



Kedu Chickens Exhibit Better Resilience to Thermal Stress During the Two Weeks Prior to Market Age Compared to Broiler Chickens

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ABSTRACT

Information about resilience to thermal stress is important for establishing the optimum rearing temperature for chickens. This study investigated the effects of heat stress on the physiological and hematological responses of Kedu and broiler chickens. Based on a 2×2 factorial arrangement, 192 male Kedu and broiler chickens were raised at temperatures of $23.83 \pm 0.23^\circ\text{C}$ or $35.33 \pm 1.78^\circ\text{C}$. Sampling and data measurement were conducted after two weeks of rearing. Heat stress had no effect on the weight gain of the Kedu chickens, whereas the broiler weight gain fell ($p \leq 0.05$). Heat stress reduced feed consumption, while increasing water intake, feed conversion ratio (FCR), rectal temperature, heart rate, and respiratory rate ($p \leq 0.05$). The heterophil-to-lymphocyte ratio of the Kedu chickens was higher than that of broilers ($p \leq 0.05$), while heat stress increased glucose and uric acid levels in the broilers ($p \leq 0.05$), but not in the Kedu chickens. Heat stress also increased heterophils. In addition, mean corpuscular volume and mean corpuscular hemoglobin levels were higher in the Kedu chickens than in the broilers, while platelet counts were higher in the broilers ($p \leq 0.05$). Erythrocyte, hemoglobin, and hematocrit levels decreased during heat stress ($p \leq 0.05$), while platelet distribution width (PDW) in the broilers increased ($p \leq 0.05$). Blood proportion to live chicken body weight was higher in the Kedu chickens than in the broilers, while heat stress reduced the blood and feather proportions in the broilers ($p \leq 0.05$). Heat stress also reduced the liver weight of the Kedu chickens, as well as that of the thymus and *Bursa of Fabricius* in both types of chicken ($p \leq 0.05$). The Kedu chickens had higher gizzard, spleen, and *Bursa of Fabricius* weights than the broilers ($p \leq 0.05$). In conclusion, compared to the broilers, the Kedu chickens demonstrated greater tolerance to heat stress, as indicated by their unaffected weight gain, blood glucose levels, PDW, and feather proportion.

Keywords: broiler; immune; native chicken; physiological response; stress

INTRODUCTION

Climate change has negatively impacted poultry production in tropical regions, primarily due to increases in temperature, which cause heat stress. Poultry exhibit a rapid metabolic rate and limited capacity to dissipate excess body heat due to the lack of sweat glands, which makes them highly sensitive to heat stress (Nawaz *et al.*, 2021). Economically, climate change has significant impacts, as heat stress can compromise growth rates and reduce feed efficiency. In this case, energy derived from feed, normally used to support growth, is diverted to restoring and maintaining homeostasis under stressful conditions (Vandana *et al.*, 2020).

Heat stress in poultry can trigger an imbalance between heat production and the body's ability to dissipate heat. Under these conditions, chickens

demonstrate various adaptive physiological and hematological changes, including increased rectal temperature, respiratory and heart rates, blood glucose levels, and changes in their erythrocyte, hemoglobin, hematocrit, and differential leukocyte count (Brugaletta *et al.*, 2022). When these changes exceed the physiological range, chickens can be highly susceptible to health problems, weakened immune function, decreased productivity, and compromised welfare (Oluwagbenga & Fraley, 2023).

Indonesia is a country with abundant and diverse genetic resources, one of which is the native chicken. The Kedu is a type of native chicken with potential as a meat producer (Setiaji *et al.*, 2025). In the current poultry industry, feed efficiency and environmental sustainability have recently become important trends and are a shared concern for farmers. Although the

growth rate of the Kedu chicken is relatively slower than that of modern broiler chickens, the advantage of this local chicken breed is its better adaptability to local environmental conditions (a hot and humid tropical climate). This ability is invaluable in ensuring sustainable production in the face of climate change and the increasingly serious threat of disease in commercial breeds (Maharani *et al.*, 2021; Kpomasse *et al.*, 2023).

To mitigate the adverse effects of heat stress on the growth performance and health of modern broiler chickens, producers typically construct chicken houses with controlled internal environmental conditions. However, the cost of constructing these is very high. This situation makes it unaffordable for indigenous chicken farmers, who typically operate on a small scale with very minimal capital. With specific regard to Kedu chickens, Mushawwir *et al.* (2024) note that they demonstrate excellent tolerance to high tropical temperatures. Owing to this characteristic, they are likely to require different environmental conditions than modern broiler strains. Chicken body weight is one factor that determines the severity of heat stress during rearing. Under typical conditions, higher body weight increases the risk of heat stress. Gogoi *et al.* (2021) document that commercial broiler chickens with higher body weights are more vulnerable to heat stress compared to those with lower weights. In line with this, native chickens with higher body weights have also been shown to be more vulnerable to heat stress (Boonkum *et al.*, 2024). More specifically, Malila *et al.* (2022) found that chickens approaching market age (harvesting age) were more prone to heat stress than younger chickens.

