International Journal of Biometeorology
https://doi.org/10.1007/s00484-024-02744-z

ORIGINAL PAPER

Comparative assessment of climate resilient potential in four poultry genotypes reared in hot-humid tropical environment: a preliminary evaluation

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Received: 20 January 2024 / Revised: 20 June 2024 / Accepted: 18 July 2024

Abstract

The general objective of this study is to comparatively assess the climate-resilient potential of four different poultry genotypes-Giriraja ($n=8$), Country chicken ($n=8$), Naked neck ($n=8$), and Kadaknath ($n=8$)—reared in a hot-humid tropical environment. Birds from all genotypes had *ad libitum* access to feed and water and were exposed to identical environmental temperatures in the experimental shed. Diurnal meteorological data were recorded inside and outside the shed daily. Blood biochemical, hormonal, and endocrine variables were monitored monthly until the birds reached 12 weeks of age. Significant variations ($P<0.01$) were observed at different intervals in variables, including total protein, albumin, globulin, triglycerides, and cholesterol. Genotype-specific differences were noted in triglycerides ($P<0.01$), albumin ($P<0.01$), total protein ($P<0.05$), and cholesterol ($P<0.05$). Inter-genotype variations ($P<0.05$) were also observed in serum cortisol, T_3, and T_4 levels. Distinct variations ($P<0.05$) were also observed during specific intervals, particularly in cortisol and T_3 levels. The study of hepatic mRNA expression of *HSP*s and *HSF-1* revealed a significant breed difference ($P<0.05$) in the expression pattern of *HSP60*, *HSP70*, *HSP90*, and *HSP110*, while no difference was observed between genotypes for *HSP40* and *HSF-1*. The study highlights the Naked Neck breed as an exemplar of resilience, showcasing its distinctive ability to maintain homeostasis under heat stress compared to other genotypes. The genetic and physiological insights gained from this investigation offer prospective pathways for aligning sustainable poultry farming with environmental exigencies.

Keywords Heat stress · Heat shock proteins · Backyard poultry farming · Nacked neck

Introduction

The global human population is expected to reach 9.6 billion by 2050 (Mottet and Tempio 2017). In parallel, Alexandratos and Bruinsma (2012) anticipate a 70% growth in demand for animal-derived food from 2005 to 2050. While beef and pork production anticipate increases of 66% and 43%, poultry meat stands out with a remarkable 121% growth projection, complemented by a 65% surge in egg demand (Mottet and Tempio 2017). However, the specter of climate change, which has garnered substantial attention due to its pervasive consequences across sectors, including livestock (Sejian et al. 2018; Shashank et al. 2023), poses a critical threat to poultry production. The IPCC Fifth Assessment Report foresees a rise in global average surface temperatures by 2.2 to 3.5 ºC by 2100, resulting in increased heat stress affecting not only human populations but also the poultry industry. Notably, heat stress inflicts substantial economic losses, with the U.S. poultry industry alone witnessing annual losses of $128 to $165 million out of the $1.69 to $2.36 billion in the broader livestock industry (St-Pierre et al. 2003). In light of the escalating heat stress challenges faced by poultry production due to climate change, the exploration and development of adapted poultry breeds represent a crucial avenue for sustaining and securing the industry's future resilience.

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Published online: 31 July 2024

Indigenous chickens are well known for their adaptability to local climatic and geographical conditions. These indigenous fowls are active, resistant to diseases, and require minimal care and management. They play a great role in increasing the income and livelihood of rural families. Indigenous chicken is better adapted to scavenging systems of backyard rearing (Ahlawat et al. 2004). As per the resources, the organized sector of the poultry industry in India meets about 67% of the total output and the remaining 33% comes from the unorganized sector (backyard poultry farming) (Kumar et al. 2021). Although backyard poultry farming has low productivity, it still plays an important role in the food and nutritional security of rural people living in fragile and resource-poor ecosystems (Chaiban et al. 2020; FAO and IFAD 2022).

Backyard poultry farming, a practice deeply rooted in many communities, has been a reliable source of nutrition, income, and cultural significance for households around the world (Singh et al. 2022). Backyard poultry is a source of scarce animal protein in the form of meat and eggs (FAO 2013). Backyard poultry helps in pest control, provides manure, converts kitchen waste into good-quality protein, and is required for religious and social ceremonies (Alders and Pym 2009). However, in the face of contemporary challenges such as climate change, the backyard poultry farming faces new and evolving dynamics that necessitate a closer examination of its sustainability and resilience. The intrinsic connection between these locally adapted poultry breeds and climate change resilience underscores the critical importance of research in unraveling the unique attributes that can fortify backyard poultry farming against the evolving challenges posed by a changing climate.

