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Comparative analysis of physiological, biochemical and immunological responses to heat stress in hybrid and purebred ducks in tropical environments

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ABSTRACT

Heat stress is a major environmental constraint compromising poultry productivity, welfare, and health in tropical regions. This study evaluated the adaptive responses of five duck genotypes, including local Rupali, a BLRI-improved common duck with white plumage; Pekin, an exotic duck; F1, a crossbred (two-way crossing: Pekin ♂ x Rupali ♀); Muscovy duck, an exotic and H1, the first hybrid (three-way crossing: Muscovy ♂ x Pekin ♂ x Rupali ♀) under heat stress in tropical Bangladesh. A total of 200 ducklings (40 ducklings/genotype) were used in the study and allocated into 4 replicate groups per genotype (10 ducklings/group) housed in individual pens. The ducklings were exposed to elevated temperature in a controlled environmental chamber for 21 days. Respiration and panting rate, skin and rectal temperature, plasma corticosterone, immunological Y and M (IgY, IgM), cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), hemoglobin (Hb), heterophil to lymphocyte ratio (H/L ratio), white and red blood cells (WBC and RBC) count parameters were assessed at baseline (day 21), during acute heat stress (day 35), and after prolonged heat stress (day 42) period. Significant genotypic differences were observed in heat tolerance. H1 hybrid ducks exhibited comparatively balanced physiological and biochemical responses relative to the exotic genotypes under heat stress. These findings suggest that heterosis in H1 ducks may support partial metabolic stability and adaptive responses under heat stress conditions. The results support the potential use of H1 hybrids to enhance sustainable duck production in tropical environments.

Introduction

Living organisms adapt to tropical environments through structural, physiological, morphological, and behavioral specializations (Yakubu et al., 2009). This adaptability, defined as the capacity to survive and reproduce under specific environmental conditions through physiological plasticity and genetic potential, is essential for efficient animal production in hot and humid climates (Barker, 2009). In poultry systems, heat stress (HS) is one of the most critical environmental constraints, directly reducing growth performance, feed efficiency, and survivability, and ultimately causing substantial economic losses (Lara & Rostagno, 2013). According to Lin et al. (2006) and Ramnath et al. (2008), heat stress resulting from factors such as temperature, humidity, radiant heat, and airflow negatively impacts poultry meat quality, body weight, and overall production.

Under thermoneutral conditions, birds maintain core body temperature through balanced heat production and dissipation without activating additional thermoregulatory mechanisms (Howard, 2012). In

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tropical and subtropical environments, however, ambient temperatures often exceed the upper critical limit of the thermoneutral zone, forcing birds to activate adaptive responses such as increased respiration, elevated heart rate, and higher core body temperature, which disrupt normal homeostasis (Oguntunji et al., 2019 and Mashaly et al., 2004) . High relative humidity further aggravates this condition by reducing the efficiency of evaporative cooling mechanisms like panting, thereby increasing physiological strain (Freeman et al., 2024).

At the systemic level, HS induces wide-ranging alterations in hematological, metabolic, endocrine, and immune functions. The heterophil-to-lymphocyte (H/L) ratio is widely recognized as a sensitive biomarker of stress in poultry due to stress-induced leukocyte redistribution (Howard, 2012 and Zulkifli et al., 2003). Consistent with findings by Taylor et al. (2012), the heterophil/lymphocyte (H/L) ratio in blood leucocytes is a key indicator of stress in birds, with stressed individuals exhibiting increased heterophils and decreased lymphocytes. Metabolically, HS elevates circulating triglycerides, cholesterol, and corticosterone while reducing glucose and red blood cell counts, reflecting disrupted energy metabolism and oxygen transport (Belhadj et al., 2016; Swain et al., 2023). In hybrid meat-type ducks, HS has been shown to increase triglycerides, cholesterol, and stress hormone levels while decreasing HDL-C, glucose, and IgY, indicating metabolic dysregulation and immune suppression (Park et al., 2018). Collectively, these changes impair growth performance, compromise immune competence, and negatively affect meat quality (Lara & Rostagno, 2013 and Zhang et al., 2012). Recent studies have further highlighted that heat stress negatively affects the chemical composition and overall quality of broiler meat (Dai et al., 2012; Imik et al., 2012).

Genetic variation and crossbreeding strategies are increasingly recognized as effective approaches to improving heat tolerance, productivity, reproductive performance, and meat quality in tropical poultry systems (Matitaputty et al., 2015; Padhi & Sahoo, 2012). Indigenous breeds generally exhibit superior thermotolerance but lower production efficiency, whereas exotic breeds often show higher productivity under optimal conditions but reduced resilience in hot and humid environments. To overcome these limitations, a structured three-way crossbreeding program involving Rupali, Pekin (*Anas platyrhynchos*), and Muscovy (*Cairina moschata*) ducks was developed to combine adaptive and productive traits. The present study therefore evaluates and compares the physiological, biochemical, immunological, and hormonal responses to heat stress in hybrid progeny (H1) relative to their parental lines under tropical conditions in Bangladesh. By integrating multi-system indicators, this study provides novel insights into genotype-specific resilience and highlights the potential of three-way crossbreeding strategies to enhance sustainable duck production in tropical climates.

Materials and methods

Experimental design and heat chamber unit

This study utilized 200 ducklings (40 ducklings/genotype) with five genotypes, including local Rupali (*A. platyrhynchos*), a BLRI-improved indigenous common duck with white plumage; Pekin duck (*A. platyrhynchos*) as an exotic purebred line; F1, a two-way crossbred (50% Pekin ♂ x 50% Rupali ♀); Muscovy duck (*C. moschata*), an exotic purebred line and H1, the first hybrid produced through three-way crossing (50% Muscovy x 25% Pekin ♂ x 25% Rupali ♀).

Each genotype, comprising 40 ducklings, was housed in a separate chamber subdivided into four pens, with each pen serving as a replicate containing 10 ducklings. The pen was considered the experimental unit, while individual ducklings within each pen were treated as subsamples for analysis. All ducks were exposed to the same controlled heat stress conditions throughout the experimental period, and no separate non-heat-stressed control group was included. Baseline (non-heat stress, NHS) physiological measurements were recorded in the morning before daily heat exposure and compared with heat stress (HS) values obtained during peak exposure in the same ducks. In contrast, biochemical, hormonal, and immunological parameters were assessed only under heat stress conditions to compare genotype-specific responses, rather than to quantify changes relative to a non-stressed control.

From hatch to day 14, ducklings were brooded at 32–33 °C, after which the brooding temperature was gradually reduced to 25 °C under controlled ventilation. Genotypes were housed separately and fed ad libitum a crumble starter diet (days 1–14) followed by a grower diet (days 15–42), with continuous access to water. On day 15, ducklings were transferred to a heat chamber unit (1000 sq ft; 10 ft width x 100 ft length) featuring an elevated slatted floor (wood, plastic, and metal bars; 3 ft above ground) for cleanliness and disease prevention, providing 2.5 sq ft/duckling during heat stress (days 22–42).

Heat exposure was the sole intended stressor; ammonia accumulation was minimized by hourly removal of droppings using a hose-pipe. Feed and water were provided ad libitum throughout the experiment. Chambers were polythene-covered with minimal ventilation, each equipped with five hovers (four 200-watt bulbs/hover) and gas brooder backup. Heat acclimation was applied progressively as follows: 2 h/day (days 15–17), 4 h/day (days 18–19), and 6 h/day (days 20–21). From days 22–42, ducklings experienced 34–36 °C and 60–80% relative humidity for 6 h/day (10:00–16:00 h), following Badmus et al. (2022), with ambient conditions otherwise. Humidity was adjusted manually using water trays and exhaust fans. Temperature and relative humidity were recorded hourly in both the heat chamber and the outside environment over the experimental period (21st to 42nd day). The temperature–humidity index (THI) was calculated for each observation, and for diurnal analysis, values at each time point were averaged across days to obtain the mean hourly THI. Temperature and humidity were monitored continuously at duck level via an electronic display, supplemented by wet- and dry-bulb thermometers.