A number of studies have been conducted to compare the resilience to heat stress and physiological changes between native chicken breeds from tropical countries and modern broiler chickens (Tirawattanawanich *et al.*, 2011; Malila *et al.*, 2022; 2023). However, to date, little research has focused on the comparative effect of heat stress on Kedu and modern broiler chickens. Therefore, a study comparing the impact of heat stress on the two strains as a basis for considering whether or not it is necessary to build environmentally controlled broiler houses for Kedu chickens is vital. Taking all these facts into consideration, this study was conducted to investigate the effects of thermal stress during the two weeks prior to market age on the physiological and hematological responses of Kedu and broiler chickens.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Indonesia (Approval No. 61-08/A-17/KEP-FPP).

In Vivo Trial

The study was conducted using a 2×2 factorial design, with chicken type (Kedu and modern broiler

strains) as the first factor, and temperature (23.83 ± 0.23 °C and 35.33 ± 1.78 °C) as the second. A total of 192 male chickens were used, consisting of 96 Kedu chickens aged 10 weeks (body weight 1090.89 ± 13.47 g/chick) and 96 3-week-old modern broiler strains (Cobb; body weight 854.01 ± 2.17 g/chick). Given that chickens approaching market age are highly susceptible to heat stress (Malila *et al.*, 2022), the Kedu chickens and broiler strains were both studied two weeks before market age. Typically, Kedu chickens are harvested at 12 weeks (Setiaji *et al.*, 2025), and modern broiler strains at 5 weeks (Gogoi *et al.*, 2021). The chicks were raised in an environmentally controlled broiler house facility at the Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (Semarang, Indonesia) for two weeks under two different environmental temperature conditions; that is, 23.83 ± 0.23 °C (no temperature-induced heat stress) and 35.33 ± 1.78 °C (temperature-induced heat stress). The broiler house was divided into two rooms for the trial. To maintain a temperature of 23.83 ± 0.23 °C (throughout the day), one room was equipped with air conditioning, while the other was equipped with only a blower fan. To reach and establish a temperature of 35.33 ± 1.78 °C, heating lamps were placed in the room. To ascertain the established microclimatic conditions inside the broiler house, temperature and humidity were regularly monitored using a digital thermometer and hygrometer.

Both chicken breeds were allotted equally into the two temperature groups, with 48 birds per group. Each treatment was replicated six times, with eight birds per replication. The heat-stress treatment was applied daily from 10:00 a.m. to 3:00 p.m. (the hottest time of day in the Semarang area), after which the temperature was returned to tropical levels (28-30 °C). This heat-stress regimen was continuously applied for two weeks. The chickens were housed in 1×1 m² pens. Feed was provided in mash form (Table 1), without antibiotics, antioxidants, enzymes, coccidiostats, or antifungal agents. The crude protein content in the feed for the Kedu chickens and broiler strains was formulated differently, with consideration of the lower crude protein requirements of indigenous chickens compared to those of modern broiler strains (Kingori *et al.*, 2003; Manyelo *et al.*, 2020). Throughout the experimental period, both feed and drinking water were offered *ad libitum*.

Sample Collection and Analysis

Body weight, feed consumption, and the feed conversion ratio (FCR) were recorded throughout the study. At the end of the period (when the Kedu chickens were 12 weeks old and the broiler chickens 5 weeks old), two chickens in each replication or pen with body weights close to the average pen weight were selected. The rectal temperature of these chickens was measured using a digital rectal thermometer inserted into the chicken's cloaca. The respiratory rate of the selected chickens was also measured by counting chest movements over a minute, while heart rate was measured directly using a stethoscope placed on the

left-hand side chest of the birds. Subsequently, the same chickens had blood drawn from the brachial vein on the wings. The sample collections were conducted at around 1:00 p.m.

A 1 mL blood sample was placed in a blood tube containing ethylenediaminetetraacetic acid (EDTA) for complete blood count analysis, and 2 mL of blood was placed in a non-EDTA blood tube for serum glucose and uric acid analysis. Complete blood counts were analyzed using a Prima fully automated hematology analyzer, manufactured by PT. Prima Alkesindo Nusantara, Jakarta, Indonesia. The non-EDTA blood was centrifuged at 500 × g for 10 minutes to produce serum. The levels of glucose were determined using spectrophotometric techniques, while uric acid analysis was conducted based on enzyme-based colorimetric

techniques according to the manufacturer’s protocols (DiaSys Diagnostic System GmbH, Holzheim, Germany).