As climate change introduces new variables such as altered temperature patterns, unpredictable precipitation, and the proliferation of novel diseases, the need to comprehend and harness the innate resilience of these breeds become increasingly apparent. Research in this realm is not merely an academic pursuit; it is a proactive measure to safeguard food security, preserve cultural heritage, and empower communities reliant on small-scale poultry farming. Considering the aforementioned factors, this study aims to explore the adaptive responses of four indigenous poultry breeds to severe heat stress. Through an integrated analysis of meteorological variables, biochemical markers, endocrine dynamics, and molecular expressions, we seek to uncover breed-specific strategies for coping with climatic challenges. By unraveling the physiological and genetic aspects of these breeds that contribute to their resilience in the face of environmental challenges, we aim to develop a robust understanding of these adaptive mechanisms. This knowledge, in turn, can be utilized to formulate targeted propagation strategies for these breeds among farmers and empower the financial well-being of farmers.

Materials and methods

Study site

The study was conducted at the grower house of the Department of Livestock Farm Complex (LFC), Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India, which is located in the semi-arid region of the country with a latitude and longitude of 11° 94' N and 79° 80' E and, at an elevation of 15 m above mean sea level. The average annual rainfall is 67.83 mm depends mostly on the North-East monsoon. The annual mean temperature of around 30 °C and 70–85% relative humidity. The annual rainfall in this area is around 600–750 mm (About District, 2024).

Birds and accommodation

The study involved the examination of four distinct poultry genotypes: Giriraja (GR), Country Chicken (CC), Naked Neck (NN), and Kadaknath (KN). These birds were sourced from the hatchery facility affiliated with LFC, RIVER. The study was conducted for a period of 90 days with straight run chicks. All birds were housed in a homogenous, well-ventilated, open-sided deep litter facility with asbestos roofing, characterized by an east-to-west orientation and variable height parameters (4.26 m at the center and 3.6 m at the sides). Uniform concrete flooring and paddy husk bedding were provided, and the birds had access to *ad libitum* feed and water throughout the study. The experimental facility, including the house, walls, and floor, underwent thorough washing and fumigation with formaldehyde before the arrival of the chicks and the commencement of the experiment. Chicks of all genotypes from the same hatch were selected at one day of age, individually weighed, and wing-banded before allocated to their respective groups. The experimental birds had ad libitum access to good-quality drinking water. Additionally, all birds were housed in a lighting regimen of 12 L:12D. Care and management practices for all treatment groups were uniform and consistent throughout the experimental period. Stringent hygienic measures and a sanitation program were implemented during the study period. Furthermore, the birds were vaccinated against diseases according to the vaccination schedule followed at LFC, RIVER, ensuring that the birds maintained healthy condition throughout the study.

Experimental details

The study was conducted for a period of 90 days during the summer season (June - August). The birds were randomly divided into four groups i.e. GR ($n=50$), CC ($n=50$), NN ($n=50$), and KN ($n=50$), having 2 replicates of 25 birds each, according to a completely randomized design. All four poultry genotypes were exposed to the same environmental temperatures inside the experimental shed. All experimental birds were assigned to two different dietary schedules during the trial period (0–8 weeks- Layer starter; 9–12 weeks- Layer grower) (layer starter- CP: 21.36%, TDN: 85%, and ME: 3827Kcal/kg; layer grower- CP: 15.30%, TDN: 90%, and ME: 3751Kcal/kg). Table 1 describes the chemical composition of the feed offered to the birds. All adaptation variables were recorded at monthly intervals (30, 60, and 90 days, respectively). At the end of the experiment, the birds ($n=8$/ group) were sacrificed and liver samples were collected for comparative analysis of various adaptive molecular variables. The experiment was conducted after obtaining due approval from Institute Animal Ethics Committee (IAEC approval number: 2/RIVER/2023).

Variables studied

Meteorological variables

Micro and macro environment climatic data such as dry and wet bulb temperature in degrees Celsius (ºC) was measured at 0730 h and 1430 h by analogue hygrometer (Zeal, UK) throughout the experimental period both outside and inside the shed. Temperature-Humidity Index (THI) was calculated from the dry bulb and wet bulb temperature using the following formula Zulovich and DeShazer (1990).

