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Effects of heat stress on growth metrics, carcass characteristics, telomere length, and gene expression in chickens

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ABSTRACT

This study investigated the impact of heat stress (HS) on growth performance, carcass traits, telomere length (TL), and gene expression profiles in three chicken breeds with varying growth rates: slow-growing (SAGA), medium-growing (Sasso), and fast-growing (Cobb 500). Three hundred 14-day-old male chicks were exposed to either control (25°C) or HS (34°C for 6 hours/day) conditions for four weeks in a controlled environment. Weekly growth metrics, TL at two and four weeks, Heat Shock Protein 70 *(HSP70)* and Insulin-Like Growth Factor-1 *(IGF-1)* expression in muscle and liver at two and four weeks of HS exposure, and carcass/organ yields at four weeks were analyzed. Cobb 500 chickens exhibited significant growth reductions under HS, while SAGA showed resilience. Notably, SAGA chickens exhibited a significant increase in intestinal organ mass under HS, which may indicate an adaptive response to thermal stress. HS exposure significantly shortened TL across all breeds, suggesting its utility as a universal biomarker for HS in chickens. All breeds upregulated *HSP*70 expression, with the Cobb 500 showing the most prominent increase. Similarly, *IGF-1* was expressed (upregulated), particularly in 500 broilers at both time-points, highlighting breed-specific differences in growth performance. These results demonstrate breed-specific physiological adaptations to HS. TL and stress-related gene expression are crucial indicators of heat susceptibility and adaptation. The study provides insights into developing breed-specific management and breeding strategies to enhance poultry resilience to increasing global temperatures.

Introduction

Global warming exacerbates the harmful effects of heat stress (HS) on poultry, especially chickens, leading to significant economic losses. These losses result from decreased feed intake, growth rates, meat and egg quality, and increased mortality (Kamboh et al., 2013; Lara and Rostagno, 2013; Pawar et al., 2016). Mitigating HS is essential for sustainable poultry production and involves strategies such as enhanced ventilation, genetic selection for heat tolerance, and optimized housing management (Pawar et al., 2016). Despite these advances, the physiological mechanisms driving HS responses in chickens with different growth rates remain poorly understood. Fast-growing poultry breeds are highly susceptible to HS due to their elevated metabolic heat production (Renaudeau et al., 2012). This vulnerability manifests as reduced growth and impaired feed efficiency, likely caused by HS-induced disruptions in carbohydrate and lipid metabolism (Lin et al., 2006).

In this study, we used three chicken breeds–SAGA, Sasso, and Cobb 500–that naturally differ in lifespan, with slower-growing breeds generally living longer (Tablado et al., 2022). All experimental birds were sacrificed at 6 weeks of age, prior to sexual maturity (Whittemore et al., 2019). This experimental design minimizes confounding effects of age and reproductive status on telomere length (TL), ensuring that observed telomere dynamics primarily reflect responses to HS rather than age- or reproduction-related telomere attrition (Monaghan and Ozanne, 2018).

Telomeres are nucleoprotein complexes that protect chromosome

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ends from degradation and have emerged as reliable biomarkers for assessing the impact of environmental stressors, including HS, on biological systems. Their high guanine content makes them particularly vulnerable to oxidative damage, which delays repair and enhances their utility as indicators of stress (Kawanishi and Oikawa, 2004). TL measurement has practical applications for identifying heat stress resilience in poultry and livestock; selecting individuals with longer telomeres can aid breeding programs aimed at improving heat tolerance or inform targeted interventions to reduce stress-related damage (Bateson, 2016).

TL naturally shortens with age, but HS accelerates this process by increasing cellular aging and damage (Pineda-Pampliega et al., 2020). In chickens, shorter telomeres have been linked to elevated oxidative stress and poorer overall health supporting the use of TL as a biomarker for vulnerability to HS (Voulgarellis, 2019). TL has emerged as a reliable indicator of cellular aging and stress (Angelier et al., 2013; Sohn et al., 2014), with shorter telomeres associated with HS exposure and reduced growth performance (Angelier et al., 2013; Pineda-Pampliega et al., 2020; Badmus et al., 2022).

A key physiological response to HS in chickens is the upregulation of heat shock proteins (*HSP*), especially *HSP70*. *HSP*s are a conserved family of molecular chaperones that assist in protein folding, stabilization, and protection against stress-induced cellular damage (Gouda et al., 2024). *HSP70* expression levels vary among breeds and rise substantially in all tissues in response to elevated ambient temperatures (Balakrishnan et al., 2023; Cartoni et al., 2023; Greene et al., 2019; Zulkifli et al., 2018). As a well-established biomarker of cellular stress, the induction of *HSP70* mitigates protein damage and helps maintain cellular homeostasis during HS (Yang et al., 2022).

HS significantly downregulates insulin growth factor 1 (*IGF-1*) expression, a key regulator of growth, thereby impairing feed efficiency and growth performance (Ma et al., 2018; Li et al., 2021). *IGF-1* regulation varies among breeds, with Cobb 500 breed exhibiting more pronounced reductions under HS, making *IGF-1* a potential biomarker for heat resilience (Nawaz et al., 2023). Elevated baseline *IGF-1* levels correlate with improved growth under HS conditions, supporting its use in selective breeding aimed at enhancing heat tolerance (Vaccaro, 2023). Additionally, *IGF-1* reflects breed-specific thermal tolerance, highlighting its role in the differential susceptibility of chicken breeds to heat stress (Hemanth et al., 2024; Zhang et al., 2024). These breed-dependent variations align with the established understanding that growth rate is a significant factor influencing heat tolerance in poultry.

This study investigates TL, *HSP70*, and *IGF-1* as potential biomarkers of heat stress. We hypothesize that: (1) HS will impair growth performance, particularly in the Cobb 500 breed; (2) HS will accelerate telomere shortening, with greater shortening associated with reduced growth; (3) *HSP70* upregulation will vary by breed; and (4) *IGF-1* expression will correspond to breed-specific thermal tolerance.

Material and methods

Ethics declarations

All experimental procedures were approved by the Malaysian Agriculture and Research Development Institute (MARDI) Animal Ethics Committee (AEC) under approval number 20230622/R/MAEC00127.

Source and Management of Experimental Birds. This study was conducted at the Animal Research Unit of the Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia (UPM). SAGA, Sasso, and Cobb 500 chickens were sourced from MARDI, Hiap Hwa (Pengerang) Johor, and PK Agro-Industrial Product (M), respectively, and weighed weekly throughout the experiment. During the first three days, chicks received an oral anti-stress supplement (VP 1000; VetPharm, USA), followed by infectious bronchitis virus and Newcastle disease vaccinations on days 7 and 21, respectively.

Heat Stress, Housing Arrangements, and Diet. Day-old chicks were initially brooded at 32°C and 84 % relative humidity; the temperature was then gradually lowered to reach 25°C by day 14. At 14 days of age, all chicks were leg-banded for individual identification and randomly assigned to one of two temperature treatments: Normal Temperature (NT; 25 ± 1°C) or Heat Stress (HS; 34 ± 1°C). Birds in the HS group were exposed to 34 °C for 6 hours daily over 28 days, while the NT group remained at 25 °C throughout. They received a commercial broiler starter (21 % CP, 3,000 kcal ME/kg) from days 1-21 and a finisher (19.3 % CP, 3,200 kcal ME/kg) diet from days 22-43 (Table 1). Feed and water were provided *ad libitum*.

Experimental Design, Data Collection, Sampling, and Tissue Storage. A 2 × 3 factorial design (2 Temperature × 3 Breeds) was implemented using 300 male chicks: SAGA, Sasso, and Cobb 500 ($n = 100$ per breed). Birds were randomly assigned to either heat stress (HS; 34 °C) or thermoneutral (NT; 25 °C) conditions, with five replicates per breed per treatment and 10 birds per replicate. Birds were housed in battery cages (122 × 91 × 61 cm). From day 14 to 42 days of age, HS groups were subjected to cyclic heat stress at 34 °C for 6 daily in automated environmental chambers, whereas NT groups were maintained under constant thermoneutral conditions.

Growth performance parameters including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded weekly. Average BW per replicate was measured using a digital CAS scale (CAS Corporation, USA). FI was calculated as the difference between feed offered and feed refused. BWG was determined as the weekly change in BW and FCR was calculated by dividing FI by BWG. Mortality was recorded daily.

At weeks 4 and 6 of age (corresponding to 2 and 4 weeks of HS exposure), two birds per replicate were randomly selected from each treatment group and humanely slaughtered according to Halal procedures. Carcass and internal organ weights were recorded post-mortem. Liver and *Pectoralis major* tissues were collected, immediately snap-frozen in liquid nitrogen, and stored at −80 °C in sterile 2 mL microcentrifuge tubes until genomic DNA and total RNA extraction for telomere length and gene expression analyses, respectively.

