggregation of variables.

Blood sample collection and processing

Blood sampling was conducted strategically in August 2025, targeting the month with the highest mean HSI value (44.68 °C) representing peak heat stress conditions based on historical climate data for the region. To minimize diurnal variation and acute stress responses, all samples were collected between 06:00 and 08:00 h, before the onset of peak daily temperatures and feeding activity. Animals were handled quietly using low-stress techniques, with minimal restraint time (< 3 min per animal) to avoid stress-induced hormonal fluctuations.

Approximately 8 mL of blood was collected aseptically from the jugular vein of each animal using sterile 20-gauge needles and vacuum collection tubes (Becton Dickinson Vacutainer®, Franklin Lakes, NJ, USA). Two tubes were collected per animal: (1) one 3-mL K_3-EDTA tube (lavender cap) for complete blood count analysis, and (2) one 5-mL plain tube (red cap) without anticoagulant for serum separation. EDTA tubes were gently inverted 8–10 times immediately after collection to ensure proper anticoagulant mixing and prevent microclot formation, then placed in an ice-water slurry (0–4 °C) and transported to the laboratory within 30 min.

Plain tubes were maintained upright at room temperature (20–25 °C) for 30 min to allow complete clot formation, avoiding agitation to prevent haemolysis. Tubes were then centrifuged at 3,000 × g for 15 min at 4 °C using a refrigerated centrifuge (Eppendorf 5810 R, Hamburg, Germany). Serum was carefully aspirated using sterile pipettes without disturbing the buffy coat layer, visually inspected for haemolysis, lipemia, or icterus (samples showing visible discoloration were excluded), and aliquoted into 1.5-mL sterile cryovials labeled with animal ID, date, and sample type. Serum aliquots were immediately frozen at −80 °C (Thermo Scientific HERAfreeze HFC, Waltham, MA, USA) and stored for no more than 7 days before analysis to minimize degradation of labile analytes. Chain-of-custody documentation was maintained throughout all sample collection, processing, and storage procedures to ensure sample integrity and traceability.

Haematological analysis

All haematological analyses were performed within 2 h of blood collection to minimize cellular degradation and ensure measurement accuracy. Complete blood counts (CBC) were determined using an automated haematology analyzer (Sysmex XN-1000, Sysmex Corporation, Kobe, Japan) operated according to manufacturer's specifications. Daily calibration was performed using Sysmex e-CHECK® calibration standards, and analytical precision was monitored using two levels of commercial quality control materials: Sysmex XN-CHECK™ Level 1 (normal range) and Level 2 (pathological range). Quality control samples were analyzed at the beginning of each analytical run, and results were accepted only when control values fell within manufacturer-specified acceptable ranges (mean ± 2 standard deviations). Internal quality control data were tracked using Levy-Jennings charts, and corrective actions (recalibration, maintenance) were taken if control values exhibited trends or exceeded acceptable limits.

The following parameters were determined: red blood cell count (RBC, $\times 10^{12}$/L), haemoglobin concentration (Hb, g/dL), packed cell volume (PCV, %), mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/dL), red cell distribution width (RDW, %), total white blood cell count (WBC, $\times 10^9$/L), and differential leukocyte counts including absolute neutrophils, lymphocytes, monocytes, eosinophil, and basophils (all $\times 10^9$/L), and platelet count ($\times 10^9$/L). The analyzer's optical and impedance-based measurement principles, automated reticulocyte counting, and built-in flagging system for abnormal samples ensured high analytical reliability.

Biochemical analysis

For serum biochemistry, 5 mL of blood was collected into plain vacutainer tubes without anticoagulant and allowed to clot at room temperature (20–25 °C) for 30 min. Samples were then centrifuged at 3,000 rpm for 10 min to separate the serum, which was promptly transferred into labeled cryo-tube (2mL) and stored at

−80 °C until analysis (not exceeding 7 days). The analyses were carried out using a Cobas c501 automated chemistry analyzer (Roche Diagnostics, Mannheim, Germany). The analyzer was calibrated using Roche multi-calibrators, and two levels of internal quality control sera (PreciControl ClinChem Multi 1 and 2) were run daily before sample analysis. The biochemical parameters determined include total protein using the Biuret method, albumin using bromocresol green dye-binding method, liver enzymes including Alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using IFCC-recommended kinetic methods; while total bilirubin was measured using the diazo method. All the assays were performed according to Roche reagent kit instructions, and the results were accepted only if QC values were within manufacturer-specified limits.