$THI = 0.6\ T_{db} + 0.4\ T_{wb}$.

Where T_{db} = Dry bulb temperature (ºF) and T_{wb} = Wet bulb temperature (ºF).

Serum variables

Blood samples were collected at monthly intervals (30, 60, and 90 days, respectively) from all the groups ($n=8$/ group) (same birds selected for slaughter were utilized for blood sample collection, ensuring consistency in the dataset) in sterile serum collection tubes with clot activator (BD, Franklin Lakes NJ, USA) through wing vein puncture, posing minimal disturbance to the birds. Blood samples were placed on ice until centrifugation (2,500 rpm for 25 min to separate the serum), and stored at -20 °C for further analysis of serum biochemical and hormonal variables.

Biochemical variables such as Total protein, Albumin, Globulin, Cholesterol, and Triglycerides were estimated using the Coral Clinical System kit, Tulip Diagnostics (P) Ltd. U. K. India. Serum Cortisol, Triiodothyronine (T_3), and Thyroxine (T_4) were estimated by enzyme-linked immunosorbent assay (ELISA) using a microplate reader (Thermo Scientific, Finland) by ELISA kits (Puregene, Genetix Biotech Asia Pvt. Ltd). The analytical sensitivity of the kits was 0.016ng/mL; 0.013ng/mL and 0.24ng/mL for cortisol, T_3 and T_4, respectively. The intra-assay and inter-assay coefficients were $<8\%$ and $<10\%$ for all the ELISA kits.

RNA isolation, cDNA synthesis, and qPCR from liver samples

At the end of the experiment (i.e., 90th day) the birds ($n=8$/ group) were sacrificed for liver tissues. Liver samples (approximately 200 mg) were rinsed in saline before being placed in a micro-centrifuge tube with 1 mL of RNAlater (Ambion, Darmstadt, Germany). Subsequently, they were processed and stored at -80 °C for later mRNA extraction and analysis. Total RNA was extracted from liver samples ($n=3$ in each group) using Trizol reagent, following the guidelines provided by the manufacturer (Invitrogen, Corp., CA, USA). The concentration and purity of the RNA were assessed using a NanoDrop ND-1000 spectrophotometer

Table 1 Chemical composition of the diet offered during the study period

Attributes	Experimental diets	
	Layer starter (0–8 weeks)	Layer grower (9–12 weeks)
Chemical composition (%)		
Dry Matter	89.45	87.46
Crude protein	21.36	15.30
Crude fiber	5.38	8.28
Ether extract	3.49	7.36
Total ash	7.34	10.02
Acid insoluble ash	2.39	3.09
Calcium	0.70	1.20
Phosphorus	0.86	1.08
Salt	0.39	0.42
Moisture	10.55	12.54

%Percentage

(Thermo Scientific, USA) and an Experion Bio-analyzer (Bio-Rad, USA). The OD 260/280 absorption ratios for the various samples ranged from 1.90 to 2.10. Further, cDNA was synthesized using the Revertaid First strand cDNA synthesis kit (Thermo Scientific, CA, USA), according to the manufacturer's protocols. The primers for gene expression analysis were designed using Primer 3.0 software, and the primer sequences are detailed in Table 2. Before using them as templates for qPCR, each of the cDNA samples was diluted 1:5 (v: v) with DNase/ RNase-free water. The qPCR reactions were carried out in duplicate with a total reaction volume of 20 µL, including 50 ng of template, 10 µL (2X) Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Thermo Fisher Scientific), 0.5 µM primer concentration, and the remaining volume made up by DNase/RNase-free water per sample. The real-time qPCR reactions comprised an initial step of 10 min at 95°C, followed by 40 amplification cycles of 15 s at 95°C (denaturation), annealing at 60°C for 30 s, and extension at 72°C for 30 s. The $2-\Delta\Delta CT$ method (Livak and Schmittgen 2001) was employed for the analysis of relative gene expression data.