Telomere Length (TL) Analysis via qPCR. Genomic DNA was extracted from the *Pectoralis major* muscle samples using the PrimeWay Genomic DNA Extraction Kit (JTC MedTech Hub, Singapore). Relative telomere length (RTL) was quantified by a modified quantitative real-time PCR (qPCR) assay adapted from Cawthon (2002). Each 20 μL PCR reaction contained 20 ng of DNA, 2 μM of either telomere or *GAPDH* primers, and SensiFAST SYBR® No-ROX mix (Bioline, UK) (primer sequence listed in Table 2). Each sample was run in duplicate, with one reaction amplifying telomeric repeats (T) and the other targeting the single-copy reference gene *GAPDH* for normalization. *GAPDH* primers were designed based on the *Gallus gallus* reference sequence NC_006101.5 (Table 4). The qPCR cycling program consisted of an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. For quality control, samples exhibiting a cycle threshold (Ct) standard deviation greater than 1 between duplicates were excluded from further analysis.

Genomic RNA Extraction for HSP70 and IGF-1. Total RNA was extracted from the *Pectoralis major* and liver tissues using the PrimeWay Total RNA Extraction Kit (JTC MedTech Hub, Singapore) following the manufacturer's protocol (qPCR master mix composition for gene

Table 1
Nutritional composition of commercial broiler starter and finisher diets.

Composition (%)	Starter (%)	Finisher (%)
Crude protein	21.0	19.30
Crude fibre	5.00	5.00
Crude fat	4.50	5.00
Moisture	13.0	13.00
Ash	8.00	8.00
Calcium	0.80	0.80
Phosphorous	0.40	0.40

Table 2
qPCR master mix composition for telomere length analysis.

Reagents Concentration	Volume per 20 μL Reaction	Final Concentration
SYBR green, 2x	10 μL	1x
Telomere Primer F, 2 μM	2 μL	0.1 μM
Telomere primer R, 2μM	2 μL	0.1 μM
Nuclease-free H_20 -	4.5 μL	-
DNA template, 20 ng/μL	1.5 μL	20 ng

Note: Final reaction volume: 20-μL reaction. Cycling conditions: 95°C for 10 min; 40 cycles of 95°C for 15 sec, 60°C for 1 minute.

expression analysis listed in Table 3). *HSP70* and *IGF-1* primers (Table 4) were adopted from Nawaz et al. (2023). Extracted RNA was immediately stored at −80°C. cDNA was synthesized from the RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific™, US) following the manufacturer's protocol and stored at −20°C until further analysis.

q-PCR for the Determination of Gene Expression (HSP70 and IGF-1). Each 20 μL qPCR reaction contained a cDNA template, gene-specific primers, and SYBR Green qPCR mix (Fisher Scientific, UK) as detailed in Table 4. The qPCR protocol included an initial denaturation at 94°C for 3 minutes, followed by cycles of 94°C for 10 seconds and 58°C for 30 seconds. *GAPDH* was used as a reference gene for normalization due to its established stability under HS.

Statistical Analyses. Growth performance metrics, relative carcass traits, and TL were analyzed using a general linear model. Mortality data were evaluated using a chi-square test in R software (version 4.2.1). The relative TL was calculated as the T/S ratio using the comparative ΔCt method: 2ˆ-(Ct_telomere - Ct_reference). Gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method for visualization. ΔCt values were analyzed by two-way ANOVA to assess the effects of breed and environmental conditions (HS vs NT). Tukey's HSD test was applied for multiple comparisons among breed, while paired t-tests was used to evaluate within-breed differences between the NT and HS groups. Effect sizes were reported using Cohen's d.

Results

Growth performance and mortality

The results of the growth performance and mortality are presented in Table 5. Throughout the HS period, no significant change was observed in any of the measured parameters between the HS and NT groups in SAGA chickens. In Sasso chickens, a significant difference in BWG was observed after 2 weeks of heat stress exposure (weeks 4-6 of age). HS significantly ($P < 0.05$) impaired all measured parameters in Cobb 500 chickens. Specifically, Cobb 500 chickens exposed to HS exhibited a 12.4 % reduction in feed intake (1,061.5 g vs. 1,212.0 g) and 13.8 % decrease in body weight (1,288.9 g vs. 1,496.6 g) compared to those reared under NT conditions. Additionally, Cobb 500 chickens under HS showed a higher mortality rate (11.66 % vs. 3.31 %), indicating increased systemic stress. Table 6 presents the interaction effects. The breeds factor had highly significant effects ($P < 0.001$) on all measured parameters across the evaluated periods (weeks 2-4, weeks 4-6, and overall), except for FCR at week 4-6 of age.

Table 3
qPCR master mix composition for gene expression analysis (HSP70 and IGF-1).

Reagents Stock Concentration	Volume per 20 μL Reaction	Final concentration Purpose
SYBR green Master Mix 2x	10 μL	1x Fluorescent DNA detection
Forward Primer (*HSP70/IGF-1*) 2 μM	0.8 μL	0.08 μM Target gene amplification
Reverse Primer (*HSP70/IGF-1*) 2 μM	0.8 μL	0.08 μM Target gene amplification
Nuclease-free H_20 -	6.4 μL	- Adjust final volume
cDNA Template 20 ng/μL	2 μL	2 μL Sample DNA

Note: Primer sequences and annealing temperatures are listed in Table 4. Cycling conditions: 94°C for 3 min; 40 cycles of 94°C for 10 sec, 58°C for 30 sec. GAPDH was used as the reference gene for normalization.

Relative Carcass Yields. The result of relative carcass yields is presented in Table 7. The results revealed that HS significantly ($P < 0.05$) increased wing weight (WW; 7.24 g vs. 5.74 g) and shank weight (SW; 5.10 g vs. 4.69 g) compared to NT group for SAGA chicken. In Sasso chicken, HS significantly ($P < 0.05$) lowered carcass weight (CW; 66.73 g vs 74.10 g), drumstick weight (DS; 8.09 g vs 9.49 g), but increased in WW (7.94 g vs 7.18 g). In Cobb 500, HS significantly ($P < 0.05$) lowered carcass weight (CW; 77.94 g vs 99.73 g), breast meat (BM; 25.13 g vs 27.75 g), thigh weight (TW; 9.91 g vs 11.40 g), and drumstick (DS; 7.31 g vs 9.59 g). Table 8 summarizes the effects of interaction on relative carcass yields. Significant ($P < 0.05$) interactions were observed for CW, DS, WW, and SW.

Effect of Heat Stress on the Relative Internal Organ Yields. Table 9 depicts the mean (±SE) weights of various internal organs: liver (LV), heart (HT), kidney (KD), adipose tissue (AT), intestines (IN), and spleen (SP). In SAGA chicken, HS significantly increased ($P < 0.05$) the weights of liver, heart, kidney, and intestine compared to controls. For Sasso chicken, HS resulted in a significant decrease ($P < 0.05$) only in the spleen (SP), and gizzard (GD). Cobb 500 chickens also showed a significant reduction ($P < 0.05$) in both liver weight (LV), kidney weight, and SP. Table 10 presents the interaction effects between breeds and temperature on relative internal organ weights due to HS. Breeds show a significant ($P < 0.05$) difference in all parameters evaluated except heart samples. Significant interaction ($P < 0.05$) effects were observed for heart and intestine. There is spleen atrophy in Cobb 500 chickens (0.06 g vs. 0.08 g).

Telomere Length as a Biomarker of Heat Stress and Aging. Table 11 delineates the effects of HS on relative TL in SAGA, Sasso, and Cobb 500 chicken breeds at weeks 2 and 4, with TL quantified in kilobases (kb) per diploid chicken genome. HS and NT groups differed significantly in TL across all breeds. (SAGA, Sasso, Cobb 500) and at both points (weeks 2 and 4). Statistically significant variations in TL were also noted among the breeds at week 2 and week 4 ($P < 0.05$), indicating that genetic differences between the breeds play a role in influencing TL under HS.

Table 12 depicts the effects of breed, temperature, and their interaction on relative TL (measured in kilobases per diploid chicken genome) in SAGA, Sasso, and Cobb 500 chickens at weeks 2 and 4. Significant inter-breed variation in TL was detected at week 2 by breed ($P < 0.01$) and both by breed ($P < 0.01$) and temperature ($P < 0.001$) at week 4. However, the interaction between breed and temperature was not significant at either time point. Faster TL shortening in Cobb chickens is likely associated with higher oxidative stress observed in fast-growing breeds. In contrast, SAGA chickens showed no significant change in TL during 2-4 weeks of HS exposure.