The temperature-humidity index (THI) was calculated as:

$$\mathbf{THI} = \mathbf{db} - - [(0.31 - - 0.31\mathbf{xRH}) \mathbf{x} (\mathbf{db} - 14.4)]$$

Where db = dry-bulb temperature (°C) and RH = relative humidity (proportion), according to Marai et al. (2007).

Physiological indices

Physiological responses were measured twice daily. Baseline (non-heat stress, NHS) values were recorded in the morning (07:00–08:30 h) before heat exposure in the chamber, while heat stress (HS) values were obtained in the afternoon (12:00–13:30 h) under peak chamber conditions. For each genotype, the same 12 ducks (three randomly selected from each of the four replicate pens) were repeatedly measured over 7 days (days 35–42). Therefore, comparisons between NHS and HS conditions represent within-subject responses, as paired measurements were obtained from the same individuals. All measurements were conducted following the protocol described by Oguntunji et al. (2020) (Table 1).

Table 1
Measurement procedures for physiological parameters under heat stress.

Physiological parameters	Measurement procedure
Respiratory rate (breaths/min) (RR)	Abdominal/vent movements were counted for 1 min using a stopwatch.
Skin temperature (°C) (ST)	Temperatures were measured at a shaved area under the wing using an infrared thermometer until the device alarm sounded.
Rectal temperature (°C) (RT)s	A sterilized digital thermometer probe was inserted into the cloaca until the device alarm sounded.
Panting rate (pants/min) (PR)	Panting episodes were counted for 1 min using stopwatch.

Blood sampling and processing

Blood samples were collected from 3 ducks per replicate group per genotype on day 21st, 35th, and 42nd during peak heat-stress exposure (approximately 3–4 h after the onset of the heat challenge). Ducks were caught and sampled consecutively to minimize stress (Bello et al., 2018; Romero & Reed, 2005). Blood was obtained from the brachial (wing) vein with a syringe and needle during a single venipuncture (AVMA, 2020). Approximately 5 mL of blood was collected and immediately divided into two portions: 2 mL into Ethylenediaminetetraacetic acid (EDTA) coated tubes (gently inverted 4–5 times) for whole blood analysis, and 3 mL into plain tubes for serum separation. Serum was obtained by centrifugation at 3000 × g for 15 min at 4 °C and stored at −20 °C, while EDTA-treated samples were stored at 4 °C until analysis (Mitin et al., 2022).

Blood biochemical parameters

Serum (stored at −20 °C) was analyzed for triglycerides (TG), total cholesterol (Chol), low-density lipoprotein (LDL), high-density lipoprotein (HDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) using an automated analyzer (Bioassays A-15; Biosystems S.A., Spain) and a precision microplate reader, according to the manufacturer's protocols.

Blood hematological parameters

EDTA blood (equilibrated at 5 °C) was analyzed within 1 h using a VetScan I-STAT 1 analyzer (CA, USA) for white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), heterophil (H), lymphocyte (L) and H/L ratio.

Serum immunoglobulin Y and M (IgY, IgM) and stress hormone (Corticosterone)

Serum IgY, IgM, and corticosterone were quantified using chicken-specific Enzyme-linked immunosorbent assay (ELISA) kits (IgY: ECK10065, IgM: ECK10045, and corticosterone: ECKC0073; 96-well/kit; ABclonal, USA) according to the manufacturer's instructions. Absorbance was read at 450 nm (microplate reader). Standard curves and regression equations are shown in Fig. 1.

Statistical analysis

Data were analyzed using SPSS (version 26.0) and RStudio (version 4.3.1). Descriptive statistics are presented as mean ± SEM. Data normality was assessed using the Shapiro–Wilk test ($p > 0.05$). The experiment followed a completely randomized design (CRD), with the pen considered the experimental unit. Individual ducks within each pen were treated as subsamples for physiological, hematological, biochemical, hormonal, and immunological measurements. For physiological traits measured repeatedly over time (days 35–42), repeated-measures analysis was performed using a mixed-model approach, where the individual duck was treated as the subject factor and time (measurement day/condition) as the repeated factor. Ducks were nested within pens to account for the subsampling structure, and pen was included as a random effect in the model. Genotype, heat-stress condition (NHS vs. HS), age/day, and their interactions were treated as fixed effects. For biochemical, hematological, hormonal, and immunological parameters measured at days 21, 35, and 42, repeated-measures two-way ANOVA was performed to evaluate the effects of genotype, age, and genotype × age interaction. When significant effects were detected, means were separated using Duncan's Multiple Range Test (DMRT). Statistical significance was declared at $p \leq 0.05$.

Results

Thermohygrometric parameters during the experimental period

The temperature–humidity index (THI) inside the heat chamber increased from morning and exhibited a bimodal pattern with two peaks (12:00 and 14:00), reaching a maximum of 34 before declining thereafter (Fig. 2). This pattern likely reflects active environmental control to maintain the target temperature range (34–36 °C), resulting in short-term fluctuations. In contrast, the outside THI followed a natural diurnal trend, with a single peak around 13:00 and a comparatively smoother pattern (Fig. 2). From the 21st to 42nd day as monitoring period, hourly chamber THI remained relatively stable (typically 30–32, with brief peaks up to 34; Fig. 3A), whereas outside THI showed greater variability (approximately 28–32) with a unimodal peak between 12:00 and 15:00 (Fig. 3B).

Physiological indices

Skin temperature (ST) increased significantly under heat stress (HS) compared to non-heat stress (NHS) ($p < 0.05$; Fig. 4A). Rectal temperature (RT), respiratory rate (RR), and panting rate (PR) also increased under HS ($p < 0.05$; Fig. 4B-D). Under HS, Pekin RT was the highest (42.18 °C), exceeding Rupali (40.91 °C), F1 (41.67 °C), (41.72 °C), and Muscovy (42.09 °C; $p < 0.05$). RR increased under heat stress, rising from 44.10 to 50.7 breaths/min in the baseline condition to 96.3–142.3 breaths/min during heat stress. The lowest RR was observed in Rupali, whereas the highest was recorded in Pekin ducks. PR was negligible under baseline conditions but increased significantly under heat stress ($p < 0.001$). Pekin showed the highest rate (65 pants/min), followed by Muscovy (55), F1 (37), and Rupali (22).

Serum biochemical parameters

The principal component analysis (PCA) revealed a clear

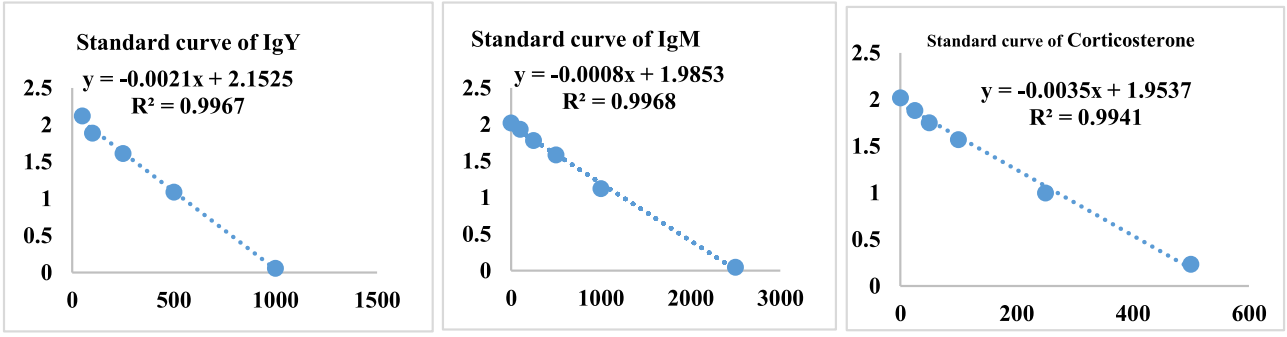

Fig. 1. Standard curves for IgY, IgM, and corticosterone, with corresponding regression equations, X-axis: IgY/IgM/Corticosterone concentration (μg/ml); Y-axis: Optical density (OD) at the measured wavelength (commonly 450 nm for ELISA).