Statistical analysis

The data were analyzed by the general linear model (GLM) repeated measurement analysis of variance (SPSS 20.0). The effect of fixed factors, namely breed (GR, CC, NN, and KN), was considered as between subjects' factors, and days (day 30, 60, and 90, respectively) were considered as within-subjects factor. Also, the interaction between breed and days was analyzed on various variables studied. Comparison of means of the different subgroups was made by Duncan's multiple range tests as described by Kramer (1957). The changes in the relative expression of hepatic *HSP70*, *HSP40*, *HSP60*, *HSP90*, *HSP110*, and *HSF-1* mRNA to *GAPDH* were analyzed by One-way analysis of variance (ANOVA) with Tukey's post hoc test to compare the means across the genotypes. The significant level was set at $P < 0.05$.

Results

Meteorological variables

Figure 1 illustrates the average THI values throughout the study period. Experimental birds were subjected to the prevailing microenvironment, with mean values of approximately 90.90 and 89.34 units inside and outside the experimental shed, respectively, during the afternoon, indicating severe heat stress. In the morning, the THI values were 85.71 and 84.38 units inside and outside the experimental shed, respectively.

The highest THI was recorded in June, reaching 93.02 and 94.49 units during the morning and afternoon outside the shed, respectively. According to Zulovich and DeShazer (1990), THI can be categorized into four levels: comfort (THI < 70), alert (THI 70–75), danger (THI 76–81), and emergency (THI > 81) zones. Considering the aforementioned data, we can classify our THI values under the emergency category, indicating the substantial impact of heat load on the birds.

Biochemical variables

Table 3 summarizes the impact of heat stress on serum biochemical variables in experimental birds. Significant differences ($P < 0.01$) were observed during different intervals in variables such as total protein, albumin, globulin, triglycerides, and cholesterol. Furthermore, noteworthy variations

Table 2 Primer sequences, and accession numbers in chicken

Target Genes	Sequence	Accession number	T_m (°C)	Species
HSP70	F: AATGAACCCCACCAACACCA R: TTCATGTCCGACTGCACTGT	NM_001006685.2	60 °C	*Gallus gallus domesticus*
HSP40	F: AGTGGCACCCTGACAACTTT R: TTTCCGCCTCATTTCTGGGT	NM_001008437.3	60 °C	
HSP60	F: AAGGTTGGACGGAAAGGTGT R: TCACATTTCTGCCCTTTGGC	NM_001012916.3	60 °C	
HSP90	F: TAGCGCGTTTGCTGATTCTG R: GCCCAAAGGTTCACCGTTAT	NM_001109785.2	60 °C	
HSP110	F: ATCCCAGATGTCAAGAAAACAAGTG R: CTGCCAAACCAAGTTAGCTTCA	NM_001159698.2	60 °C	
HSF-1	F: AGCACCCCTATTTCATCCGT R: TTTATGCTGGACACGCTGGT	NM_001305256.1	60 °C	
GAPDH	F: ATGGTCCACATGGCATCCAA R: TCCAACAAAGGGTCCTGCTT	NM_204305.2	60 °C	

HSP70 Heat shock protein 70, HSP40 Heat shock protein 40, HSP60 Heat shock protein 60, HSP90 Heat shock protein 90, HSP110 Heat shock protein 110, HSF-1 Heat shock factor-1, GAPDH Glyceraldehyde 3-phosphate dehydrogenase, °C Degree Celsius, *F* Forward, *R* Reverse, T_m Melting temperature