Gene Expression Profiles: HSP70 and IGF-1 Dynamics. Tables 13 and 14 depict the differential expression patterns of the molecular chaperone *HSP70* under chronic HS conditions in three chicken breeds with distinct growth rates. Increased *HSP70* expression, a marker of cellular stress response, was detected in both liver and muscle tissues across all breeds, confirming the activation of cellular stress mechanisms due to HS. Cobb 500 chicken, which exhibits rapid growth rates, showed the most significant upregulation of *HSP70*, particularly in liver tissue. In contrast, SAGA chickens demonstrated the lowest levels of *HSP70* induction. These breed-specific differences in *HSP70* expression are likely to have arose from the genetic and metabolic adaptations to thermal stress. The significant upregulation of *HSP70* in Cobb 500 and Sasso chickens under HS conditions likely signifies heightened cellular stress and protein damage, potentially attributable to their elevated metabolic rate. Conversely, the comparatively lower *HSP70* expression in SAGA chickens may suggest an intrinsically enhanced basal thermotolerance.

Tukey's HSD test confirmed breed-specific differences in *HSP70*

Table 4
Primer sequences, annealing temperatures, and amplicon sizes for qPCR Analysis of TL, *HSP70, IGF-1,* and GAPDH.

Gene	Primer Sequence (5′→3′)	Annealing Temp (°C)	Amplicon Size (bp)	Gene ID
TL	5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′ F 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′R	60	79	N/A
GAPDH	5′-ACTATGCGGTTCCCAGTGTC −3′ F 5′-TGCCACCATCAGAAAAATGA −3′ R	60	215	374193
HSP70	5′-GCGCCAGGCCACCAAAGATG −3′ F 5′-GCCCCCTCCCAAGTCAAAGATG-3′R	58	58	423504
IGF-1	5′-ACCTTGGCCTGTGTTTGCTTAC-3′ F 5′-GAGGGTAGTGAAGGCTGCTG-3′ R	58	111	418090
GAPDH	5′-GAGGGTAGTGAAGGCTGCTG −3′ F 5′-CATCAAAGGTGGAGGAATGG −3′ R	58	58	374193

Note: Primers for TL and reference gene (*GAPDH*) were adapted from Cawthon (2002) and NCBI (NC_006101.5), respectively. Gene-specific primers (**HSP70, IGF-1*) follow Nawaz et al. (2023).

Table 5
Effects of heat stress (HS) on growth performance metrics (feed intake, body weight, body weight gain, feed conversion ratio, and mortality) in SAGA, Sasso, and Cobb 500 chickens.

Parameters	Variables						p-values		
	SAGA		Sasso		Cobb 500				
	HS	NT	HS	NT	HS	NT	SG	SS	CB
FI (g/bird)									
Week 2 to 4	299.50±14.84	283.20±5.03	438.00± 29.87	456.40±29.43	1061.50±39.3	1212.00±40.64	0.74	0.21	0.00***
Week 4 to 6	474.37+35.24	447.6 + 43.20	763.75+51.53	788.00+71.80	1698.2 + 29.10	1820.8 + 15.80	0.08	0.36	0.00***
BW (g/bird)									
Week 2 to 4	495.50±32.58	508.30±34.70	727.50±52.48	702.80± 47.26	1288.90±76.50	1496.60±67.30	0.79	0.73	0.01*
Week 4 to 6	972.50+12.95	981.30+8.79	1498.65+81.50	1563.75+82.54	2173.60+37.20	2444.00+ 39.30	0.57	0.16	0.04*
BWG (g/bird)									
Week 2 to 4	169.10±8.69	183.60±9.13	276.30±13.37	256.10±13.66	526.50±14.50	736.30±31.60	0.49	0.30	0.01*
Week 4 to 6	213.75±15.63	205.3 ± 13.90	324.87±20.10	382.37±24.31	899.30±45.50	1073.05±41.30	0.69	0.05*	0.01*
FCR (g/g)									
Week 2 to 4	1.77±0.57	1.54±0.08	1.58±0.07	1.78± 0.13	2.01± 0.09	1.64±0.04	0.40	0.21	0.01*
Week 4 to 6	2.21+ 0.20	2.18+ 0.37	2.35+ 0.30	2.06+ 0.18	1.88+ 0.20	1.69+ 0.07	0.89	0.34	0.05*
Mortality									
Week 2 to 4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00± 0.13	1.00± 0.01	NA	NA	0.03*
Week 4 to 6	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	7.60± 0.13	2.1 ± 0.10	NA	NA	0.01*
Overall (2-6)									
FI (g/bird)	773.80± 18.69	730.84±40.10	1201.75±51	1244.43±52.8	2756.60±43.70	3032.80±63.23	0.48	0.76	0.05*
BW (g/bird)	1468.00±18.60	1489.6 ± 18.20	2226.15±31.50	2266.55±41.52	3462.50±69.10	3940.60±66.80	0.89	0.88	0.05*
BWG (g/bird)	382.75±11.99	388.94±12.10	601.17±20.17	638.3 ± 24.31	1425.80±38.50	1809.35±50.20	0.84	0.40	0.01*
FCR (g/g)	2.02± 0.42	1.87± 0.27	1.99± 0.30	1.98± 0.18	1.93± 0.18	1.67± 0.07	0.54	0.64	0.01*
Mortality	0.00± 0.00	0.00± 0.00	0.00±0.00	0.00±0.00	11.66±0.01	3.31± 0.01	NA	NA	0.05*

Note: FI, feed intake; BW, body weight; BWG, body weight gain; FCR, feed conversion ratio. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

upregulation under heat stress ($P < 0.05$), with Cobb 500 exhibiting the highest expression, followed by Sasso and SAGA (all groups distinct; Cohen's d indicated large effects, strongest in Cobb 500) as summarized in Table 17.

Tables 15 and 16 illustrate the differential response of the growth-related hormone *IGF-1* following a two-week exposure to HS. Cobb 500 chickens displayed a significant increase in *IGF-1* levels in both muscle and liver tissues, suggesting a continued effort to sustain growth under HS. Sasso chickens showed a moderate rise in *IGF-1*, limited to muscle tissue, while the SAGA breed exhibited no significant changes in *IGF-1* levels, potentially indicating a prioritization of stress adaptation overgrowth. *HSP70* expression is markedly upregulated in Cobb 500 chickens, while *IGF-1* expression in muscle remains sustained.

Breed-specific responses were observed: only Cobb 500 showed significant hepatic *IGF-1* upregulation ($P < 0.05$; large effect size), while SAGA and Sasso remained unchanged. In muscle, expression followed Cobb 500 > Sasso > SAGA, with Cohen's d supporting robust inter-breed differences (Table 17).

Discussion

Growth performance

In this research, SAGA chickens showed consistent growth performance HS conditions, unlike Cobb 500 chickens. This finding is consistent with previous research on African Indigenous chickens, which demonstrated that slower-growing breeds exhibit greater resilience to HS than their faster-growing counterparts (Gheyas et al., 2021). The relative resilience of SAGA, the moderate impact on Sasso, and the pronounced negative effects on Cobb 500 underscore the phenotypic and physiological differences between these breeds. These differences are likely rooted in their genetic backgrounds, metabolic rates, and evolutionary adaptations to environmental stressors.

HS markedly impairs growth performance in chickens by reducing feed intake, disrupting digestive processes, and diminishing nutrient absorption (Ahmad et al., 2022). These effects are particularly pronounced in Cobb 500 breed, which exhibited a substantial decrease in feed intake under HS conditions compared to normal temperature (NT) conditions. This reduction in feed intake is a survival mechanism to limit metabolic heat production, but it compromises growth performance, as evidenced by slower weight gain and lower feed conversion efficiency (Abdel-Moneim et al., 2021). In contrast, slow-medium chickens

Table 6
Interaction effects between breed and temperature on growth performance metrics in chickens.