After the final body weight was measured, the chicken from which blood had been drawn was slaughtered following halal convention. The blood was collected in a special container and weighed to determine the percentage of blood in the chicken’s body. The feathers were then plucked and their weight measured to determine their percentage to live weight. The chicken was then dissected, and the internal organs were removed and weighed to determine their relative weight to the chicken’s live weight.

Statistical Analysis

Data were treated according to a 2 (Kedu and broiler chickens) × 2 (23.83 ± 0.23 °C and 35.33 ± 1.78 °C) factorial arrangement on the basis of the general linear models procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Significant interactions between the main factors were included in the statistical model, whereas any insignificant interaction effect was eliminated. The results are presented as least-squares means (LSMEANS) and standard error (SE) for the treatment groups. Duncan’s Multiple Range Test was conducted to show the differences in LSMEANS (p≤0.05).

RESULTS

Growth Performance of the Chickens

The data on the growth performance of the Kedu and broiler chickens raised in temperatures of 23.83 ± 0.23 °C and 35.33 ± 1.78 °C are presented in Table 2. There was a significant interaction (p≤0.05) between chicken type and temperature for the final BW, weight gain, feed intake, and water intake, while no interaction was observed between the two factors concerning the FCR of the broiler chickens. In addition, there was no significant difference in weight gain between the Kedu chickens raised at the two temperatures, while the weight gain in the broiler chickens was significantly lower (p≤0.05) at 35.33 ± 1.78 °C compared to 23.83 ± 0.23 °C. At 35.33 ± 1.78 °C, feed consumption decreased (p≤0.05) and water intake increased (p≤0.05) for both types of chicken. The broiler chickens had a lower FCR (p≤0.05) than the Kedu ones, while the temperature of 35.33 ± 1.78 °C significantly increased (p≤0.05) FCR values for both.

Table 1. Ingredients and nutritional composition of diets for Kedu and broiler chickens

Ingredients	Proportion (%)	
	Kedu chickens ¹	Broiler strains ²
Yellow corn	65.05	60.44
Palm oil	2.06	2.55
Soybean meal	28.05	33.25
DL-methionine	0.20	0.20
Bentonite	1.00	0.75
Limestone	1.35	1.00
Calcium monophosphate	1.55	1.00
Premix ³	0.27	0.34
Choline chloride	0.07	0.07
Salt	0.40	0.40
Nutrient Composition		
ME ⁴ (kcal/kg)	3000	3000
Crude protein	18.0	20.0
Crude fat	4.80	5.12
Crude fiber	5.14	5.03
Ca	1.31	1.05
P (available)	0.64	0.53
Lysine	0.88	1.02
Methionine	0.44	0.49

Notes: ¹The ingredients and composition of diets for Kedu chickens were taken from Sugiharto *et al.* (2021). ²The ingredients and composition of diets for broiler chickens were taken from Tentravinata *et al.* (2023). ³Vitamin and mineral composition per kg premix: 50,000 IU vitamin D₃; 0.5 mg vitamin B₁₂; 32.5% calcium (Ca); 1% phosphorus (P); 6 g iron (Fe); 4 g manganese (Mn); 0.075 g iodine (I); 0.3 g copper (Cu); 3.75 g zinc (Zn). ⁴Metabolizable energy (ME) was calculated according to the formula: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5}

Table 2. Growth performance of Kedu and broiler chickens at normal (23.83 ± 0.23 °C) and high (35.33 ± 1.78 °C) temperatures

Variables	23.83 ± 0.23 °C		35.33 ± 1.78 °C		SE	p value		
	Kedu	Broiler	Kedu	Broiler		C	T	C*T
Final BW (g/bird)	1503 ^c	2055 ^a	1391 ^c	1705 ^b	28.8	<0.01	<0.01	<0.01
Weight gain (g/bird)	412 ^c	1201 ^a	300 ^c	851 ^b	29.4	<0.01	<0.01	<0.01
Feed intake (g/bird)	1087 ^c	1880 ^a	990 ^d	1670 ^b	16.2	<0.01	<0.01	0.02
Water intake (mL/bird)	3419 ^d	4595 ^b	3957 ^c	5779 ^a	110	<0.01	<0.01	0.05
FCR	2.73	1.57	3.55	2.01	0.19	<0.01	0.03	0.49

Notes: ^{a,b,c,d} Means within the same row with different superscripts differ significantly at p≤0.05. BW = body weight; FCR = feed conversion ratio; SE = standard error; C = chicken type; T = temperature; C*T = interaction between chicken type and temperature.