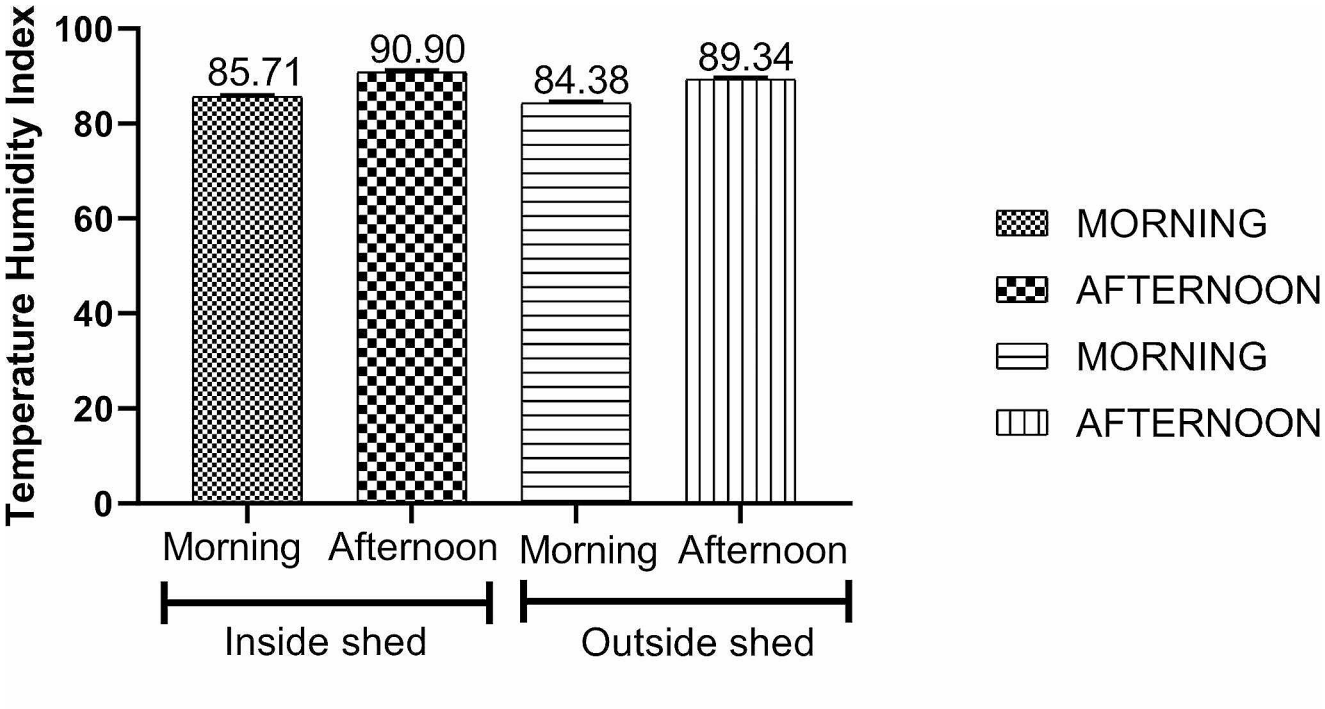

Fig. 1 The Temperature-humidity index (THI) values for both inside and outside the shed

within genotypes were identified in variables such as triglycerides ($P<0.01$), albumin ($P<0.01$), total protein ($P<0.05$) and cholesterol ($P<0.05$). Furthermore, considerable differences in the interaction between genotype and interval were noted in variables such as albumin ($P<0.01$), triglycerides ($P<0.01$), and cholesterol ($P<0.05$).

At cursory look, it was observed that in variables such as total protein, albumin, and triglycerides, the NN genotype exhibited significantly ($P<0.05$) higher values compared to the other experimental groups, while the GR genotype demonstrated significantly ($P<0.05$) lower values. Notably, no significant ($P>0.05$) differences were observed between genotypes in globulin levels; however, numerically, NN displayed the highest value while GR exhibited the lowest. Conversely, in the case of cholesterol levels, significantly ($P<0.05$) higher values were observed in the GR genotype, with the lowest values recorded in the NN genotype. However, the results underscore genotype-specific responses to heat stress, revealing significant variations in serum biochemical variables. The intricate interplay between genotype and interval further accentuates the dynamic nature of these responses, providing valuable insights into the physiological impact of heat stress on poultry health.

Endocrine variables

Table 4 provides a comprehensive summary of the effects of heat stress on serum endocrine variables in the experimental avian subjects. Statistical analyses revealed significant differences ($P<0.05$) between genotypes, particularly in variables such as cortisol, T_3, and T_4. Moreover, noteworthy variations ($P<0.05$) were discerned during distinct intervals, specifically in cortisol and T_3 levels. Furthermore, the interaction between genotype and interval significantly influenced T_4 levels, highlighting the nuanced dynamics of endocrine responses under varying conditions.

Upon meticulous scrutiny, it is discernible that the GR genotype exhibited the highest levels of cortisol, succeeded by KN, CC, with the lowest levels observed in the NN genotype. Conversely, concerning T_3 levels, the NN genotype demonstrated the highest concentrations, whereas CC exhibited notably ($P<0.05$) lower concentrations compared to NN. Concerning T_4 levels, both GR and NN genotypes displayed nearly equivalent concentrations, significantly ($P<0.05$) distinct from CC and KN. Nonetheless, numerically superior concentrations were noted in NN, while the lowest were observed in CC. This nuanced comprehension of endocrine reactions elucidates the distinct hormonal adjustments among different genotypes in response to heat stress.