Parameters	Variables					P-values		
	Breeds			Temperature				
	SG	SS	CB	HS	NT	Breeds	Temp	Breeds*Temp
FI (g/bird)								
Week 2 to 4	516.30± 6.21	796.98+ 5.51	589.40 ± 5.48	589.72 ± 8.46	618.73 ± 3.19	0.00***	0.21	0.74
Week 4 to 6	641.56± 5.52	1299.60±14.10	681.31 ± 17.67	691.80 ± 8.79	656.51 ± 13.13	0.00***	0.36	0.08
BW(g/bird)								
Week 2 to 4	1409.40±08.92	1964.90 ± 4.48	1586.50± 4.48	1625.36 ± 9.83	1702.9 0 ± 16.03	0.00***	0.21	0.68
Week 4 to 6	1935.64±24.77	2863.69 ± 7.35	2261.16± 5.13	2384.81± 21.45	2455.52 ± 13.90	0.00***	0.08	0.11
BWG (g/bird)								
Week 2 to 4	335.64± 6.20	663.69 ± 7.22	576.16 ± 6.11	384.81 ± 8.58	455.52 ± 5.20	0.00***	0.08	0.11
Week 4 to 6	486.45± 8.32	1007.01 ± 6.25	645.91 ± 6.43	411.33± 3.25	553.92 ± 6.99	0.00***	0.13	0.60
FCR (g/g)								
Week 2 to 4	1.53 ± 0.17	1.20± 0.07	1.23± 0.15	1.53 ± 0.18	1.34 ± 0.19	0.00***	0.53	0.91
Week 4 to 6	1.31± 0.25	1.29± 0.43	1.05± 0.28	1.67 ± 0.31	1.18 ± 0.34	0.45	0.17	0.64
Mortality								
Week 2 to 4	0.00± 00	0.15 ± 0.11	0.00± 0.00	0.1 ± 0.09	0.00 ± 00	0.02*	0.04*	0.02*
Week 4 to 6	0.0 ± 0.00	0.50 ± 0.22	0.00± 0.00	0.26 ± 0.18	0.06 ± 0.08	0.00***	0.03*	0.01*
Overall (2-6)								
FI (g/bird)	1,157.86± 6.75	2,096.58±27.60	1,270.71± 16.20	1,281.52±24.74	1,274.24± 8.58	0.00***	0.89	0.09
BW(g/bird)	3,345.04±27.68	4,828.59±30.80	3,847.66±27.39	4,010.17±17.41	4,158.42±28.9	0.00***	0.36	0.80
BWG (g/bird)	822.09± 20.37	1,670.70± 17.60	1,222.07± 10.40	796.14± 12.03	1,009.44±21.3	0.00***	0.90	0.98
FCR (g/g)	1.40 ± 0.21	1.25± 0.35	1.30± 0.27	1.60± 0.26	1.26± 0.31	0.04*	0.36	0.78
Mortality	0.00 ± 0.00	0.65± 0.18	0.00± 0.00	0.34± 0.15	0.06 ± 0.06	0.00***	0.00*	0.00**

Note: Breed*Temp, interaction between breed and temperature. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

Table 7
Relative carcass yields (%) of SAGA, Sasso, and Cobb 500 chickens under HS and NT conditions.

Parameters	Variables						P-values		
	SAGA		Sasso		Cobb 500				
	HS	NT	HS	NT	HS	NT	SG	SS	CB
CW (g)	70.49±1.30	67.65±1.40	66.73±3.27	74.10±1.16	77.94±2.09	99.73±0.66	0.15	0.04*	0.04*
BM (g)	12.69±0.54	15.34±1.27	15.55±0.78	15.79±0.50	25.13±1.21	27.75±1.27	0.07	0.79	0.03*
BC (g)	23.52±0.72	25.53±0.51	22.35±1.83	18.27±1.22	21.73±0.84	21.99±0.69	0.03	0.08	0.81
TW (g)	9.72±1.30	8.90±0.17	9.81±0.47	10.40±0.30	9.91±1.29	11.40±0.27	0.08	0.31	0.02*
DS (g)	9.46±0.34	8.61±0.29	8.09±0.40	9.49±0.46	7.31±0.46	9.59±0.08	0.07	0.03*	0.03*
WW (g)	7.24±0.89	5.74±0.60	7.94±0.33	7.18±0.20	5.88±0.38	5.89±0.17	0.03*	0.05*	0.96
SW (g)	5.10±0.17	4.69±0.07	4.57±0.22	4.78±0.10	3.67±0.16	4.53±0.07	0.04*	0.41	0.04*
AF (g)	1.06±0.40	1.02±0.32	1.10±0.05	1.50±0.02	2.16±0.09	2.05±0.07	0.10	0.05*	0.34

Note: CW, carcass weight; BM, breast muscle; BC, back cut; TW, thigh weight; DS, drumstick; WW, wing weight; SW, shank weight; AF, abdominal fat. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

Table 8
Effect of interaction between breed and temperature on relative carcass yields in chickens.

Parameters	Variables					P-values		
	Breeds			Temperature				
	SG	SS	CB	HS	NT	Breeds	Temp	Breeds*Temp
CW (g)	69.07±1.39	70.41±2.67	78.83±1.54	73.35±1.88	72.19±2.78	0.00***	0.55	0.017*
BM (g)	14.02±1.04	15.67±0.64	25.44±1.21	18.20±0.54	18.542±1.01	0.00**	0.83	0.09
BC (g)	20.31±0.69	21.86±1.65	24.52±0.75	22.69±1.18	21.78±1.29	0.00**	0.37	0.07
TW (g)	9.31±0.33	10.11±0.40	10.65±0.94	10.40±0.81	9.64±0.36	0.10	0.14	0.14
DS (g)	9.04±0.75	8.79±0.30	9.45±0.55	9.08±0.42	9.10±0.46	0.23	0.94	0.00**
WW (g)	6.49±0.32	7.56±0.45	5.88±0.23	6.78±0.43	6.51±0.54	0.00***	0.44	0.01*
SW (g)	4.90±0.43	4.67±0.21	3.85±0.24	4.51±0.12	4.44±0.23	0.00***	0.67	0.03*
AF (g)	1.14±0.08	2.11±0.09	1.16 ± 0.06	1.48±0.09	1.46±0.10	0.00***	0.87	0.11

Note: Breed*Temp, interaction term. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

maintained similar weight and growth performance regardless of temperature conditions, highlighting their greater resilience to HS. This aligns with previous studies showing that slower-growing breeds are less affected by HS due to their lower metabolic rates and better thermoregulatory ability (Kpodo et al., 2020; Fathi and Mardani, 2024).

HS diverts energy toward thermoregulation, further impairing growth in Cobb 500 breed. Chickens expend more energy to regulate their body temperature in hot environments, diverting resources away from growth and other essential physiological functions (Nawab et al., 2018). This energy reallocation is particularly detrimental to Cobb 500

Table 9
Relative internal organ weights of SAGA, Sasso, and Cobb 500 chickens exposed to HS and NT.

Parameters	Variables						P-values		
	SAGA		Sasso		Cobb 500				
	HS	NT	HS	NT	HS	NT	SG	SS	CB
LV (g)	1.83± 0.06	1.56± 0.03	1.79±0.06	1.77 ± 0.04	2.44±0.14	1.88±0.03	0.00**	0.78	0.00**
HT (g)	0.55± 0.02	0.48± 0.01	0.49±0.03	0.47±0.02	0.46±0.03	0.49±0.02	0.00**	0.57	0.30
KD (g)	0.80± 0.01	0.05± 0.00	0.03±0.00	0.04±0.00	0.03±0.00	0.02±0.00	0.00**	0.11	0.03*
IN (g)	3.43± 0.10	2.77±0.07	3.51±0.17	3.47±0.12	2.79±0.16	2.86±0.08	0.04*	0.85	0.72
SP (g)	0.16± 0.01	0.14± 0.01	0.14±0.01	0.17±0.02	0.06±0.00	0.08±0.00	0.06	0.01**	0.01*
GD (g)	2.47± 0.28	2.28± 0.16	1.21±0.07	1.91±0.09	1.72±0.24	1.89±0.08	0.57	0.00***	0.50

Note: LV, liver weight; HT, heart weight; KD, kidney weight; IN, intestine weight; SP, spleen weight; GD, gizzard weight. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

Table 10
Interaction effects between breed and temperature on relative internal organ weights in chickens.

Parameters	Variables					P-values		
	Breeds			Temperature				
	SG	SS	CB	HS	NT	Breeds	Temp	Breeds*Temp
LV (g)	1.69±0.60	1.78±0.15	2.16±0.14	1.86±0.10	1.90±0.12	0.00***	0.60	0.24
HT (g)	0.32±0.04	0.28±0.04	0.28±0.02	0.29±0.02	0.29±0.03	0.12	0.91	0.00**
KD (g)	0.27±0.01	0.22±0.00	0.23±0.20	0.24±0.01	0.24±0.03	0.00***	0.60	0.49
IN (g)	3.10±0.90	3.49±0.12	2.83±0.89	3.19±0.12	3.09±0.13	0.00***	0.42	0.02*
SP (g)	0.35±0.12	0.26±0.86	0.27±0.54	0.33±0.98	0.33±0.16	0.00***	0.91	0.71
GD (g)	2.37±0.23	1.56±0.16	1.80±0.18	1.83±0.22	2.00±0.21	0.00***	0.34	0.58

Note: Breed*Temp, interaction term. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

Table 11
Relative telomere length (RTL) in (kb/genome) in SAGA, Sasso, and Cobb 500 chickens at weeks 2 and 4 of heat stress exposure.

parameters	Variables						P-values		
	SAGA		Sasso		Cobb 500				
RTL (T/S Ratio)	HS	NT	HS	NT	HS	NT	SG	SS	CB
Week 2	337.80±38.50	413.52±47.40	293.10±27.62	487.80±33.21	309.88±39.41	549.1 ± 47.40	0.04*	0.04*	0.02*
Week 4	339.68±36.71	426.83±39.73	265.30±23.54	350.97±28.53	250.44±34.27	435.45±41.53	0.05*	0.01*	0.03*

Note: RTL; Relative telomere length, T/S ratio represents relative telomere length. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

Table 12
Effect of interaction between breed and temperature on (RTL) (Kb/Genome) in chickens at weeks 2 and 4.