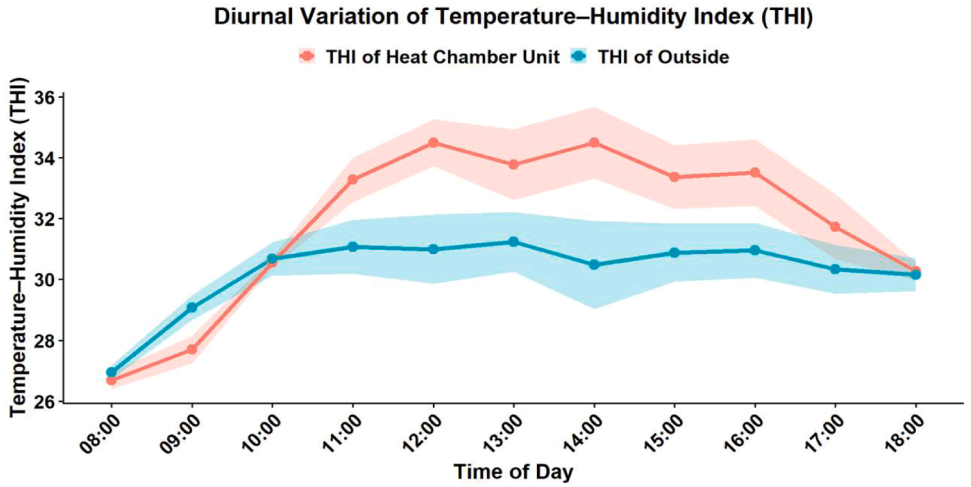

Fig. 2. Diurnal variation of temperature–humidity index (THI) in the heat chamber (red) and outside environment (blue). Values represent the mean of hourly measurements recorded across the entire (21st to 42nd day) experimental period; shaded areas indicate variability.

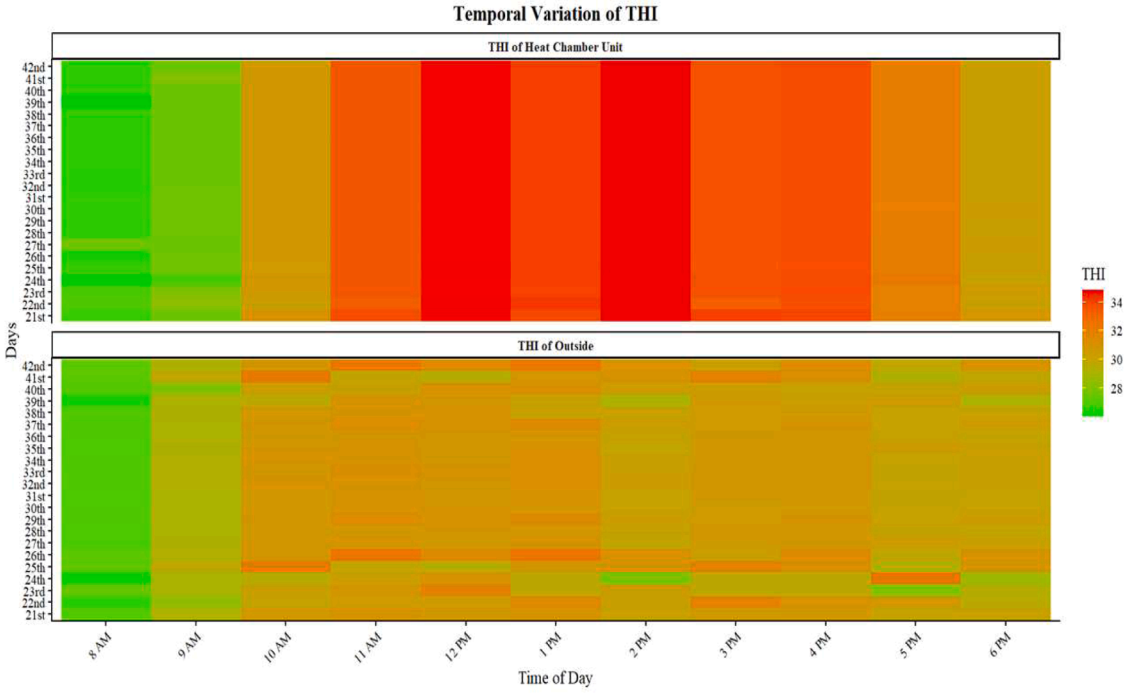

Fig. 3. Hourly THI heatmaps over the experimental period (21st to 42nd day); (A) heat chamber; (B) outside environment.

multivariate pattern in lipid and liver enzyme responses among duck genotypes under heat stress conditions (Fig. 5). The first two principal components explained a substantial proportion of the total variance (PC1: 59%; PC2: 13.2%), together accounting for over 72% of the variability, indicating strong model reliability. The PCA biplot demonstrated that PC1 was the primary axis of separation, largely driven by lipid profile and hepatic enzyme activity. Specifically, cholesterol and LDL exhibited strong positive loadings along PC1, while HDL showed a negative association, suggesting a clear metabolic divergence between protective and stress-related lipid fractions. Similarly, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) clustered closely together on the positive side of PC1, indicating a strong positive correlation and reflecting coordinated hepatic stress responses. The opposing orientation of HDL relative to LDL, cholesterol, ALT, and AST suggests an inverse relationship, where increased metabolic stress and lipid dysregulation are associated with reduced levels of protective HDL.

Genotype-wise distribution revealed partial clustering, although with noticeable overlap, indicating that while genetic background contributes to variation, environmental heat stress exerts a dominant influence across all groups. Notably, Rupali (green) ducks were predominantly positioned along the negative axis of PC1, suggesting a more favorable lipid profile and lower hepatic stress, also suggesting comparatively better metabolic stability under heat stress. In contrast, H1 (red) and Muscovy (yellow) genotypes were more closely associated with the positive PC1 axis, reflecting elevated LDL cholesterol and liver enzyme activities, indicative of increased metabolic strain and possible hepatic dysfunction. However, the distribution of H1 ducks remained less pronounced than that of Muscovy ducks, suggesting intermediate or partially buffered responses under heat stress rather than clear superiority in resilience. Pekin (Purple) and F1 (blue) genotypes displayed an intermediate distribution, suggesting moderate responses to heat stress. Furthermore, age-related effects were not distinctly separated in the

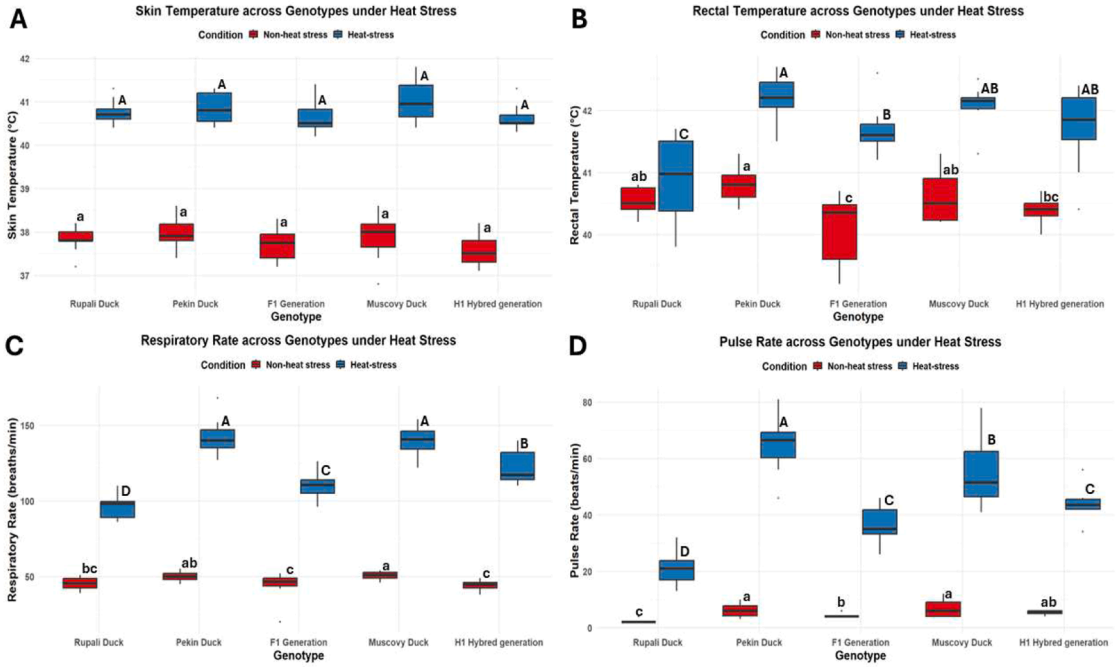

Fig. 4. Physiological responses of duck genotypes under (red) and HS (blue: (A) Skin temperature (°C), (B) Rectal temperature (°C), (C) Respiratory rate (breaths/min), and (D) panting rate (pants/min) Different superscripts (a-c and A-D) denote differences among genotypes within condition (DMRT; $p < 0.05$).