Physiological Variables of the Chickens

Data on the physiological variables of the chickens are presented in Table 3. Significant interactions ($p \leq 0.05$) were observed for rectal temperature, respiratory rate, heart rate, and glucose levels. Rectal temperature and heart rate increased significantly ($p \leq 0.05$) in both chicken species due to exposure to the temperature of 35.33 ± 1.78 °C for two weeks of rearing. The respiratory rate increased ($p \leq 0.05$) by approximately 2.68 times in the Kedu chickens and 4.40 times in the broiler chickens following exposure to 35.33 ± 1.78 °C. No significant interaction ($p > 0.05$) was observed between the two factors in terms of the H/L ratio. However, the ratio of the Kedu chickens was higher ($p \leq 0.05$) than that of broiler chickens, irrespective of rearing temperature. Rearing at 35.33 ± 1.78 °C increased ($p \leq 0.05$) glucose levels in the broiler chickens, but these levels did not change in the Kedu chickens. Regardless of chicken type, uric acid levels increased ($p \leq 0.05$) due to the high temperatures.

Hematological Profiles of the Chickens

The hematological profiles of the Kedu and broiler chickens raised under the two temperatures are

presented in Table 4. A significant interaction ($p \leq 0.05$) was observed for PDW, but other parameters showed no interaction between the two factors. Heterophils, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels were higher ($p \leq 0.05$) in the Kedu chickens compared to the modern broilers, while platelet counts were higher ($p \leq 0.05$) in the broilers than in the Kedu chickens. Irrespective of chicken type, erythrocytes, hemoglobin, and hematocrit decreased ($p \leq 0.05$) with exposure to 35.33 ± 1.78 °C. Platelet distribution width (PDW) in the broiler chickens increased ($p \leq 0.05$) at 35.33 ± 1.78 °C, but not in the Kedu chickens.

Blood, Feather, and Internal Organ of the Chickens

The proportions of blood and feathers, and the relative weight of the chickens subjected to the two temperatures are presented in Table 5. A significant interaction ($p \leq 0.05$) between chicken type and temperature was observed for the proportions of feathers and liver relative weight, while no interaction was observed for the other parameters. The proportion of blood was higher ($p \leq 0.05$) in the Kedu chickens than in the broilers, while rearing at 35.33 ± 1.78 °C significantly decreased ($p \leq 0.05$) the proportion of blood

Table 3. Physiological variables of Kedu and broiler chickens at normal (23.83 ± 0.23 °C) and high (35.33 ± 1.78 °C) temperatures

Variables	23.83 ± 0.23 °C		35.33 ± 1.78 °C		SE	p value		
	Kedu	Broiler	Kedu	Broiler		C	T	C*T
Rectal temperature (°C)	41.3 ^c	40.7 ^d	41.6 ^b	42.0 ^a	0.07	0.53	<0.01	<0.01
Respiratory rate (breaths/min)	35.1 ^c	46.1 ^c	94.2 ^b	203 ^a	7.41	<0.01	<0.01	<0.01
Heart rate (beats/min)	259 ^{cd}	247 ^{cd}	304 ^b	331 ^a	3.54	0.16	<0.01	<0.01
H/L ratio	0.06	0.05	0.07	0.05	0.01	0.04	0.68	0.53
Glucose (mg/dL)	259 ^c	286 ^b	277 ^{bc}	355 ^a	5.22	<0.01	<0.01	<0.01
Uric acid (mg/dL)	4.52	4.18	5.12	5.35	0.23	0.88	0.01	0.39

Notes: ^{a,b,c,d} Means within the same row with different superscripts differ significantly at $p \leq 0.05$. H/L ratio = heterophil-to-lymphocyte ratio; SE = standard error; C = chicken type; T = temperature; C*T = interaction between chicken type and temperature.

Table 4. Hematological profiles of Kedu and broiler chickens at normal (23.83 ± 0.23 °C) and high (35.33 ± 1.78 °C) temperatures

Variables	23.83 ± 0.23 °C		35.33 ± 1.78 °C		SE	p value		
	Kedu	Broiler	Kedu	Broiler		C	T	C*T
Leukocytes ($\times 10^3/\mu\text{L}$)	83.98	84.46	84.08	82.92	1.57	0.88	0.75	0.72
Lymphocytes (%)	79.53	80.76	79.02	79.29	1.49	0.72	0.64	0.82
Heterophils (%)	4.46	3.69	5.07	3.63	0.37	0.04	0.61	0.53
Erythrocytes ($\times 10^6/\mu\text{L}$)	2.17	2.24	2.03	2.13	0.04	0.12	0.02	0.76
Hemoglobin (g/dL)	8.88	8.92	8.73	8.24	0.14	0.27	0.04	0.20
Hematocrit (%)	36.63	37.14	33.84	34.90	0.56	0.33	<0.01	0.73
Platelets ($\times 10^3/\mu\text{L}$)	19.75	21.42	20.42	25.89	1.19	0.04	0.14	0.27
MCV (fL)	169.35	166.13	167.50	164.55	0.76	<0.01	0.11	0.90
MCH (pg)	40.98	39.89	43.13	39.18	0.67	0.02	0.46	0.15
MCHC (g/dL)	23.70	23.57	25.27	23.34	0.39	0.07	0.23	0.11
RDW-SD (fL)	50.50	47.45	48.33	46.56	0.91	0.07	0.25	0.62
RDW-CV (%)	10.28	9.86	9.98	9.77	0.17	0.22	0.43	0.66
MPV (fL)	9.86	10.36	9.72	10.03	0.15	0.08	0.29	0.67
PDW (%)	9.04 ^b	9.06 ^b	8.18 ^b	11.78 ^a	0.46	<0.01	0.17	<0.01