Parameters	Variables					P-values		
	Breeds			Temperature				
RTL (T/S Ratio)	SG	SS	CB	HS	NT	Breeds	Temp	Breeds*Temp
Week 2	375.63±38.53	390.11±42.71	429.48±41.46	313.60±32.10	483.20±39.23	0.74	0.00**	0.51
Week 4	433.20±42.55	258.14±28.56	342.91±31.33	251.81±20.50	437.75±43.41	0.00**	0.00***	0.99

Note: RTL; Relative telomere length, Breed*Temp, interaction term. Data are presented as mean ± SE. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. HS, heat stress; NT, normal temperature; SG, SAGA; SS, Sasso; CB, Cobb 500.

chickens, which require a high energy intake to sustain their rapid growth. The significant decline in growth metrics and increased mortality is observed in Cobb 500 chickens under HS conditions are consistent with findings from previous studies (Awad et al., 2020). These results highlight the vulnerability of Cobb 500 breeds to HS and underscore the importance of breed-specific management strategies in poultry production.

Breed-specific responses to HS, the differential responses of slow-fast chickens to HS can be attributed to their distinct growth rates and genetic backgrounds. SAGA chicken, which are SAGA and have been selectively bred in hot climates, exhibit minimal changes in growth metrics under HS. This resilience is likely due to their lower metabolic rates, reduced oxidative stress due to the lower metabolic rate, leading to less heat production and fewer reactive oxygen species (ROS) from mitochondria, and efficient thermoregulatory mechanisms (Awad et al., 2020). Similarly, Sasso chicken, which is medium-growing, shows moderate resilience to HS, with no significant reduction in final weight or growth performance. In contrast, Cobb 500 chickens, which are Cobb 500 and bred for high productivity, are more susceptible to HS due to their higher metabolic demands (Cartoni et al., 2023).

Table 6 shows biologically relevant numerical differences in key performance metrics between heat-stressed (HS) and thermoneutral (NT) groups, despite these differences not reaching statistical significance. Notably, HS exposure was associated with an 18 % reduction in

Table 13
Mean fold-change of *HSP70* gene expression in chicken liver under HS versus NT Conditions at weeks 2 and 4 of HS exposure.

Breed	Week	Condition	Fold-Change	P-value (NT vs. HS)	Cohen's d	Tukey's Group (HS)
SG	2	BL (NT)	0.99	0.04*	0.80	-
SG	2	HS	1.60			†
SG	4	BL (NT)	1.00	0.01**	1.20	-
SG	4	HS	1.90			†
SS	2	BL (NT)	1.00	0.01**	1.50	-
SS	2	HS	2.00			‡
SS	4	BL (NT)	0.98	0.001***	2.00	-
SS	4	HS	2.40			‡
CB	2	BL (NT)	1.00	0.001***	2.80	-
CB	2	HS	3.00			§
CB	4	BL (NT)	1.00	0.001***	3.20	-
CB	4	HS	3.50			§

Note: Fold-changes are relative to NT controls (baseline = 1.0). ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. BL, Baseline; NT, normal temperature; HS, heat stress; SG, SAGA; SS, Sasso; CB, Cobb 500. Cohen's d values indicate effect size (small = 0.2, moderate = 0.5, large = 0.8+). Groups with the same symbol (†, ‡, §, and #) do not differ significantly based on Tukey's HSD post-hoc test ($P < 0.05$) among breeds under HS.

Table 14
Mean fold-change of *HSP70* gene expression in chicken liver under HS versus NT conditions at weeks 4 and 6 of HS exposure.

Breed	Week	Condition	Fold-Change	P-value (NT vs. HS)	Cohen's d	Tukey's Group (HS)
SG	2	BL (NT)	1.00	-0.03*	0.70	-
SG	2	HS	1.55			†
SG	4	BL (NT)	0.97	-0.01**	-1.00	-
SG	4	HS	1.75			†
SS	2	BL (NT)	1.00	-0.01**	-1.30	-
SS	2	HS	1.85			‡
SS	4	BL (NT)	0.96	-0.001***	-1.80	-
SS	4	HS	2.30			‡
CB	2	BL (NT)	1.00	-0.001***	-2.50	-
CB	2	HS	2.75			§
CB	4	BL (NT)	1.00	-0.001***	-3.00	-
CB	4	HS	3.20			§

Note: Fold-changes are relative to NT controls (baseline = 1.0). ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. BL, Baseline; NT, normal temperature; HS, heat stress; SG, SAGA; SS, Sasso; CB, Cobb 500. Cohen's d values indicate effect size (small = 0.2, moderate = 0.5, large = 0.8+). +). Groups with the same symbol (†, ‡, §, and #) do not differ significantly based on Tukey's HSD post-hoc test ($P < 0.05$) among breeds under HS.

both feed intake (FI; Weeks 4-6) and body weight gain (BWG; Weeks 2-4) (P = 0.087). These trends align with established HS physiology, where metabolic trade-offs prioritize thermoregulation overgrowth, even without formal statistical significance. Such deviations may still affect production efficiency and therefore warrant consideration in poultry management strategies.

Significant disparities in body weight gain (BWG) among chicken breeds during weeks 4-6, following two weeks of HS exposure–highlight inherent differences in thermal adaptability and metabolic efficiency across genetic lines. Cobb 500 chickens, characterized by their rapid-growth phenotype, experienced a marked reduction in BWG under HS. This observation concurs with previous studies reporting compromised feed intake and inefficient thermoregulation in hot environments for this breed (Lin et al., 2006; Nawab et al., 2018).

Conversely, SAGA chicken, an indigenous breed, maintained relatively stable BWG despite HS. This suggests breed-specific resilience, potentially attributable to lower basal metabolic rates, enhanced mitochondrial efficiency, and reduced muscle mass. These physiological traits collectively reduce metabolic heat production and oxidative stress

Table 15
Mean fold-change of *IGF-1* gene expression in chicken liver under HS versus NT conditions at weeks 2 and 4 of HS exposure.

Breed	Week	Condition	Fold-Change	P-value (NT vs. HS)	Cohen's d	Tukey's Group (HS)
SG	2	BL (NT)	1.00	-0.15	-0.10	-†
SG	2	HS	0.95			
SG	4	BL (NT)	1.00	-0.20	-0.10	-
SG	4	HS	0.95			†
SS	2	BL (NT)	1.00	-0.50	-0.00	-
SS	2	HS	1.00			†
SS	4	BL (NT)	1.00	-0.10	-0.20	-
SS	4	HS	1.10			†
CB	2	BL (NT)	1.00	-0.01**	-1.50	-
CB	2	HS	1.80			‡
CB	4	BL (NT)	1.00	-0.001***	-2.20	-
CB	4	HS	2.30			§

Note: Fold-changes are relative to NT controls (baseline = 1.0). ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. BL, Baseline; NT, normal temperature; HS, heat stress; SG, SAGA; SS, Sasso; CB, Cobb 500. Cohen's d values indicate effect size (small = 0.2, moderate = 0.5, large = 0.8+). +). Groups with the same symbol (†, ‡, §, and #) do not differ significantly based on Tukey's HSD post-hoc test ($P < 0.05$) among breeds under HS.

Table 16
Mean fold-change of *IGF-1* gene expression in chicken liver under HS versus NT conditions at weeks 4 and 6 of HS exposure.

Breed	Week	Condition	Fold-Change	P-value (NT vs. HS)	Cohen's d	Tukey's Group (HS)
SG	2	BL (NT)	1.00	0.40	0.00	-
SG	2	HS	0.98			†
SG	4	BL (NT)	1.00	0.30	0.10	-
SG	4	HS	1.05			†
SS	2	BL (NT)	1.00	0.04*	0.40	-
SS	2	HS	1.20			‡
SS	4	BL (NT)	1.00	0.01**	0.90	-
SS	4	HS	1.45			‡
CB	2	BL (NT)	1.00	0.001***	1.80	-
CB	2	HS	2.00			§
CB	4	BL (NT)	1.00	0.001***	2.50	-
CB	4	HS	2.60			#

Note: Fold-changes are relative to NT controls (baseline = 1.0). ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant. BL, Baseline; NT, normal temperature; HS, heat stress; SG, SAGA; SS, Sasso; CB, Cobb 500. Cohen's d values indicate effect size (small = 0.2, moderate = 0.5, large = 0.8+). +). Groups with the same symbol (†, ‡, §, and #) do not differ significantly based on Tukey's HSD post-hoc test ($P < 0.05$) among breeds under HS.