Molecular variables

Figure 2 presents the impact of heat stress on hepatic mRNA expression in the experimental poultry subjects. A significant breed difference ($P<0.05$) was evident in the hepatic

Table 3 Impact of heat stress on serum biochemical variables in Giriraja, Country chicken, Naked neck, and Kadaknath birds

Attributes	Months	Treatments				Effects		
		GR	CC	NN	KN	Genotype	Interval	G*I
Total Protein (mg/dL)	1	2.64	2.93	3.12	3.15	*	**	ns
	2	3.17	3.51	3.49	3.50			
	3	3.89	3.96	4.34	3.83			
	Mean	**3.24^{b}**	**3.47^{ab}**	**3.65^{a}**	**3.49^{ab}**			
	Pooled SE	± 0.36	± 0.30	± 0.36	± 0.20			
Albumin (g/dL)	1	1.35	1.40	1.47	1.61	**	**	**
	2	1.52	1.58	1.84	1.67			
	3	1.62	1.79	2.03	1.54			
	Mean	**1.50^{b}**	**1.59^{b}**	**1.78^{a}**	**1.60^{b}**			
	Pooled SE	± 0.08	± 0.11	± 0.16	± 0.04			
Globulin (g/dL)	1	1.28	1.52	1.65	1.43	ns	**	ns
	2	1.83	1.92	1.77	1.74			
	3	2.26	2.16	2.31	2.27			
	Mean	**1.79^{a}**	**1.87^{a}**	**1.91^{a}**	**1.81^{a}**			
	Pooled SE	± 0.28	± 0.18	± 0.20	± 0.24			
Triglycerides (mg/dL)	1	114.48	145.42	155.32	157.67	**	**	**
	2	106.00	102.63	128.19	106.17			
	3	64.88	72.34	80.64	64.38			
	Mean	**95.12^{c}**	**106.80^{b}**	**121.38^{a}**	**109.41^{b}**			
	Pooled SE	± 15.32	± 21.20	± 21.83	± 26.98			
Cholesterol (mg/dL)	1	90.79	82.40	92.41	94.68	*	**	*
	2	86.09	81.73	78.83	85.90			
	3	129.62	118.51	99.09	111.74			
	Mean	**102.17^{a}**	**94.21^{b}**	**90.11^{b}**	**97.44^{ab}**			
	Pooled SE	± 13.79	± 12.15	± 5.96	± 7.58			

Values bearing different superscripts within a row differ significantly from each other ($P < 0.05$)

mg/dL Milligram per deciliter, *g/dL* Gram per deciliter, *GR* Giriraja Chicken, *CC* Country Chicken, *NN* Naked Neck Chicken, *KN* Kadaknath Chicken, *G*I* Genotype and Interval Interaction

** $P < 0.01$, * $P < 0.05$, *ns* Non-significant; *SE* Standard error

Table 4 Impact of heat stress on serum endocrine variables in Giriraja, Country chicken, Naked neck, and Kadaknath birds

Attributes	Months	Treatments				Effects		
		GR	CC	NN	KN	Genotype	Interval	G*I
Corticosterone (ng/mL)	1	0.56	0.56	0.53	0.74	*	*	ns
	2	1.04	0.62	0.48	0.59			
	3	0.88	0.61	0.64	0.79			
	Mean	**0.83^{a}**	**0.60^{b}**	**0.55^{b}**	**0.71^{ab}**			
	Pooled SE	± 0.14	± 0.02	± 0.05	± 0.06			
T_3 (ng/mL)	1	0.58	0.40	0.55	0.62	*	*	ns
	2	0.58	0.42	1.01	0.49			
	3	0.67	0.59	0.73	0.54			
	Mean	**0.61^{ab}**	**0.47^{b}**	**0.76^{a}**	**0.55^{b}**			
	Pooled SE	± 0.03	± 0.05	± 0.13	± 0.03			
T_4 (ng/mL)	1	67.38	33.86	56.26	37.01	*	ns	**
	2	60.15	41.92	64.10	40.94			
	3	49.03	49.39	57.07	53.24			
	Mean	**58.86^{a}**	**41.72^{b}**	**59.14^{a}**	**43.73^{b}**			
	Pooled SE	± 5.33	± 4.48	± 2.49	± 4.88			