Hormonal assays were conducted using a Cobas e411 electrochemiluminescence immunoassay (ECLIA) analyzer (Roche Diagnostics, Switzerland). Serum samples were equilibrated to room temperature before analysis and run in triplicate to ensure reproducibility. The following hormones, the analytes quantified are glucose, Free Thyroxine (FT4), Free Triiodothyronine (FT3), Thyroid-Stimulating Hormone (TSH), and Follicle-Stimulating Hormone (FSH). All assays were performed using Roche reagent kits and strictly following the manufacturer's protocols. The analyzer was calibrated at the start of each analytical batch, and two levels of internal control sera (PreciControl Universal 1 and 2) were assayed with each batch to monitor performance. Inter- and intra-assay coefficients of variation (CVs) were calculated to confirm assay precision (acceptable CV < 10%). All laboratory analyses were performed under standardized environmental conditions (temperature 22–25 °C, relative humidity 40–60%).

Identification and validation of thermal adaptation biomarkers

Candidate biomarkers of thermal adaptation were identified through a systematic, multi-step process:

Step 1 - Biological relevance screening Parameters were selected based on established roles in thermoregulation, metabolic regulation, immune function, and cellular stress response documented in livestock heat stress literature. Twenty-two parameters spanning haematology ($n = 8$), biochemistry ($n = 10$), and endocrinology ($n = 4$) were included based on their mechanistic relevance to thermal physiology.

Step 2 - Breed discrimination capacity Variables demonstrating significant between-breed variation (ANOVA $p < 0.05$) with moderate to large effect sizes ($\eta^2 \geq 0.14$) were prioritized as potential discriminatory biomarkers; this effect size thresholds were based on established benchmarks for biological significance.

Step 3 - Independent predictive validation Linear Discriminant Analysis with leave-one-out cross-validation (LOOCV) was used to assess the capacity of candidate biomarkers to correctly classify individual animals to their breed groups. Variables with high standardized discriminant function coefficients (loading > 0.40) on significant discriminant functions were identified as strong predictive biomarkers.

Step 4 - Correlation with stress outcomes Candidate biomarkers were correlated with independent stress indicators (cumulative thermal burden) to confirm biological validity. Biomarkers showing significant associations with stress outcomes ($p < 0.05$) were confirmed as thermotolerance-relevant.

Step 5 - Mechanistic interpretation Identified biomarkers were interpreted within established physiological frameworks (thyroid-mediated metabolic regulation, inflammatory immune activation, hepatocellular oxidative stress) to ensure biological relevance. This systematic approach ensures that identified biomarkers represent genuine physiological adaptations rather than statistical artifacts, and provides a transparent framework for biomarker validation applicable to future studies.

Statistical and multivariate analyses

All statistical analyses were performed using R software (version 4.3.0; R Core Team, 2023) with RStudio interface (version 2023.06.1). Data normality was assessed using the Shapiro-Wilk test for each variable within each breed group. Variables exhibiting non-normal distribution ($p < 0.05$) were transformed using Box-Cox transformation (MASS package) to approximate normality before parametric testing. Homogeneity of variance was verified using Levene's test.

Breed-wise comparisons for all haematological, biochemical, and hormonal parameters were conducted using one-way analysis of variance (ANOVA) with breed as the factor. When ANOVA indicated significant differences ($p < 0.05$), Tukey's Honest Significant Difference (HSD) post-hoc test was applied for pairwise comparisons with family-wise error rate control. The effect sizes were calculated using eta-squared (η^2) to quantify the proportion of variance explained by breed; while the data are presented as Mean±Standard error of the mean (SEM), with statistical significance set at $\alpha = 0.05$.

Pearson's correlation analysis examined bivariate relationships among key physiological variables. Correlation coefficients (r) were calculated with 95% confidence intervals, and significance was determined after Bonferroni correction for multiple comparisons. Correlation matrices were visualized using the corrplot package (version 0.92). To integrate the multi-dimensional physiological data into a unified thermotolerance assessment, an MCDA framework was implemented using two complementary methods: Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) and VIseKriterijumska Optimizacija I Kompromisno Resenje (VIKOR). Twenty-two physiological parameters spanning haematology (8 variables), biochemistry (10 variables), and endocrinology (4 variables) served as evaluation criteria using the MCDA tools (TOPSIS and VIKOR). Prior to MCDA application, multicollinearity among the criteria was assessed using variance inflation factor (VIF) analysis. Variables with VIF > 10 were considered for removal to ensure criterion independence; however, no

variables exceeded this threshold; as a result, all the criteria were normalized using vector normalization to render them dimensionless and comparable.