Table 17
Summary table: *HSP70* and *IGF-1* expression analysis.

Table	Gene	Tissue	Breed Effect (Tukey)	Effect Size (Cohen's d)	Main Insight
13	*HSP70*	Liver	SG < SS < CB (†<‡<§)	SG: 0.8-1.2, CB: 3.2	Strong HS induction in CB
14	*HSP70*	Muscle	SG < SS < CB (†<‡<§)	SG: 0.7-1.0, CB: 3.0	Mirrored pattern in muscle
15	*IGF-1*	Liver	SG=SS < CB (†<‡/§)	SG: 0.1, CB: 2.2	Only CB showed upregulation
16	*IGF-1*	Muscle	SG < SS < CB (†<‡<§)	SG: 0.0, CB: 2.5	Strongest in the CB muscle

Note: SG, SAGA; SS, Sasso; CB, Cobb 500. Groups with the same symbol (†, ‡, §, and #) do not differ significantly based on Tukey's HSD post-hoc test ($P < 0.05$) among breeds under HS.

(Yahav, 2009). The superior thermotolerance of indigenous breed like SAGA chickens has been supported by studies demonstrating their ability to sustain productivity under chronic thermal stress through

adaptive behavioural and physiological mechanisms (Mignon-Grasteau et al., 2015; Tona et al., 2022).

Sasso chicken, representing a medium-growth hybrid, exhibited an intermediate response. Although BWG was reduced under HS, the decline was less severe than in Cobb 500 chickens. This finding aligns with reports suggesting that moderate-growth broilers retain partial resilience due to optimal balance between growth performance and heat tolerance (Franco-Jimenez & Beck, 2007).

The marked decline in feed intake and growth metrics in Cobb 500 chickens under HS conditions is consistent with the findings of Lin et al. (2006), who reported that heat-stressed broilers experience a substantial decline in feed intake, leading to decreased weight gain. This reduction in feed intake is compounded by the compromised integrity of the gastrointestinal tract and reduced activity of digestive enzymes, which further impair nutrient absorption (Sohail et al., 2012). Additionally, the increased energy expenditure associated with thermoregulation diverts resources away from growth, exacerbating the negative effects of HS on Cobb 500 breeds (Mujahid et al., 2007).

HS also induces a cascade of physiological and metabolic disruptions that impair growth performance in chickens. The suppression of growth-related hormones, such as *IGF-1* and thyroid hormones, further exacerbates the growth impairment in heat-stressed chickens (Shehata et al., 2022). *IGF-1* plays a critical role in muscle development, and its reduction under HS conditions contributes significantly to stunted growth.

HS significantly elevates mortality rates in chickens, particularly in Cobb 500 breeds like Cobb 500. The physiological strain of thermoregulation and immune suppression creates a synergistic effect that increases susceptibility to mortality (Wlaźlak et al., 2023). The gastrointestinal (GI) tract is profoundly affected by HS, with increased gut permeability allowing pathogenic bacteria and endotoxins to enter the bloodstream, inciting systemic inflammation and further compromising health (Basiouni et al., 2023). This "leaky gut" phenomenon impairs nutrient absorption and exacerbates energy deficits, diminishing the chicken's overall physiological resilience. However, the cardiovascular system is also strained under HS conditions, with increased heart rate and cardiac output potentially leading to cardiovascular collapse in severe cases (Wideman, 2000). These disruptions, along with hyperthermia-induced cellular damage, increase mortality in heat-stressed chickens (Apalowo et al., 2024). The extent of HS-induced mortality is influenced by factors such as the intensity and duration of heat exposure, the age and breed of the chickens, and the overall health status of the flock (Lara and Rostagno, 2013).

HS significantly impacted the growth performance and mortality of Cobb 500 chickens, whereas SAGA chickens demonstrated notable resilience. By Week 4, Cobb 500 birds exposed to HS showed significant reductions in feed intake (FI), body weight (BW), and body weight gain (BWG) ($P < 0.05$; Table 5). Specifically, FI decreased by 12.4 % (1,061.5 g under HS vs. 1,212.0 g in the NT group), reflecting the metabolic trade-off in which energy is redirected from production towards thermoregulation. The 13.8 % reduction in BW observed in HS-exposed Cobb 500 chickens (1,288.9 g vs. 1,496.6 g) suggests impaired nutrient utilization, likely caused by HS-induced gut dysfunction, as supported previously (Abdel-Moneim et al., 2021). Moreover, mortality rates were markedly higher in Cobb 500 chickens under HS (11.66 % vs. 3.31 % in NT), indicating systemic stress, potentially resulting from oxidative damage and cardiovascular strain (Wideman, 2000). In contrast, SAGA chickens maintained stable in FI and BW, consistent with their inherently lower metabolic rates and possible evolutionary adaptations–such as enhanced *HSP70* efficiency, which mitigate the detrimental impacts of HS (Gheyas et al., 2021).

Significant breed-by-temperature interaction effects were observed for mortality ($P < 0.05$; Table 6), highlighting clear genetic differences in susceptibility to HS. The pronounced increase in mortality among Cobb 500 chickens between Weeks 2 and 4 emphasized their heightened vulnerability relative to the more resilient Sasso/SAGA strains.

Relative Carcass Yields. SAGA breed, with their lower metabolic demands and efficient thermoregulation, exhibit greater tolerance to HS. Sasso breeds display intermediate susceptibility, while Cobb 500 breed suffers the most severe impacts.

HS, a pervasive environmental challenge in contemporary poultry production, disrupts physiological homeostasis, leading to a cascade of adverse effects on growth performance, metabolic processes, and ultimately, carcass quality (Quinteiro-Filho et al., 2012).The primary mechanisms driving these effects include reduced feed intake, impaired nutrient absorption, altered energy partitioning (Sohail et al., 2012). These physiological perturbations directly translate into reduced protein deposition, altered muscle fiber composition, and diminished carcass yields.

SAGA demonstrate enhanced resilience to HS, which is attributed to their genetic adaptation to diverse environmental stressors, lower metabolic rates, and efficient thermoregulatory mechanisms (Mutibvu et al., 2017). The observed increase in carcass and breast muscle weight in SAGA chickens under HS conditions, likely due to maintained feed intake and reduced metabolic heat production, aligns with previous findings demonstrating the superior thermotolerance of indigenous chicken breeds (Teyssier et al., 2022). Furthermore, the predominance of slow-twitch muscle fibers in the drumsticks of these breeds, known for their oxidative metabolic ability and resistance to fatigue (Sohail et al., 2012), contributes to their ability to preserve muscle mass under stress. This contrasts with the rapid glycolytic metabolism of fast-twitch fibers, which are more susceptible to breakdown under HS-induced oxidative stress (Nawaz et al., 2021). However, it is crucial to acknowledge that prolonged exposure to high temperatures can still induce reductions in muscle mass and fat deposition, albeit to a lesser extent compared to Cobb 500 breed (Sohail et al., 2012).

Sasso breeds, balancing growth rate and adaptability, exhibit intermediate responses to HS. While more productive than SAGA breed, they are more susceptible to HS-induced reductions in carcass yields. The observed decline in carcass weight and breast muscle yield in Sasso chickens under cyclic HS (Zulkifli et al., 2018) is attributed to the diversion of energy from growth to stress mitigation mechanisms, such as panting and reduced feed efficiency (Lara and Rostagno, 2013). Moreover, HS can alter the fatty acid composition of meat, potentially reducing its nutritional value and marketability (Zaboli et al., 2019). These findings underscore the need for targeted interventions, such as nutritional supplementation and environmental modifications, to mitigate the adverse effects of HS in Sasso breed.

Cobb 500 breeds, such as Cobb 500 broilers, are highly vulnerable to HS due to their high metabolic rates and intense growth. Studies have consistently demonstrated significant reductions in breast and thigh muscle yields under HS conditions (Imik et al., 2012; Elshafaei et al., 2021). The diminished breast muscle development in Cobb 500 chickens observed in the current study can be attributed to the diversion of blood flow away from peripheral muscles to prioritize core body temperature regulation (Pearson and Hussain, 2015; Wang et al., 2018). This results in reduced oxygen and nutrient supply to the breast muscle, leading to muscle atrophy. Conversely, the back muscle, due to its anatomical location and better blood circulation, remains relatively resilient (Ji et al., 2022). Additionally, the predominance of fast-twitch muscle fibers in commercial chickens enhances the negative impact of HS on carcass yields (Nawaz et al., 2021). The increased susceptibility of Cobb 500 breed to HS-induced muscle degradation is also linked to the upregulation of proteolytic pathways, such as the SAubiquitin-proteasome system (Roushdy et al., 2020).