Notes: ^{a,b} Means within the same row with different superscripts differ significantly at $p \leq 0.05$. MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; RDW-SD = Red Cell Distribution Width – Standard Deviation; RDW-CV = Red Cell Distribution Width – Coefficient of Variation; MPV = Mean Platelet Volume; PDW = Platelet Distribution Width; SE = standard error; C = chicken type; T = temperature; C*T = interaction between chicken type and temperature.

Table 5. Proportion of blood, feathers, and internal organs (% of body weight) of Kedu and broiler chickens at normal (23.83 ± 0.23 °C) and high (35.33 ± 1.78 °C) temperatures

Variables (% live BW)	23.83 ± 0.23 °C		35.33 ± 1.78 °C		SE	p value		
	Kedu	Broiler	Kedu	Broiler		C	T	C*T
Blood	4.39	3.65	3.65	3.58	0.13	0.03	0.04	0.10
Feathers	6.81 ^a	3.71 ^b	7.43 ^a	2.99 ^c	0.17	<0.01	0.84	0.01
Heart	0.48	0.47	0.46	0.43	0.02	0.38	0.24	0.48
Liver	1.95 ^a	1.72 ^b	1.78 ^b	1.85 ^{ab}	0.04	0.13	0.65	<0.01
Gizzard	2.20	1.69	2.05	1.63	0.05	<0.01	0.17	0.62
Proventriculus	0.36	0.40	0.37	0.37	0.01	0.24	0.60	0.24
Thymus	0.25	0.30	0.15	0.25	0.03	0.07	0.04	0.52
Spleen	0.38	0.09	0.26	0.08	0.03	<0.01	0.11	0.17
<i>Bursa of Fabricius</i>	0.08	0.05	0.06	0.04	0.01	<0.01	0.04	1.00

Notes: ^{a,b,c} Means within the same row with different superscripts differ significantly at p≤0.05. SE = standard error; C = chicken type; T = temperature; C*T = interaction between chicken type and temperature.

in the chickens' bodies. Rearing at 35.33 ± 1.78 °C caused a decrease (p≤0.05) in the proportion of feathers in the broilers, but this did not occur in the Kedu chickens. The higher rearing temperature reduced (p≤0.05) the relative weight of the liver in the Kedu chickens, but not in the modern broilers. Irrespective of rearing temperature, the Kedu chickens had a higher relative gizzard weight (p≤0.05) compared to the broilers. The high temperature decreased (p≤0.05) the relative weight of the thymus and *Bursa of Fabricius* in both chicken species. Compared to broiler chickens, the Kedu type had higher relative spleen and *Bursa of Fabricius* weights (p≤0.05).

DISCUSSION

In general, poultry are highly susceptible to heat stress due to their limited ability to dissipate excess body heat (Nawaz *et al.*, 2021; Oluwagbenga & Fraley, 2023). However, unlike modern broiler strains, native chickens have better tolerance to high temperatures (Mushawwir *et al.*, 2024). The study found no significant difference in weight gain between the Kedu chickens raised at 23.83 ± 0.23 °C and 35.33 ± 1.78 °C. Unlike the Kedu chickens, weight gain in the broilers decreased with increased rearing temperature. The study data further indicate that Kedu chickens, a native Indonesian breed, have a higher tolerance to heat stress due to their innate genetic adaptation to hot tropical environmental conditions, as evidenced by their more effective and efficient physiological stress responses and physical structures that facilitate heat loss (Tirawattanawanich *et al.*, 2011). In contrast, modern broiler chickens lack a thermoregulatory system that aligns with their rapid growth. As a result, as they age and gain weight, broiler chickens become increasingly sensitive and vulnerable to heat because their heat dissipation area decreases relative to their body mass (Malila *et al.*, 2022). Heat stress is a factor that can alter homeostasis in poultry. Loss of body fluids is a consequence of heat stress due to increased panting frequency (Sugiharto, 2020). To compensate for this fluid loss, both chicken species studied increased their water intake at high rearing temperatures. Interestingly, broiler chickens had a higher increase in drinking water consumption (20.5%) compared to the Kedu type (13.6%). Reduced feed