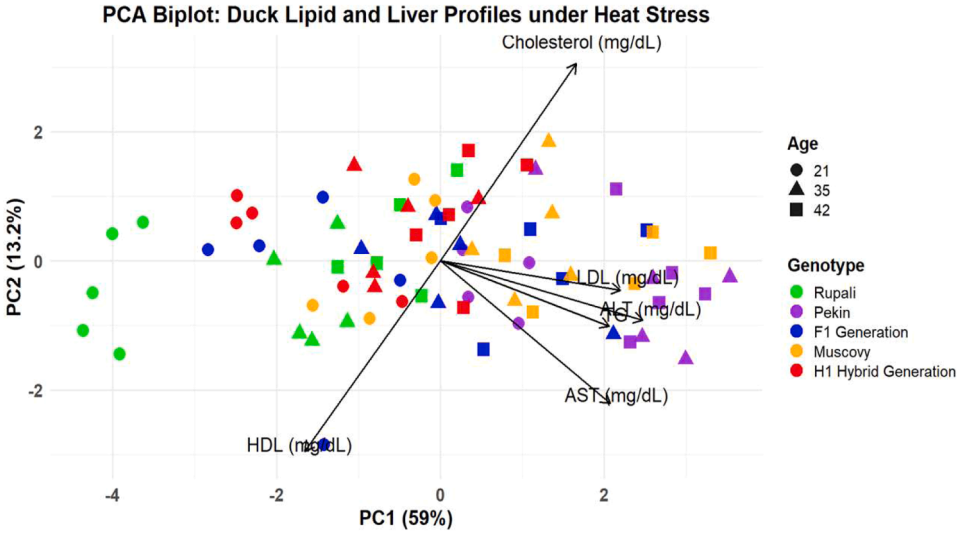

Fig. 5. The figure illustrates the Biplot of Principal Component Analysis (PCA) for lipid and liver profiles of five duck genotypes under heat stress. This biplot displays the first two principal components (PC1, 59% of the variance explained; PC2, 13.2% of the variance explained) for various duck genotypes and ages. Variables for Cholesterol, TG, HDL, LDL, ALT, and AST are shown as vectors, indicating their direction and the strength of their contribution to the components. Data points are differentiated by color for genotype (Rupali, Pekin, F1 Generation, Muscovy, and H1 Hybrid Generation) and shape for age (day 21 circle, 35 triangle, 42 square).

PCA space, as samples from different age groups (21, 35, and 42 days) were intermingled, implying that physiological responses to heat stress were more strongly influenced by metabolic and biochemical factors than by age. Overall, these findings suggest that heat stress induces a dysregulated lipid metabolism characterized by elevated atherogenic lipids and liver enzymes, reduced HDL levels, and genotype-specific differences in adaptive capacity.

At day 42, significant genotype differences were observed across biochemical markers ($p < 0.05$; Fig. 6, Table 2). Pekin had the highest cholesterol (250.03 mg/dL), ALT (58.7 mg/dL), AST (54.94 mg/dL), and LDL (210.8 mg/dL). Rupali maintained the highest HDL (69.75 mg/dL). H1 displayed moderate cholesterol (231.01 mg/dL) and triglycerides (81.86 mg/dL)

Pearson correlation analysis (Fig. 7) revealed significant associations among lipid and liver enzyme parameters. Cholesterol was positively correlated with triglycerides (TG) ($r = 0.40$, $p < 0.01$), low-density lipoprotein (LDL) ($r = 0.42$, $p < 0.001$), and aspartate aminotransferase (AST) ($r = 0.31$, $p < 0.01$), whereas it showed a significant negative correlation with high-density lipoprotein (HDL) ($r = –0.37$, $p < 0.001$). Triglycerides (TG) exhibited a significant positive correlation with LDL ($r = 0.60$, $p < 0.01$), AST ($r = 0.52$, $p < 0.001$), and alanine aminotransferase (ALT) ($r = 0.65$, $p < 0.001$), while showing a negative association with HDL ($r = –0.34$, $p < 0.01$). LDL was positively correlated with ALT ($r = 0.75$, $p < 0.001$) and AST ($r = 0.52$, $p < 0.01$), and

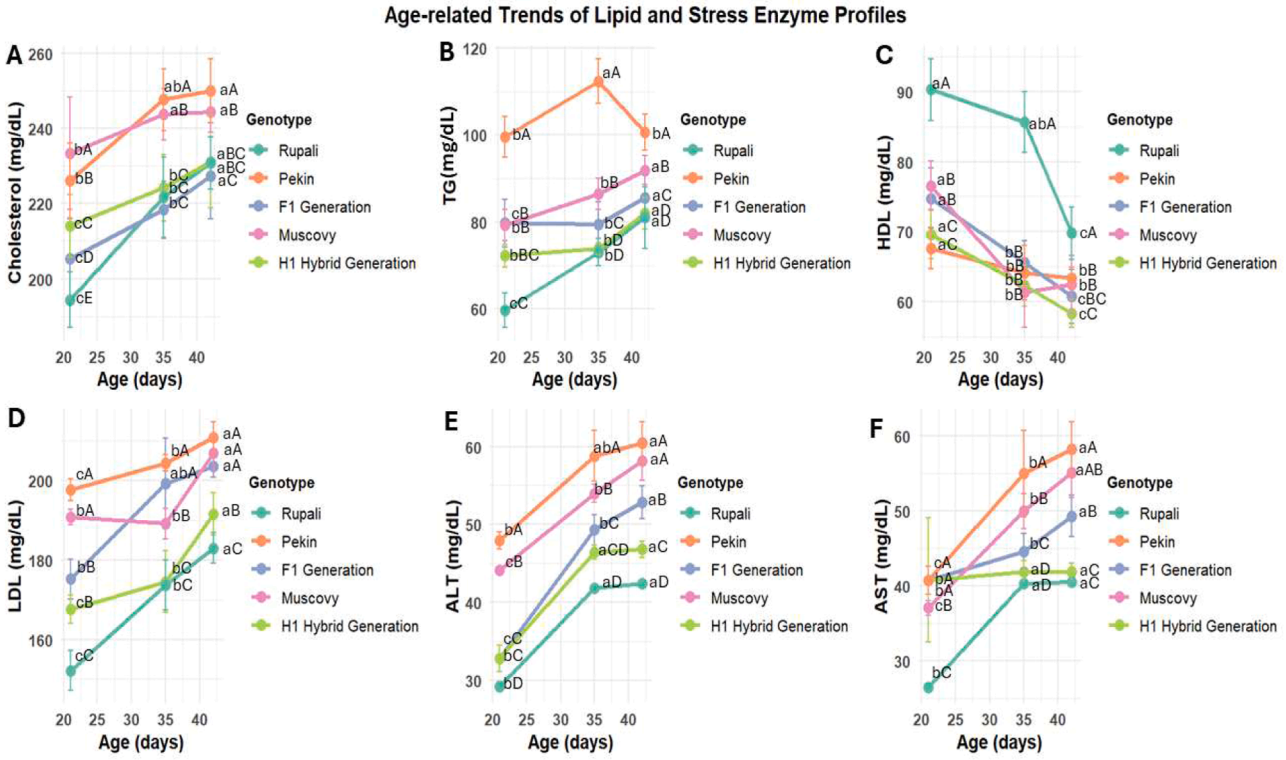

Fig. 6. Age-related lipid/liver enzyme trends (mg/dL; days 21/35/42): A. Cholesterol; B. Triglyceride (TG); C. High-density lipoprotein (HDL); D. Low-density lipoprotein (LDL); E. Alanine aminotransferase (ALT); F. Aspartate aminotransferase (AST). Superscript (a-c within genotype by age; A-D within age by genotype) denotes differences (DMRT; $p < 0.05$).