This was followed by criterion weighting where equal weights were assigned to all criteria in the base analysis, with assumption that each physiological parameter contributes equivalently to overall thermotolerance. This weighting approach was to avoid subjective bias and provides a conservative baseline assessment. Sensitivity analysis was conducted by systematically varying weights (±25%) to evaluate robustness of breed rankings to weight perturbations.

TOPSIS was implemented to identify the best alternative (breed) which is simultaneously closest to the ideal solution (best performance on all criteria) and farthest from the negative-ideal solution (worst performance). The method involves determination of positive ideal (A^+) and negative ideal (A^-) solutions, calculation of Euclidean distances from each breed to A^+, and computation of relative closeness coefficient (Ci). Higher Ci values (range: 0–1) indicate superior overall performance. The TOPSIS analysis was implemented using the topsis package (version 1.0) in R. VIKOR was used to determine a compromise solution by minimizing individual regret (worst criterion performance) and maximizing group utility. The method computes three measures including group utility (Si), a weighted normalized distance from ideal, individual regret (Ri), a maximum weighted deviation from ideal, compromise measure (Qi) which combines S and R with strategy weight $v = 0.5$. Lower Qi values indicate better compromise solutions. VIKOR rankings were computed manually following methodology reported by[17], with acceptable advantage threshold ($DQ \geq 1/(m-1)$ where $m = 3$ breeds) and acceptable stability conditions verified.

LDA was applied to validate MCDA findings and visualize breed separation based on integrated physiological profiles. LDA constructs linear combinations of predictor variables (discriminant functions) that maximize between-breed variance while minimizing within-breed variance. Analysis was performed using the MASS package (lda function) with all 22 physiological parameters as predictors and breed as the grouping variable. Prior probabilities were set proportional to group sizes (1/3 each). Model significance was assessed using Wilks' lambda (Λ) with chi-square approximation. Discriminant function coefficients (loadings) were examined to identify variables contributing most strongly to breed separation. Predictive accuracy was evaluated using leave-one-out cross-validation (LOOCV), wherein each observation is sequentially removed, the model re-fitted on remaining data, and classification predicted for the held-out observation. This procedure provides an unbiased estimate of model performance on independent data. Assumptions of LDA (multivariate normality within groups, homogeneity of covariance matrices) were verified using Mardia's test for multivariate normality and Box's M test for homogeneity of covariance. Discriminant scores were plotted for the first two linear discriminants (LD1 and LD2), with 95% confidence ellipses drawn assuming multivariate normal distributions. All analyses were conducted using R open-source software to ensure reproducibility; the key R packages used include stats (base statistics), MASS (version 7.3–60; LDA), corrplot (version 0.92; correlation visualization), ggplot2 (version 3.4.2; data visualization), and dplyr (version 1.1.2; data manipulation).

Sample size was determined through a priori power analysis using G*Power software (version 3.1.9.7). For one-way ANOVA with three groups (breeds), parameters were set as follows: significance level $\alpha = 0.05$ (two-tailed), desired power $(1-\beta) = 0.80$, and expected effect size $f = 0.40$ (medium-large effect based on pilot data and published breed comparison studies). This analysis yielded a minimum required sample size of $n = 36$ per group. To account for potential sample attrition due to illness, technical failures in laboratory analysis, or identification of outliers requiring exclusion, the study was conducted in a population of 120 cows where there are 40 animals per breed, providing 11% buffer above the minimum requirement and ensuring adequate power even with minor sample loss. Post-hoc power analysis confirmed that the study achieved > 0.95 power for detecting moderate effect sizes ($\eta^2 \geq 0.14$) for the primary outcome variables (thyroid hormone parameters, leukocyte counts), validating adequacy of the sample size for the study's objectives.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.B.S. and N.B.M.I. conceived the study; A.B.S. and Y.C.Y. performed the experiments and data collection; A.B.S. and H.A. performed the statistical and MCDA analysis; A.B.S. wrote the original draft; N.B.M.I. and H.A. provided supervision and reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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