consumption during heat stress is a way for chickens to reduce their metabolic heat production (Nawaz *et al.*, 2021). Consistent with Nawaz *et al.*, both chicken species in our study reduced their feed consumption under high temperature conditions. One interesting finding was that the broiler chickens displayed a greater decrease in feed consumption (11.17%) than the Kedu ones (8.92%). Although increased water consumption and reduced feed intake can be indicators of stress in poultry, it was difficult to establish which type of chicken was more stressed at temperatures of 35.33 ± 1.78 °C, as the Kedu type exhibited a higher water-to-feed ratio (4.00) than the broiler chickens (3.46). The FCR value in this study increased in reaction to high temperatures, indicating that feed efficiency decreased due to heat stress. The increase in the FCR value in the Kedu chickens was significantly greater (23.09%) than that of the broiler chickens (21.90%). This seems to be associated with the relatively smaller decrease in feed intake in the Kedu chickens caused by heat stress compared to the broilers. Overall, the conditions discussed above suggest that although heat stress did not notably affect weight gain, it compromised feed efficiency in the Kedu chickens.

Many parameters are commonly used to indicate heat stress in chickens, one of which is rectal temperature. In this study, rectal temperature in both chicken breeds increased in response to high temperatures during two weeks of rearing. However, the increase was greater in the broilers (1.3 °C increase) compared to the Kedu chickens (0.3 °C increase). This suggests that modern broiler strains are more prone to heat stress. Indeed, increased body temperature in chickens can indicate an imbalance between metabolic heat production and heat dissipation (Oke *et al.*, 2021; Rostagno, 2020; Sugiharto, 2020). Respiration rates also increased significantly in response to heat stress, especially in the broilers. This suggests that these chickens are more responsive to heat stress than native ones. In line with our study, He *et al.* (2019) noticed an elevation in respiratory rate from 47.8 breaths/min at 23 °C to 127 breaths/min at 32 °C. In addition, Kim *et al.* (2025) found an increase from 59.1 breaths/min at 21 °C to 104.0 breaths/min at 25 °C, and even up to 162.7 breaths/min at 33 °C. In poultry, an increased respiration rate (panting) is the principal evaporative

mechanism for cooling the body (Oluwagbenga & Fraley, 2023). In line with this rate, the heart rate also increased in both chicken types following the increase in environmental temperature, with a greater increase found in the modern broilers compared to the Kedu chickens. Xu *et al.* (2018) demonstrated that heat-stressed chickens had respiratory rates of 120-160 breaths/min, in conjunction with elevated heart rates. Kim *et al.* (2021) also observed that the heart rate rose from 253-287 beats/min at 22 °C to 327-352 beats/min at 32 °C. Such an increase is a physiological response by chickens to increased oxygen demand during stress and their effort to maintain homeostasis conditions (Gogoi *et al.*, 2021). In addition to the effects of high temperatures during two weeks of rearing, increases in rectal temperature, respiratory rate, and heart rate in chickens may also indicate physiological responses to elevated body temperatures caused by high protein levels in feed, potentially exacerbating heat stress. Furlan *et al.* (2004) reported that high-protein feeds are often attributed to increased heat gain (heat increments) and metabolic heat production, suggesting that such diets may lead to higher body heat production. In our study, the crude protein content in the broiler feed was 2% higher than that in the Kedu feed. Considering all of these facts, the observed increases in rectal temperature, respiratory rate, and heart rate in the broilers at a rearing temperature of 35.33 ± 1.78 °C were likely to have been influenced not only by the hot ambient temperature but also by the increased crude protein content in their feed.

As a physiological response to high temperatures, blood glucose levels increased significantly in the broiler chickens, although these remained unchanged in the Kedu chickens after exposure to high temperatures. Increased blood glucose levels indicate activation of the sympathetic nervous system and the release of glucocorticoids, which stimulate glycogenolysis and gluconeogenesis activities to supply more energy during stressful conditions (Siddiqui *et al.*, 2021; Zmrhal *et al.*, 2023). In line with our findings, Mohammadizad *et al.* (2025) reported an increase in glucose levels from 253.2 mg/dL at 21-22 °C to 261.0 mg/dL after three days at 33-34 °C, whereas Abuajamieh *et al.* (2025) noted an increase from 254.0 mg/dL at 22.6 °C to 292 mg/dL after seven days' rearing at 33.7 °C. When considering the potential effects of differing crude protein levels in diets on the blood glucose levels of broiler and Kedu chickens, it seems quite likely that variations in protein content may not have had a major impact. Supporting this view, Son *et al.* (2024) demonstrated that reducing the dietary crude protein levels (by up to 4%) in broilers did not significantly alter their blood glucose levels. Uric acid levels also increased under heat stress conditions in both chicken types. This was closely related to increased protein catabolism to supply amino acids for gluconeogenesis (energy production) in the liver during thermal stress. Indeed, protein catabolism during heat stress may cause higher levels of uric acid as a byproduct of protein breakdown (Zeng *et al.*, 2024; Gharib *et al.*, 2025).