Table 2
Key serum biochemical values (mg/dL) at day 42 under HS.

Genotype	Chol	TG	HDL	LDL	ALT	AST
Rupali	230.8^{b}	80.96^{b}	69.75^{a}	183.03^{c}	41.8^{d}	40.28^{b}
Pekin	250.03^{a}	100.58^{a}	63.28^{b}	210.8^{a}	58.7^{a}	54.94^{a}
F1	227.42^{b}	85.44^{b}	60.72^{b}	203.56^{ab}	49.3^{bc}	44.62^{b}
MD	244.34^{a}	91.88^{ab}	62.46^{b}	206.92^{a}	53.9^{ab}	49.94^{ab}
H1	231.01^{b}	81.86^{b}	58.32^{b}	191.56^{bc}	46.4^{cd}	41.86^{b}
SEM	4.2	2.49	1.54	2.4	1.59	1.77

Note: SEM, standard error of the mean.
^{a,b,c,d} means the difference among genotypes is significant ($p < 0.05$) for each trait shown with different letters.

negatively correlated with HDL ($r = –0.44$, $p < 0.001$). HDL exhibited significant negative correlations with ALT ($r = –0.49$, $p < 0.001$) and AST ($r = –0.34$, $p < 0.01$). Finally, a strong positive correlation was observed between AST and ALT ($r = 0.80$, $p < 0.001$), indicating a close association between these hepatic enzyme activities.

Hematological traits

At day 42 under HS, genotypes differed ($p < 0.05$) in Hb, WBC, RBC and H/L (Table 3). Pekin had the highest hemoglobin (14.4 g/dL), followed by Muscovy (13.3 g/dL) and H1 (12.9 g/dL). Rupali maintained the highest red blood cell count (2.65 × 10^6/μL), while H1 showed intermediate values (2.4 × 10^6/μL). Pekin also had the highest white blood cell count (2.67 × 10^6/μL), with H1 showing moderate levels (2.38 × 10^6/μL). The H/L ratio was highest in Pekin ducks (1.54) and moderate in H1 ducks (1.46). Significant age effects ($P < 0.001$) were observed for multiple hematological traits. Hemoglobin increased over time in Pekin ducks (12.4 to 14.4 g/dL by day 42), whereas only a slight increase was observed in H1 ducks (11.6 to 12.9 g/dL). WBC counts increased in Pekin ducks (2.58 to 2.67 × 10^6/μL), whereas RBC counts decreased across genotypes over time. The H/L ratio varied according to both age and genotype.

The two-way ANOVA results revealed genotype, age, and interaction effects on hematological parameters under HS (Table 4). For Hb, both genotype and age effects were significant ($p < 0.001$), with a significant genotype x age interaction ($p < 0.05$). For the H/L ratio, genotype ($p < 0.001$) and genotype x age interaction ($p < 0.001$) were significant, whereas the age main effect was not significant ($p > 0.05$). WBC was affected by genotype ($p < 0.001$) and age ($p < 0.001$), but not by genotype x age interaction ($p > 0.05$). RBC showed significant effects of genotype, age, and genotype x age interaction ($p < 0.001$), with the largest generalized effect size among the hematological traits reported ($\eta^2 g = 0.751$ for the interaction).

Serum immunoglobulins and corticosterone

Serum concentrations of IgY, IgM, and corticosterone were significantly influenced by genotype, age, and their interaction ($p < 0.05$; Fig. 8). IgY levels generally increased with age across all genotypes. Among genotypes, Rupali (and F1 ducks exhibited higher IgY concentrations, whereas Pekin showed comparatively lower values, particularly at earlier ages. The H1 hybrid displayed intermediate to relatively high IgY levels, approaching those of the local and F1 groups at later ages. A similar age-dependent increasing trend was observed for IgM. Rupali and H1 hybrid ducks showed comparatively higher IgM concentrations, particularly at 42 days. Corticosterone levels increased significantly with age in all genotypes, reflecting heat stress exposure. Pekin ducks exhibited the highest corticosterone concentrations, indicating greater stress sensitivity. In contrast, Rupali maintained comparatively lower corticosterone levels, while H1 hybrids showed intermediate levels, suggesting partial stress adaptation.

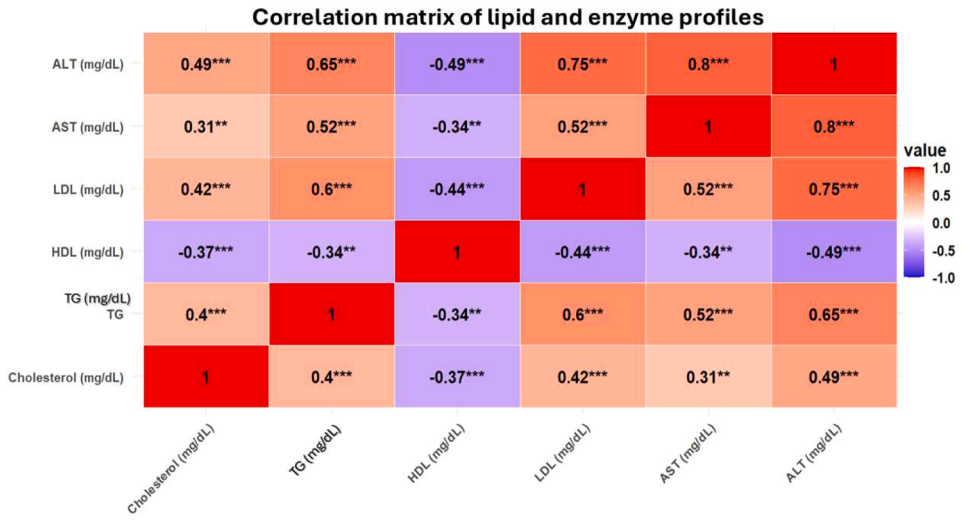

Fig. 7. Pearson correlation matrix for lipid/enzyme (Cholesterol, TG, HDL, LDL, AST, and ALT). Color scale: red/orange (positive), purple/blue (negative); asterisks denote significance (***$p < 0.001$, **$p < 0.01$).

Table 3
Effect of HS on different hematological traits of all genotypes on the 42nd day.

Genotype	Hb (g/dL)	WBC (×10⁶/μL)	RBC (×10⁶/μL)	H/L
Rupali	11.6[d]	2.11[c]	2.65[a]	1.3[c]
Pekin	14.4[a]	2.67[a]	1.8[c]	1.54[a]
F1	12.2[c]	2.23[bc]	2.5[a]	1.4[b]
MD	13.3[b]	2.58[a]	2.1[b]	1.51[a]
H1	12.9[c]	2.38[b]	2.4[ab]	1.46[ab]
SEM	0.21	0.03	0.09	.03

Note: SEM, standard error of the mean; F1, Rupali x Pekin cross; H1, first hybrid generation (Muscovy x F1 cross); Hb, Hemoglobin; H/L, Heterocyte-lymphocyte ratio; WBC, White Blood Cell; RBC, Red Blood Cell;.
[a,b,c,d] means the difference among genotypes is significant ($p < 0.05$) for each trait shown with different letters.

Integration of multi-system responses

At day 42 under heat stress, clear genotype differences were observed across physiological, biochemical, hematological, and immunological systems (Table 5). Rupali ducks showed moderate increases in rectal temperature and respiratory rate, lowest panting, favorable lipid profiles (lower cholesterol and triglycerides, highest HDL), and strong immune indices (highest IgY and IgM, lowest corticosterone). Pekin exhibited the highest rectal temperature, respiratory rate, panting, elevated cholesterol, LDL, ALT, AST, and corticosterone, alongside the lowest immunoglobulin levels, indicating poor resilience. F1 ducks displayed moderate physiological responses and RBC counts but elevated stress indices. Muscovy showed high rectal temperature and panting, elevated cholesterol and liver enzymes, and moderate immune responses, suggesting susceptibility. H1 hybrids demonstrated balanced responses with moderate physiological indices, stable RBC counts, intermediate corticosterone, and moderate immunoglobulin levels, suggesting partial retention of adaptive traits associated with crossbreeding.