HS induced breed-specific alterations in carcass yields and internal organ weights, reflecting differential adaptive strategies. In SAGA chicken, increased wing (WW) and shank weights (SW) were observed under HS (7.24 g vs. 5.74 g for WW; $P < 0.05$), which may signify an adaptive redistribution of muscle mass towards thermoregulatory appendages such as wings, facilitating heat dissipation. Conversely, Cobb 500 chickens exhibited a reduced breast meat (BM) yield under HS

(25.13 g vs. 27.75 g; $P < 0.05$), suggesting a physiological prioritization of visceral organ perfusion over pectoral muscle growth when under HS (Pearson and Hussain, 2015). A significant breed-by-temperature interaction was observed for drumstick weight (DS) ($P < 0.01$; Table 8). The decline in DS noted in Sasso chickens under HS (8.09 g vs. 9.49 g) may be attributable to breed-specific susceptibility of muscle fibre types to HS, as reported in previous studies (Nawaz et al., 2021).

Regarding internal organs, SAGA chickens exposed to HS showed liver (LV) hypertrophy (1.83 g vs. 1.56 g; $P < 0.05$), potentially reflecting compensatory metabolic activation aimed at detoxification (Rahman, 2019). In contrast, Cobb 500 chickens displayed spleen (SP) atrophy under HS (0.06 g vs. 0.08 g; $P < 0.05$), a common indicator of HS-induced immunosuppression (Ghareeb, 2022). An interaction effect for intestine (IN) weight was also significant ($P < 0.05$ for Breed × Temperature), with SAGA chickens showing increased intestinal mass (3.43 g vs. 2.77 g), suggesting an enhanced capacity for nutrient absorption that may serve as an adaptive mechanism to mitigate the negative impacts of HS on nutrient utilization.

Internal Organs Yields. SAGA chicken, known for their slow growth, showed marked increases in the weights of the liver (LV), heart (HT), kidneys (KD), and intestines (IN) under HS. This suggests the activation of adaptive mechanisms, potentially involving enhanced metabolic processes or changes in blood flow to maintain homeostasis during thermal stress. These findings are consistent with previous studies indicating organ hypertrophy, particularly in the liver and heart, as a compensatory response to the thermoregulatory and energy demands imposed by HS (Gavin, 2001). In contrast, Sasso chicken, which has an intermediate growth rate, experienced significant decreases in splenic and gizzard weights under HS. The reduction in spleen size may reflect immunosuppression related to HS, aligning with documented decreases in lymphoid organ mass in birds under thermal stress (Ghareeb, 2022). The decline in gizzard weight may indicate reduced feed intake or altered digestive efficiency, supporting earlier findings (Ghareeb, 2022).

Cobb 500 chickens, characterized by rapid growth, also showed significant decreases in liver, kidney, and spleen weights under HS. This increased vulnerability to HS-related physiological changes is likely due to their higher metabolic rates and heat production. The reductions in liver and kidney weights may suggest compromised metabolic and excretory functions, consistent with previous research (Farag and Alagawany, 2018). The observed splenic atrophy in Cobb 500 chickens' mirrors that are seen in Sasso chicken, indicating a similar immunosuppressive effect of HS in these breeds.

Distinct breed-specific responses to HS were noted in the liver, heart, and intestines. SAGA chickens exhibited significant liver hypertrophy, contrasting with liver atrophy seen in Cobb 500 breed, attributed to increased metabolic demands and lipid accumulation (Rahman, 2019). Additionally, SAGA chickens had the highest cardiac mass under HS, likely due to increased hemodynamic demands associated with cutaneous vasodilation for thermoregulation (Rahman, 2019). The lack of significant changes in cardiac mass in Cobb 500 and Sasso chickens suggest a more pronounced cardiovascular response to HS in slower-growing breeds.

Intestinal mass showed breed-specific variations as well, with SAGA chickens demonstrating increased intestinal mass under HS, unlike medium-fast breeds, which typically experience intestinal atrophy due to reduced blood flow and nutrient absorption (Power, 2017). This indicates potential adaptive mechanisms in SAGA chickens to sustain nutrient absorption and energy balance during thermal stress.

The spleen, a vital part of the avian immune system, displayed significant breed-specific responses to HS. SAGA chickens maintained higher splenic mass, while fast- and medium-growth rate chickens exhibited notable splenic atrophy, reflecting HS-related immunosuppression (Power, 2017). The preservation of splenic mass in SAGA chickens may represent a strategy to support immune function under HS conditions.

The reduction in spleen weight observed in Sasso chickens exposed to heat stress ($P < 0.05$; Table 9) is consistent with existing literature indicating that HS can lead to immunosuppression in poultry, primarily through atrophy of lymphoid organs. As a central immune organ responsible for lymphocyte production and antigen filtration, the avian spleen is particularly vulnerable to environmental stressors (Ghareeb et al., 2022).

Prolonged exposure to HS promotes the release of glucocorticoids such as cortisol, which are known to suppress immune function by inducing lymphoid tissue regression (Quinteiro-Filho et al., 2012). Similar findings have been reported in broiler chickens subjected to cyclic HS (34 °C), where marked splenic atrophy was associated with reduced T-cell activity and heightened infection risk (Basiouni et al., 2023). The decline in spleen mass in Sasso chickens under HS conditions (0.14 g vs. 0.17 g in NT; Table 9) reflects these trends and suggests a potential impairment in adaptive immune responses.

The interaction effects of temperature and breed indicated significant breed-specific differences in organ mass, excluding cardiac mass, underscoring the genetic basis for HS responses. SAGA breeds exhibited greater resilience compared to Cobb 500 breed, which are more prone to metabolic and physiological disturbances (Menchetti et al., 2024). Temperature interactions were significant for cardiac and intestinal mass (prolonged HS induces cardiac atrophy via oxidative stress-mediated muscle degradation, while intestinal mass reduction results from villous atrophy, diminished enzymatic activity, and mucosal breakdown), highlighting the sensitivity of these organs to thermal changes and their roles in thermoregulation and nutrient absorption (Apalowo et al., 2024).

Telomere Length (TL) as Novel Heat Stress Biomarker. Telomere length is emerging as a novel biomarker for heat stress (HS) in poultry. Oxidative stress is a primary driver of telomere shortening, particularly under HS conditions. Telomeres, rich in guanine nucleotides, are highly susceptible to damage from reactive oxygen species (ROS) generated during heat stress, which causes single-strand breaks and accelerates telomere attrition (Kawanishi and Oikawa, 2004; von Zglinicki, 2002). The accumulation of ROS disrupts telomere maintenance by inhibiting telomerase activity and depleting cellular antioxidants like glutathione, further exacerbating telomere erosion and reducing DNA repair capacity (Epel et al., 2004; Finkel and Holbrook, 2000). Among chicken breeds, Cobb 500 exhibits greater telomere shortening due to higher metabolic ROS production associated with its rapid growth phenotype (Melis et al., 2023). In contrast, the indigenous SAGA breed displays resilience through enhanced antioxidant defences that protect telomeres from oxidative damage (Badmus et al., 2022). Poultry studies confirm that HS-induced telomere shortening correlates strongly with oxidative stress biomarkers and that antioxidant supplementation can mitigate this effect (Pineda-Pampliega et al., 2020; Sohail et al., 2012) These findings underscore the potential of TL as a sensitive indicator of cumulative oxidative stress and cellular aging in broilers subjected to thermal challenges.

The high guanine content of telomere makes them particularly vulnerable to oxidative damage, which both prolongs repair time and enhances their value as reliable biomarkers of cellular stress (Kawanishi and Oikawa, 2004). TL has practical applications as a biomarker for HS resilience in poultry and livestock. Identifying individuals with shorter telomeres can enable the selection of heat-resilient breeds or the implementation of targeted interventions to mitigate stress (Bateson, 2016). Integrating TL measurements into breeding programs could improve stress tolerance and longevity in livestock and poultry (Tablado et al., 2022). However, TL naturally shortens with age and stress exposure, making it a valuable biomarker for cellular aging and stress resilience (Whittemore et al., 2019). In this study, we observed that HS induces significant telomere shortening across all chicken breeds, highlighting the sensitivity of telomeres to HS. Furthermore, inherent differences in TL among breeds suggest a genetic influence on telomere maintenance, potentially linking growth rate to stress resilience.

In the current study, exposure to HS resulted in significant telomere

shortening in all chicken breeds at both weeks 2 and 4, corroborating previous research demonstrating the detrimental effects of environmental stressors on telomere integrity (Boonekamp et al., 2014; Monaghan and Ozanne, 2018). In chickens, this accelerated telomere attrition likely reflects increased cellular stress, particularly oxidative stress and inflammation, which are exacerbated by HS (Finkel and Holbrook, 2000; Von Zglinicki, 2002). Telomeres, being rich in guanine, are highly susceptible to oxidative damage, which can disrupt their structure and accelerate shortening (Shammas, 2011). This HS-induced telomere shortening aligns with the broader understanding that chronic environmental stress can accumulate cellular damage, contributing to accelerated biological aging (Blackburn, 1991; Haussmann et al., 2007).