Heat stress is typically associated with an increased H/L ratio in poultry (Sugiharto *et al.*, 2017).

However, data from our study did not show such an impact on either chicken species during rearing. The exact explanation for this condition is unknown, but it is likely that the two-week heat stress treatment accustomed the chickens to high temperature conditions, rendering the impact of heat stress on the H/L ratio insignificant. Consistent with this inference, Ogundeji and Ayo (2025) reported that heat-habituated chickens showed no change in their H/L ratio during rearing at high temperatures. Irrespective of heat stress treatment, Kedu chickens had a higher H/L ratio compared to broiler chickens. Typically, the H/L ratio in chickens is strongly influenced by genetics (species/strain) and the environment. In addition to being an indicator of stress, the ratio also indicates the capacity of the chicken's immune system, as this is defined by the proportion of heterophils and lymphocytes in the blood (Stefanetti *et al.*, 2023). As an indicator of stress, the proportion of heterophils will increase and that of lymphocytes will decrease in line with the stress experienced by chickens (Sugiharto *et al.*, 2017; Stefanetti *et al.*, 2023). In this study, the impact of heat stress was not seen on the H/L ratio, so it can be inferred that the high H/L ratio in the Kedu chickens was due to a higher proportion of heterophils (irrespective of stress treatment, there was no difference in lymphocytes between the two types of chicken). Consequently, it had an impact on improving the innate immune system (first line of defense) in the chickens.

Heat stress showed varying effects on the hematological profiles of the Kedu and broiler chickens. Leukocyte counts did not change for either under high temperature conditions. Likewise, the lymphocyte percentage in both types of chicken were unaffected by thermal stress. These results are different from those reported by Sugiharto (2020), which indicated that heat stress was associated with the lower counts of leukocytes and lymphocytes. In this context, Molnár *et al.* (2021) revealed that the leukocyte and lymphocyte count of chickens may vary in response to heat stress, depending on its length and extent. Regardless of the heat stress response, the heterophil proportion was significantly different between the two chicken types, with Kedu chickens having higher values than those of the broilers. As a critical part of the innate immune system, high heterophil implies that Kedu chickens exhibit stronger immune competence than broilers. Similar findings were also made by Malila *et al.* (2023), who found that Thai native chickens exhibited a better innate immune system than modern broiler types. However, in contrast to our study, Anoh *et al.* (2025) noted no difference between Philippine native chickens and broilers with respect to the heterophil count in the blood. In another study, Duangjinda *et al.* (2017) reported that native chickens had lower heterophil counts than modern broilers. Overall, the above variations seemed to be closely related to the genetic potential of each indigenous chicken (Duangjinda *et al.*, 2017; Anoh *et al.*, 2025).

Irrespective of chicken type, the values of erythrocytes, hemoglobin, and hematocrit decreased in response to heat stress. In such cases, heat stress

inhibits the production of the hormone erythropoietin, thereby inhibiting erythrocyte production (Ayo & Ogbuagu, 2021). This causes a decrease in hematocrit and hemoglobin in chickens exposed to heat stress. Regardless of heat stress treatment, MCV and MCH levels were higher in the Kedu chickens than in the modern broilers. Similarly, the levels of MCV and MCH were found to be higher in indigenous Vanaraja chickens than in broiler chickens (Mohanty & Acharya, 2020). In contrast to our findings, Baudouin *et al.* (2021) reported that indigenous chickens had lower MCV and MCH values than broilers. MCV is a parameter used to indicate the size of erythrocytes, while MCH is used to measure the amount of hemoglobin per blood cell. Although the values of both parameters vary between chicken species, they remain within the normal physiological range (Odunitan-Wayas *et al.*, 2018). Irrespective of high temperature treatment, platelet counts were higher in the modern broilers than in the Kedu chickens. In agreement with our findings, Mabelebele *et al.* (2017) reported that Ross 308 broilers had higher platelet counts than indigenous Venda chickens. Platelets can indicate potential inflammation in chickens. This is because they release inflammatory mediators and are involved in the immune response, with levels increasing under conditions of stress and infection (Ferdous & Scott, 2023). Based on this, it can be assumed that broiler chickens have a higher potential for stress than native types. In our study, PDW increased significantly in the broiler chickens due to heat stress, but this was not observed in the Kedu chickens. PDW shows heterogeneity of platelet morphology due to the presence of large platelets alongside normal-sized ones. Under conditions of heat stress, Farran *et al.* (2025) also reported an increase in PDW in laying hens. This is because heat stress can induce inflammation and platelet activation, encouraging the chicken's body to produce younger and activated platelets, which vary more in size, thereby increasing PDW (Farran *et al.*, 2025).