Discussions

The heat chamber THI peaked at ~34 (Figs. 2,3), imposing severe thermal stress compared with moderate external conditions, consistent with tropical poultry thresholds reported by Oguntunji et al. (2014) and Marai et al. (2007). The temperature-humidity index (THI) is a widely accepted metric to assess heat stress in livestock, offering a standardized method to evaluate environmental conditions that impact animal welfare and productivity (Lallo et al., 2018; Nascimento et al., 2019). THI is especially relevant in tropical and arid regions, where it aids understanding of climate effects on livestock and informs management strategies to mitigate heat stress (Hossain et al., 2023). In the present study, the THI profile confirms that the internal chamber successfully imposed a severe thermal challenge, whereas the external environment remained within a moderate stress range during the heat-stress phase. These findings agree with previous reports and reinforce the consensus that tropical climatic conditions can compromise livestock welfare and productivity (Castanheira et al., 2010).

Genetic variation in heat tolerance among duck genotypes is an important determinant of productivity under hot environments. Local duck genotypes demonstrate enhanced thermal tolerance, evidenced by reduced physiological stress indicators such as skin temperature, rectal temperature, respiratory rate (RR), and panting rate (PR) (Oguntunji et al., 2020, 2019). In contrast, exotic ducks exhibit elevated physiological indices, indicating greater susceptibility to heat stress and poorer adaptation to tropical heat compared to local breeds (Oguntunji et al., 2019). Under high temperature conditions, poultry maintain homeostasis via behavioral, hormonal, physiological, and biochemical adaptations (Castanheira et al., 2010). Heat dissipation mechanisms such as conduction, convection, and radiation are effective at thermoneutral temperatures; however, these are overwhelmed during elevated metabolic activity and environmental heat gain. The lower levels of RR and PR observed in local and F1 ducks under heat stress, compared to exotic and H1 genotypes, align with findings by Ewuola et al. (2020), who reported lower respiratory and panting rates in local Muscovy and hybrid mule ducks than in exotic Mallard ducks.

According to Salama et al. (2014), high temperatures induce heat loss in birds mainly through evaporative cooling mechanisms such as panting and evaporation from the skin surface, which are common heat stress responses (Rolf, 2015). Since birds lack sweat glands, they cannot dissipate heat via sweating; instead, they rely on increased respiratory rates and panting. In this study, significantly elevated RR and PR in exotic ducks, Pekin and Muscovy, indicate a greater thermal burden in these genotypes than in local and F1 ducks. These findings support the observations by Fadare et al. (2013), who noted that increased panting is necessary to maintain homeostasis through respiratory evaporation. Elevated RR enhances heat dissipation via evaporative cooling, and the highest RR observed in exotic ducks reflects thermal stress and compensatory efforts to lose heat (Gupta et al., 2013).

Exotic ducks also exhibited higher RT compared to crossbreed ducks, suggesting poor thermoregulatory capacity and increased thermal stress,

Table 4
Two-way ANOVA for hematological traits under heat stress.

Parameter	Genotype Means ± SEM	Age Means ± SEM	Source of Variation	p-value	Effect size (η^2g)
Hemoglobin (g/dL)	Rupali: 12.77 ± 0.12[b] Pekin: 13.73 ± 0.11[a] F1: 12.91 ± 0.10[b] Muscovy: 13.41 ± 0.13[a] H1: 12.71 ± 0.12[b]	21 d: 12.61 ± 0.08[b] 35 d: 13.83 ± 0.09[a] 42 d: 13.79 ± 0.10[a]	Genotype Age Genotype × Age	<0.001 <0.001 0.034	0.550 0.549 0.233
H/L ratio	Rupali: 1.31 ± 0.05[b] Pekin: 1.64 ± 0.06[a] F1: 1.47 ± 0.05[ab] Muscovy: 1.51 ± 0.06[ab] H1: 1.29 ± 0.05[b]	21 d: 1.45 ± 0.04[b] 35 d: 1.59 ± 0.05[a] 42 d: 1.38 ± 0.04[b]	Genotype Age Genotype × Age	<0.001 0.048 0.013	0.255 0.090 0.265
Total WBC (×10⁵)	Rupali: 2.65 ± 0.03[a] Pekin: 2.62 ± 0.03[ab] F1: 2.64 ± 0.03[a] Muscovy: 2.63 ± 0.03[ab] H1: 2.54 ± 0.03[b]	21 d: 2.58 ± 0.02[b] 35 d: 2.68 ± 0.02[a] 42 d: 2.62 ± 0.02[ab]	Genotype Age Genotype × Age	0.036 <0.001 0.946	0.155 0.261 0.044
Total RBC (×10⁵)	Rupali: 2.01 ± 0.05[c] Pekin: 2.10 ± 0.05[b] F1: 2.39 ± 0.05[a] Muscovy: 2.33 ± 0.05[a] H1: 2.22 ± 0.05[ab]	21 d: 2.25 ± 0.04[b] 35 d: 2.30 ± 0.04[a] 42 d: 1.96 ± 0.04[c]	Genotype Age Genotype × Age	<0.001 <0.001 <0.001	0.362 0.468 0.751

Note: SEM, standard error of the mean; R, Rupali; P, Pekin; MD, Muscovy duck; F1, first filial generation (2-way crossing); H1, first hybrid generation (3-way crossing); Hb, Hemoglobin; H/L, Heterocyte-lymphocyte ratio; WBC, White Blood Cell; RBC, Red Blood Cell;.

[a,b,c] means the difference among genotypes is significant ($p < 0.05$) for each trait shown with different letters; $p > 0.05$, non-significant.

likely due to inadequate heat dissipation to maintain homeothermy (Castanheira et al., 2010). The lower RT values in local ducks under heat stress concur with Nascimento et al. (2019), who found that Cobb broiler strains effectively lower RT to manage thermal load. Elevated RT signifies heat accumulation, which can impair welfare and performance, even a rise of <1 °C in RT can reduce productivity in livestock species (McDowell et al., 1976). Compared to heavy exotic breeds, Rupali, F1 and H1 ducks showed lower RT, suggesting comparatively improved thermoregulatory responses. Hansen (2004) demonstrated that smaller body size in tropical breeds is an adaptive trait to reduce thermal susceptibility, consistent with literature showing that heavier animals experience greater difficulty dissipating heat (McDowell et al., 1976). This study confirms that smaller ducks like Rupali and F1 are physiologically more efficient at dissipating heat than heavier exotics. In H1, this apparent local advantage was only partially retained, suggesting that crossbreeding may dilute some body size-related thermoregulatory adaptation. The interaction between genotype and stress period on skin temperature was not significant, suggesting consistent changes across genotypes. However, significant interactions were observed for RT, RR and PR, indicating that heat stress effects on these indices vary among genotypes.

Biochemical and metabolic markers further distinguished genotype-specific responses to heat stress. Cholesterol, a reliable metabolic stress marker, differed significantly among genotypes, with higher levels in Pekin and Muscovy, suggesting a stronger impact of heat stress in these heavy breeds, potentially due to higher basal metabolic rates. Conversely, local Rupali ducks had significantly lower cholesterol levels, reflecting greater heat tolerance. The H1 crossbred showed cholesterol levels closer to Rupali and significantly lower than those of the exotic breeds, indicating partial inheritance of lipid stability traits from the local genotype. Nonetheless, a moderate age-related increase in cholesterol in H1 implies some susceptibility to prolonged heat stress, albeit less severe than in exotic lines. Similar patterns were reported by, underscoring the role of lipid homeostasis in heat-stressed poultry. Elevated cholesterol under prolonged heat stress may reflect increased lipolysis or compensatory mechanisms to preserve cellular integrity under oxidative stress (Cheng et al., 2022).

The elevated triglyceride (TG) levels observed in Pekin and Muscovy ducks indicate increased metabolic activity under heat stress, whereas the lower TG levels in Rupali ducks suggest more efficient energy management. The comparatively improved TG profile in H1 suggests partial metabolic stability, potentially associated with hybrid vigor. These findings align with Lan et al. (2022), who reported that heat stress in broilers elevates serum TG levels due to increased hepatic lipogenesis and abdominal fat deposition, effects that can be partially mitigated by dietary interventions such as vitamin E supplementation, implicating oxidative stress. Furthermore, TG levels significantly increased between day 21 and day 42, reflecting the cumulative impact of prolonged stress. This is consistent with Zhang et al. (2012), who demonstrated elevated TG levels in thermally stressed birds.