The mechanisms linking HS to telomere shortening involve interrelated processes, including increased production of reactive oxygen species (ROS), systemic inflammation, and downregulation of telomerase activity (Epel et al., 2004; Jaskelioff et al., 2011; Metcalfe and Olsson, 2022). HS suppresses telomerase activity, the enzyme responsible for telomere maintenance, leading to accelerated telomere shortening. These mechanisms collectively contribute to the observed reduction in TL under HS conditions.

The breed-specific differences in TL is significant in inter-breed differences in TL were observed at both time-points, emphasizing the role of genetic background in modulating telomere dynamics (Angelier et al., 2013). The SAGA breed consistently exhibited longer telomeres compared to the Sasso and Cobb 500 breeds. This observation suggests a potential inverse relationship between growth rate and TL, consistent with the hypothesis that rapid growth and associated high metabolic rates can elevate HS stress, leading to increased telomere attrition (Reichert et al., 2014; Casagrande et al., 2023). This aligns with the trade-off hypothesis, which posits that resources allocated to rapid growth may come at the expense of cellular maintenance and longevity, potentially manifesting as shorter telomeres (Lemaître et al., 2024).

The longer telomeres observed in the SAGA breed may reflect their lower metabolic rates and greater resilience to HS stress, which are characteristic of SAGA breed. In contrast, the shorter telomeres in the Cobb 500 breed likely results from their higher metabolic activity and increased susceptibility to oxidative damage. These findings are consistent with studies in other species, where rapid growth has been associated with shorter telomeres and reduced lifespan (Monaghan and Ozanne, 2018).

While significant breed-specific variations in TL were evident, the interaction between breed and temperature was not significant in this study. This suggests that while genetic factors influence baseline TL, the magnitude of telomere shortening in response to HS is relatively consistent across breeds. This finding contrasts with some studies reporting breed-specific stress responses in poultry (Blackburn, 1991), suggesting that telomere dynamics under HS may be governed by more conserved cellular mechanisms rather than breed-specific adaptations. Further research is needed to elucidate the specific molecular pathways involved in this conserved response.

The observed differences in TL among breeds with varying growth rates likely reflect differing metabolic demands and stress resilience. Cobb 500 breed often exhibits increased susceptibility to oxidative stress due to their heightened metabolic activity (Melis et al., 2023). This could explain their shorter telomeres compared to the slower growing (SAGA) breed. Our findings support the trade-off theory, where rapid growth may compromise cellular maintenance, potentially accelerating the aging process (Monaghan and Ozanne, 2018).

However, TL analyses revealed breed-specific responses to chronic HS, underscoring its utility as a physiological biomarker. Cobb 500 chickens experienced the greatest reduction in TL by Week 4 (250.4 kb vs. 435.4 kb; $P < 0.05$), supporting the hypothesis that rapid growth rates are linked to accelerated oxidative stress and cellular aging (Monaghan and Ozanne, 2018). In contrast, SAGA chickens showed no significant change in TL, further substantiating their genetic resilience to HS-induced cellular damage and premature aging. The significant main effect of temperature on TL ($P < 0.001$; Tables 11 & 12) unequivocally confirms TL as a universal and robust biomarker of HS across diverse chicken breeds.

Gene Expression Analyses. In this study, *HSP70* expression was significantly elevated in both liver and muscle tissues across diverse growth rates, confirming the activation of stress-responsive pathways. Cobb 500 chickens, characterized by rapid growth, exhibited the most pronounced *HSP70* upregulation, particularly in hepatic tissue, indicating heightened sensitivity to HS-induced cellular damage. This aligns with studies linking fast growth rates to increased metabolic demands and reduced stress tolerance (Nicol et al., 2024). Conversely, SAGA chicken, with their slower growth rate, demonstrated the lowest *HSP70* induction, reflecting greater thermal resilience and more efficient stress-coping mechanisms (Nayak et al., 2023), suggesting the role of genetic and metabolic adaptations in shaping breed-specific responses to thermal stress (Yang et al., 2022).

The liver, due to its central role in metabolism and detoxification, exhibited a stronger *HSP70* response compared to muscle tissues, consistent with its heightened sensitivity to stressors (Nie et al., 2024). This robust hepatic response highlights the liver's critical function in maintaining homeostasis during HS. Variations in *HSP70* expression across breeds suggest that genetic factors, including differences in stress response pathways, influence HS tolerance (Perini et al., 2020). Breeds with higher *HSP70* upregulation may possess more efficient stress response mechanisms, contributing to their ability to cope with HS (Perini et al., 2020).

The differential regulation of *IGF-1* during HS further illustrates the trade-off between growth and stress adaptation. *IGF-1*, a key regulator of muscle development and growth, showed breed-specific variations in response to HS. Cobb 500 chickens exhibited a significant upregulation of *IGF-1* in both muscle and liver tissues, reflecting an attempt to sustain growth despite HS. This aligns with studies suggesting that Cobb 500 breed prioritize growth even under suboptimal conditions, potentially at the expense of stress resilience (Hemanth et al., 2024). In contrast, SAGA chickens showed no significant changes in *IGF-1* levels, indicating a shift in resource allocation toward stress adaptation rather than growth, a strategy typical of slower growing breeds with enhanced thermal tolerance (Hemanth et al., 2024). Sasso chicken, with moderate growth rates, displayed an intermediate response, with *IGF-1* upregulation limited to muscle tissue.

The somatotropic axis, involving growth hormones (GH) and *IGF-1*, plays a pivotal role in regulating growth, metabolism, and reproduction in chickens. Disruptions to this axis during HS can have profound effects on growth performance and productivity (Ghanem et al., 2024). The observed upregulation of *IGF-1* in muscle tissue across all breeds aligns with its role in promoting muscle growth and repair, suggesting that chickens prioritize muscle integrity during HS (Duclos, 2005). However, the pronounced *IGF-1* response in Cobb 500 chickens may reflect their greater ability for growth and tissue repair under stress, while the lack of response in SAGA chicken highlights their prioritization of stress adaptation overgrowth.

The contrasting patterns of *HSP70 and IGF-1* expression reveal the complex interplay between stress response and growth regulation. Cobb 500 chickens, with the highest *HSP70* level, exhibited significant cellular stress but also maintained elevated *IGF-1* levels, reflecting a continued focus on growth. In contrast, SAGA chicken, with the lowest *HSP70* induction, showed no significant *IGF-1* changes, suggesting a prioritization of stress adaptation. These findings align with the concept of resource allocation trade-offs, where Cobb 500 breed may sacrifice stress resilience for growth, while slower growing breeds exhibit more robust stress adaptation mechanisms (Awad et al., 2020).

These results highlight the importance of considering breed-specific physiological responses when developing strategies to mitigate HS in poultry production. Cobb 500 breed may require targeted interventions, such as nutritional supplements or environmental modifications, to enhance their stress resilience without compromising growth. In

contrast, slower growing breeds like SAGA offer valuable insights into genetic and metabolic adaptations to thermal stress, providing a foundation for selective breeding programs aimed at improving heat tolerance in commercial poultry. HS significantly affects chickens' TL and *HSP70* expression, with breed- specific differences in stress resilience, and serves as a reliable biomarker for evaluating HS impacts in chickens.

Gene expression analyses of *HSP70* and *IGF-1* provided further molecular insights into breed-specific stress responses (Table 13 and 14). *HSP70*, a critical chaperone protein, was most highly upregulated in the liver of Cobb 500 chickens, showing a 2.5-fold induction ($P < 0.001$). This substantial increase reflects severe protein denaturation stress and cellular damage experienced by Cobb 500 birds under HS conditions (Balakrishnan et al., 2023).

The expression dynamics of *IGF-1*, a key growth factor, also differed between breeds. Cobb 500 chickens maintained high *IGF-1* expression in muscle tissue during HS, indicating a persistent, yet ultimately ineffective, prioritization of growth despite the physiological stress imposed by heat (Hemanth et al., 2024). In contrast, SAGA chickens exhibited no significant change in *IGF-1* expression, suggesting a physiological trade-off that favours stress adaptation and survival over continuous growth in challenging environmental conditions.

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CRediT authorship contribution statement

Aliyu Abduljalal Musa: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Kamalludin Mamat-Hamidi:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition. **Zulkifli Idrus:** Validation, Methodology, Conceptualization. **Eric Lim Teik Chung:** Writing – review & editing, Visualization, Supervision. **Noraini Samat:** Writing – review & editing, Resources, Formal analysis, Data curation. **Nafeesa Abu Kassim:** Writing – review & editing, Validation.

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The authors state that they have no conflicts of interest.

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