The blood proportion (relative to live body weight) was higher in the Kedu chickens than in the modern broilers. Generally, blood volume in chickens varies, largely determined by their body weight. Chickens with smaller weights typically have a higher blood percentage than those with larger body weights. Despite the Kedu chicken's smaller body weight, their higher blood percentage indicates that this type of chicken has better oxygen transport and greater heat dissipation capacity, thus significantly supporting its resilience to heat stress (Beckford *et al.*, 2020). In both types of chicken, heat stress caused a decrease in the blood percentage relative to the chicken's body weight. Consistent with our study, Kim *et al.* (2025) noted a decrease in whole blood volume in broiler chickens reared at 33 °C for 31 days, compared to broiler chickens reared at 21 °C for the same period. In this context, heat stress can cause significant loss of body fluids, primarily through increased panting frequency, which releases water vapor. Unlike Kedu chickens, high temperatures during rearing reduced the proportion of feathers in the broiler chickens. Wasti *et al.* (2020) reported that

heat stress causes broiler chickens to lose their feathers because, when heat-stressed, they frequently flap their wings to cool their bodies. Over time, this activity can potentially damage the chicken's feathers. In contrast to studies reporting an increase in relative liver weight in broiler chickens due to fat accumulation under heat stress (Ma *et al.*, 2022), the relative liver weight of Kedu chickens in fact decreased due to heat stress over the two-week rearing period. The precise reason for this decrease remains unclear, but it is highly likely that the heat stress treatment did not cause fat accumulation in the liver of Kedu chickens. Furthermore, the reduction in final body weight (although not statistically significant) in the Kedu chickens may further explain the decrease in relative liver weight, as the live body weight of the Kedu chickens serves as the denominator during calculations. This condition causes a decrease in the relative liver weight in these chickens exposed to heat stress. Although heat stress did not affect relative gizzard weight, the Kedu chickens exhibited a higher relative weight than the broilers. The higher crude fiber content in Kedu chicken feed compared to that in broiler feed was likely responsible for the greater relative gizzard weight in the Kedu chickens. Jha and Mishra (2021) explain that crude fiber in feed can stimulate the physical grinding activity of the gizzard, thereby stimulating more intensive muscle layer development and ultimately resulting in increased gizzard weight. Our study shows that Kedu chickens have a higher relative weight of immune organs, especially the spleen and *bursa of Fabricius*, compared to broilers, indicating that they have superior immune competence. Although no research has directly compared the relative weight of immune organs in native and broiler chickens, the higher relative weight of the spleen and *bursa of Fabricius* in the Kedu chickens indicates that native chickens, especially the Kedu type, have stronger immune competence than broilers (Lola *et al.*, 2016). Specifically related to heat stress treatment, high temperatures during the two-week rearing period significantly reduced the relative weight of the immune organs, especially the thymus and *bursa of Fabricius*, in both chicken types. The relative weight reduction of the two organs at high temperatures represents a thermal stress response of the immune system in both chicken species. These primary lymphoid organs are susceptible to stress, and their relative weight reduction is indicative of immunosuppression or a decrease in the proliferative capacity of T and B lymphocytes. This is consistent with the literature, which shows that heat stress can trigger the release of corticosterone, which induces atrophy in the thymus and *bursa of Fabricius*, potentially reducing the chicken's immune capacity against pathogens (Huang *et al.*, 2024; Kim *et al.*, 2025).

CONCLUSION

Heat stress for two weeks prior to market age impaired the physiological and hematological variables of the chickens, as indicated by the increases in rectal temperature, respiratory rate, heart rate, and blood levels of glucose, uric acid levels and MCHC, alongside

reductions in erythrocytes, hemoglobin and hematocrit, the proportions of blood and feathers to live body weight, and the relative weights of the thymus and *Bursa of Fabricius*. Compared to broilers, Kedu chickens showed more tolerance to heat stress, as indicated by the absence of an effect on their weight gain, blood glucose levels, PDW, and feather proportion. Therefore, farmers do not need to build environmentally controlled broiler houses for Kedu chickens, as they do not require the same environmental conditions as commercial broiler types.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

We state that generative AI and AI-assisted technologies were used for language refinement. All the content has been critically reviewed and edited by the authors.

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