Heat stress in heavier duck breeds leads to decreased HDL levels and increased LDL levels, indicative of susceptibility to oxidative damage and compromised lipid transport mechanisms. These results align with Emami et al. (2021), who reported that heat stress reduces HDL levels and elevates LDL in chickens, reflecting oxidative damage and lipid imbalances. Notably, H1 ducks exhibited HDL and LDL profiles more similar to the local Rupali breed than to exotic breeds, suggesting partial inheritance of stress-tolerant lipid metabolism. Farag and Alagawany similarly noted that crossbreeding enhances lipid transport efficiency and mitigates the negative effects of thermal stress. Age-related declines in HDL under heat stress observed in this study concur with Emami et al. (2021), who attributed reduced HDL synthesis to hepatic dysfunction and oxidative injury. Consequently, HDL may serve as a useful biomarker for heat tolerance in ducks.

Regarding liver enzymes, Pekin ducks exhibited elevated ALT and AST levels under heat stress, indicating hepatic dysfunction, while Rupali and H1 showed lower levels, reflecting greater resistance to heat-induced oxidative damage. These findings are consistent with Zhang et al. (2018), who demonstrated elevated liver enzymes in exotic breeds compared to indigenous and crossbred ducks. H1's comparatively lower enzyme levels also align with Akbarian et al. (2016), who reported that hybrid genotypes generally exhibit enhanced antioxidant capacities and better hepatic adaptation under stress compared to purebred exotics. Overall, H1 inherits favorable traits from the Rupali, including lower TG levels, improved LDL regulation, and moderate ALT and AST levels, all critical for heat stress tolerance. Bedere et al. (2022) support these findings, showing that crossbreeding local breeds with commercial lines improves heat tolerance and disease resistance, as seen in crossbred laying hens with enhanced productivity and resilience. Bradford et al. (2018) further demonstrate that crossbreeding improves heat tolerance in meat animals, such as swine and cattle, by enhancing genetic diversity related to heat response. The intermediate performance of H1 across physiological and biochemical parameters reflects

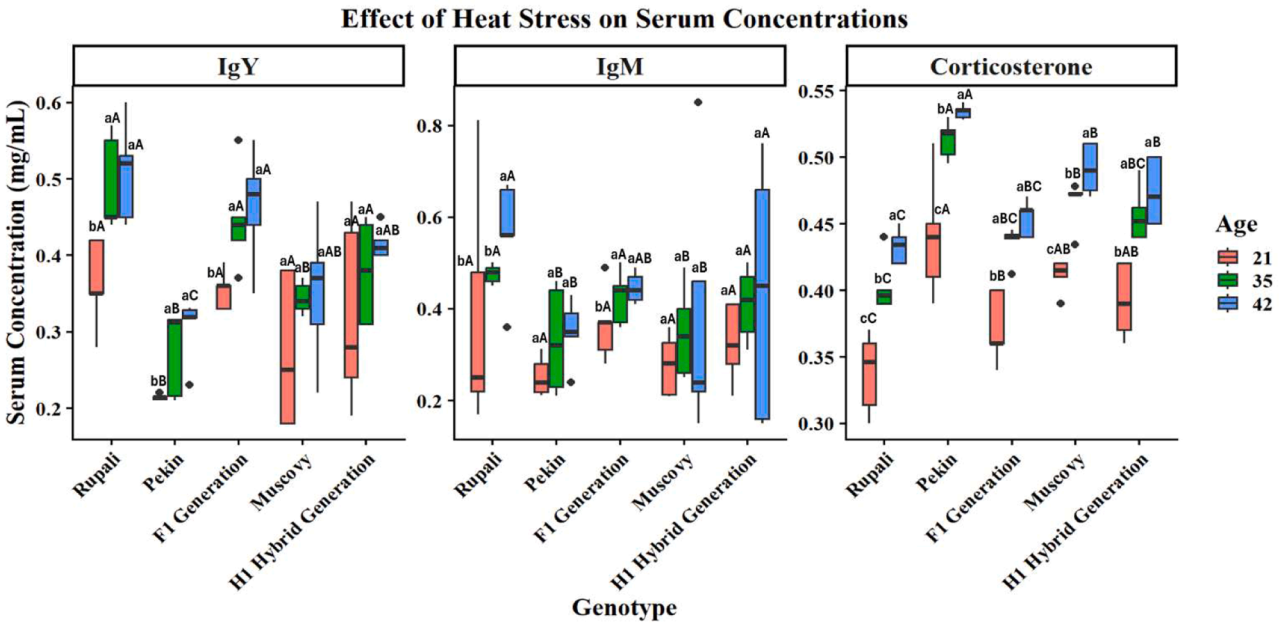

Fig. 8. Effect of heat Stress on blood serum concentrations of IgY, IgM, and corticosterone across fivs duck genotypes of Rupali, Pekin, F1 Generation, Muscovy, and H1 Hybrid Generation. Data is presented as box plots showing the median, quartiles, and range of serum concentrations (mg/mL). a-c Different lowercase letters indicate significant differences between three age groups within the same genotypes ($p < 0.05$; Tukey's test). A-C, Different uppercase letters indicate significant differences between five genotypes under the same age groups ($p < 0.05$; Tukey's test).

Table 5
Cross-system summary of heat stress responses in duck genotypes (Day 42).

Genotype	Physiological (RT, RR, PR)	Biochemical (Chol, TG, ALT/AST)	Hematological (Hb, RBC, H/L)	Immunological (IgY, IgM, Corticosterone)	Overall Resilience
Rupali	Moderate increase; lowest panting	Lower Chol/TG; highest HDL; low ALT/AST	Hb lowest; RBC highest; H/L low	Highest IgY and IgM; lower corticosterone	Good thermotolerance, strong immune response
Pekin	Highest RT, RR, PR	Highest Chol, LDL, ALT, AST	Hb highest; RBC moderate; H/L highest	Lowest IgY; IgM; corticosterone high	Poor resilience; high stress load
F1 cross	Moderate RT; higher RR	Chol moderate; TG moderate; ALT/AST elevated	RBC moderate; H/L moderate	Moderate IgY, IgM and corticosterone	Mixed profile; good RBC but high stress index
Muscovy	High RT, PR	High Chol/LDL; ALT/AST elevated	Hb moderate; RBC moderate; H/L high	IgY, IgM, corticosterone moderate (better than Pekin)	Susceptible: biochemical stress and immune suppression
H1 hybrid	Moderate RT, RR, PR	Chol/TG moderate; ALT/AST elevated	Hb moderate; RBC stable; H/L moderate	IgY moderate; IgM lower; corticosterone moderate	Balanced intermediate responses under heat stress

Note: Table 5, the cross-system summary of heat stress responses in duck genotypes (Day 42). Summary of physiological (rectal temperature, respiratory rate, panting rate), biochemical (cholesterol, triglycerides, HDL, LDL, ALT, AST), hematological (Hb, WBC and RBC counts, H/L ratio), and immunological (IgY, IgM, corticosterone) responses of five duck genotypes under heat stress. Values are interpreted from detailed results in Tables 2–4 and Figs. 4–8.

hybrid vigor, as similarly reported by Melesse et al. (2011). This study highlights the potential of hybrid genotypes to optimize the balance between productivity and adaptability in heat-stressed environments.

The observed gradual decline in Hb during prolonged heat stress aligned with previous reports (Abu-Dieyeh et al., 2006). H1 showed comparatively better hematological stability under heat stress than the pure exotic lines. The consistently low and stable H/L ratios in H1 reflect effective stress adaptation. Elevated H/L ratios are widely recognized as biomarkers of stress, and the lower H/L ratios in H1 during acute heat stress support comparatively stable stress responses, consistent with findings by Altan et al. (2003), who reported reduced H/L ratios in heat-tolerant poultry strains. This is also consistent with reports linking lower H/L ratios with enhanced heat tolerance in poultry (Gorelik et al., 2020). Although H1 exhibited slightly lower Hb levels than Pekin ducks, its RBC count remained stable during both acute and prolonged stress, indicating efficient hematological regulation. This stability is critical for maintaining oxygen transport and metabolic function under heat stress conditions (Ranjan et al., 2019). Additionally, H1 maintained stable WBC and RBC counts, reflecting a robust immune response and adaptability, in contrast to the greater fluctuations observed in the exotic strains. These results suggest that the H1 genotype inherited favorable traits from both local and exotic lineages, contributing to moderate adaptive responses. The moderate WBC response in H1, relative to Pekin and Muscovy, further suggests a more controlled stress response. Overall, these hematological findings suggest that incorporating local genetic background into exotic duck lines may enhance physiological adaptation to heat stress, likely through improved regulation of stress and immune-related parameters.

The age-associated increase in IgY and IgM indicates progressive activation of humoral immunity under heat stress, consistent with previous reports in poultry (Lara LJ & Rostagno MH, 2013; Park BS et al., 2018). However, pronounced genotype effects were evident: Rupali and F1 maintained higher immunoglobulin levels, whereas Pekin exhibited reduced IgY and IgM, suggesting heat-induced immunosuppression in exotic genotypes. This aligns with evidence that fast-growing or heavier breeds allocate fewer resources to immune function (Cheema MA et al., 2003) and are more susceptible to thermal stress (Mashaly MM et al., 2004; Zulkifli I et al., 2004). In contrast, the H1 hybrid sustained relatively high immunoglobulin concentrations, approaching those of local and F1 ducks, indicating enhanced immune resilience.

This supports the role of local genetic background in conferring thermotolerance, as indigenous genotypes are better adapted to tropical conditions, whereas exotic breeds are prone to oxidative and endocrine stress (Belhadj Slimen I et al., 2016; Fadare AO et al., 2013; Oguntunji A et al., 2019). Corticosterone increased across all genotypes, confirming activation of the HPA axis under heat stress. The highest levels in Pekin indicate poor adaptability, while lower levels in Rupali reflect inherent thermotolerance. Notably, H1 exhibited moderate corticosterone levels, suggesting a more regulated stress response (Romero LM & Reed JM, 2005). Given that elevated corticosterone suppresses immune function (Quinteiro-Filho WM et al., 2010), the ability of H1 to maintain immunoglobulin levels while maintaining controlled corticosterone levels indicates a favorable stress–immune balance. This integrated response reflects heterosis, whereby crossbred genotypes achieve improved physiological stability under stress (Isa AM et al., 2020). The contribution of locally adapted genetics, potentially complemented by the robustness of the Muscovy lineage, likely underpins the moderate adaptive responses of H1 (Guemene D, 2011; Lin H et al., 2006). Moreover, corticosterone dynamics observed here are consistent with its established role as a biomarker of thermal stress (Feng X et al., 2019; Sohail MU et al., 2012).

The cross-system analysis highlights clear genotype-dependent resilience to thermal challenge. Rupali ducks maintained favorable lipid profiles, strong immunoglobulin responses, and low corticosterone, consistent with reports that indigenous poultry exhibit superior thermotolerance and immune competence in tropical climates (Fadare et al., 2013; Oguntunji et al., 2019). In contrast, Pekin ducks showed elevated rectal temperature, respiratory rate, dysregulated lipid metabolism, and suppressed immunity, reflecting the vulnerability of fast-growing exotic breeds to heat stress (Belhadj Slimen et al., 2016; Cheema et al., 2003). Muscovy ducks also demonstrated susceptibility, with high biochemical stress and reduced immune indices, aligning with previous findings that exotic genotypes are less resilient under hot and humid conditions (Zulkifli et al., 2004). F1 crosses exhibited mixed responses, maintaining moderate RBC counts but showing elevated stress markers, suggesting partial improvement but limited stability. Importantly, the H1 hybrid achieved a balanced profile: moderate corticosterone levels coupled with sustained RBC and immunoglobulin concentrations. This supports the concept of heterosis, where crossbreeding integrates adaptive traits from local genotypes with productive traits from exotics, yielding moderate physiological stability under heat stress (Isa et al., 2020; Lin et al., 2006). The inclusion of Muscovy genetics may further enhance robustness, as Muscovy ducks are known for resilience in challenging environments (Guemene, 2011). Collectively, these findings confirm that genotype differences in thermotolerance are driven by multi-system interactions involving lipid metabolism, hematological stability, and endocrine-immune balance.

H1 hybrids demonstrated intermediate physiological, biochemical, hematological, and immunological responses under heat stress. Although some metabolic stress indicators remained elevated compared with Rupali ducks, H1 maintained comparatively balanced responses relative to the exotic parental lines, suggesting moderate tolerance and partial adaptation under heat stress conditions. These findings indicate that structured crossbreeding may help retain favorable adaptive traits while supporting sustainable duck production in tropical climates (Lara & Rostagno, 2013; Park et al., 2018)

Conclusion

This study assessed the heat stress tolerance of the H1 genotype, a hybrid derived from local Rupali and exotic Pekin and Muscovy ducks, in comparison with its parental lines. The results demonstrated that local Rupali duck ducks exhibited the greatest resilience to heat stress, whereas Pekin duck and Muscovy duck ducks appeared more vulnerable. The H1 genotype showed evidence of hybrid vigor through intermediate physiological stress responses, improved lipid stability, and relatively balanced energy metabolism under heat stress conditions. In addition, H1 ducks maintained comparatively stable hematological parameters and balanced immunological responses relative to the exotic parental lines. Overall, H1 ducks demonstrated moderate tolerance with mixed resilience, retaining certain adaptive traits from the local genotype while buffering some adverse responses observed in the exotic lines. These findings suggest that structured crossbreeding may help preserve favorable adaptive traits and improve physiological stability under tropical heat stress conditions, although indigenous Rupali ducks remained the most thermotolerant genotype in the present study.

Limitations of the study

- ❖ No separate thermoneutral control group was included; therefore, responses were compared mainly genotype-specific responses under heat stress conditions rather than absolute heat stress effects.
- ❖ The relatively short trial period may not reflect long-term adaptation or lifetime productivity.
- ❖ Economic feasibility and farmer-level profitability were not evaluated.
- ❖ Only selected duck genotypes were investigated; therefore, findings may not apply to all genetic combinations.
- ❖ Other environmental stressors, including humidity, disease pressure, and nutritional variability, were not assessed.
- ❖ Sensory and consumer preference analyses were not included in the meat quality evaluation.

Abbreviations

ANOVA	Analysis of Variance
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
°C	Degree Celsius
Cort	Corticosterone
DMRT	Duncan multiple range test
Db	Dry bulb (temperature)
EDTA	Ethylenediaminetetraacetic acid
°F	Degree Fahrenheit
F1	First filial generation
Hb	Hemoglobin
H/L	Heterophil-lymphocyte ratio
HDL	High-density lipoprotein
HS	Heat-stress
IgM	Immunoglobulin M
IgY	Immunoglobulin Y
LDL	Low-density lipoprotein
	Non heat-stress
PCA	Principal Component Analysis
PR	Panting rate
RBC	Red blood cells
RR	Respiratory rate
RH%	Relative humidity
RPM	Revolutions Per Minute
RT	Rectal temperature
ST	Skin temperature
TC	Total cholesterol
TG	Triglycerides
THI	Temperature humidity index
WBC	White blood cells

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Ethics approval

This research was approved by the Animal Care and Use Committee of the Bangladesh Livestock Research Institute (BLRI), with approval number BLRI No 33.05.2672.303.05.007.19–1469.

Consent for publication

Not applicable.

Declaration of AI and AI-assisted technologies

We did not use any AI for writing the manuscript.

CRediT authorship contribution statement

Md Abu Hemayet: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Md Sazedul Karim Sarker:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Zulkifli Bin Idrus:** Writing – review & editing, Software, Resources, Methodology, Formal analysis. **Md Zulfekar Ali:** Writing – review & editing, Software, Resources, Methodology, Formal analysis. **Awis Qurni Sazili:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Mohammad Shamsul Alam Bhuyian:** Writing – review & editing, Methodology, Formal analysis. **Halima Khatun:** Writing – review & editing, Resources. **Dela Ayu Lestari:** Writing – review & editing, Methodology. **Asep Setiaji:** Writing – review & editing. **Mamat Hamidi Kamalludin:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

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

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