Values bearing different superscripts within a row differ significantly from each other ($P < 0.05$) *ng/mL* Nanogram per milliliter, *GR* Giriraja Chicken, *CC* Country Chicken, *NN* Naked Neck Chicken, *KN* Kadaknath Chicken, *G*I* Genotype and Interval Interaction

** $P < 0.01$, * $P < 0.05$, *ns* Non-significant; *SE* Standard error

mRNA expression pattern of *HSP60*, *HSP70*, *HSP90*, and *HSP110*. However, no difference ($P > 0.05$) was observed between genotypes concerning *HSP40* and *HSF-1*. Upon closer inspection, it is noteworthy that the expression patterns of the aforementioned genes are numerically higher in the NN breed compared to the remaining groups. While statistical significance was established for certain genes, this numerical elevation in expression across multiple heat shock proteins in the NN breed suggests a potential heightened molecular response to heat stress in this specific genotype.

Discussion

In the realm of sustainable agricultural practices, backyard poultry farming emerges as a resilient and environmentally conscious endeavor, offering both economic benefits and ecological advantages (Singh et al. 2022). As global temperatures rise and weather patterns become increasingly unpredictable (Kpomasse et al. 2021), indigenous poultry breeds, known for their resilience and adaptability, face new and intensified challenges. Recognizing the intricate interplay of climate, agriculture, and biodiversity assumes paramount importance in ensuring the sustained prosperity of small-scale poultry keepers amidst a dynamically changing climate (Kpomasse et al. 2023). A comprehensive understanding of the underlying mechanisms governing poultry breeds in the context of climate change is imperative for meeting the escalating demands of the increasing global population.

The pivotal role of plasma total protein, encompassing both albumin and globulin, as a key indicator of overall protein status in the body is underscored by the contrasting profiles observed in the current study. At a cursory look, GR exhibits lower levels of total protein, along with reduced albumin and globulin concentrations, suggesting a potential concern in protein homeostasis. Conversely, NN genotype presents a contrasting scenario, with higher total protein levels, accompanied by elevated albumin and globulin levels, indicative of a state of enhanced adaptability and improved protein balance.

During heat stress, birds require more energy for survival, resulting in a noticeable reduction in feed intake (Biswal et al. 2022). Under these circumstances, birds' resort to utilizing non-carbohydrate precursors, such as proteins, for their energy needs. These protein sources are characterized by a lower heat increment, leading to reduced heat production under heat stress conditions (Mazzoni et al. 2022). Interestingly, GR had higher feed intake compared to the rest of the group, followed by NN, with the lowest intake observed in CC (Hemanth et al. 2024). Despite the higher feed intake in GR, this study found that GR had lower total protein, albumin, and globulin levels. Consequently, this utilization of proteins may contribute to the observed elevation in total protein levels in NN. Another factor contributing to increased total protein is the rise in globulin, which is associated with an enhanced immune response in birds subjected to thermal conditioning (Folarin et al. 2022).

In the current study, an increase in total protein, albumin, and globulin levels is noted especially in NN genotype, aligning with the findings of Sivaramakrishnan (2017), who observed an increase in total protein levels in birds exposed to HS. However, the GR genotype exhibits a decrease in total protein, albumin, and globulin levels, indicative of potential alterations in protein metabolism even with higher feed intake under chronic HS conditions. This observed reduction may signify diminished protein digestion, decreased protein breakdown, and attenuated protein synthesis within muscle tissues in birds exposed to HS, as outlined in previous studies (Siddiqui et al. 2020). Consequently, these changes may result in a decline in plasma protein levels, potentially compromising the immune responses of avian subjects.

On the contrary, with the exception of the NN genotype, the values observed in the CC, KN, and GR groups correspond to the findings reported by Livingston et al. (2022), who demonstrated a significant decrease in total protein levels in birds exposed to heat stress. Comparable outcomes were also noted by Hassaan et al. (2020) in groups exposed to HS when compared with unexposed group, showing variations in total protein, albumin, and globulin levels. These discrepancies may be attributed to differences in temperature, genetic makeup, and the duration of exposure to heat stress. In conclusion, it is noteworthy that the NN genotype exhibited superior performance compared to the other groups, maintaining total protein, albumin, and globulin levels in plasma without compromise. This suggests that NN possesses a remarkable capacity to maintain necessary homeostasis, enabling the birds to withstand the challenges imposed by heat stress.

In general poultry can synthesize fatty acids from non-lipid precursors, such as carbohydrates, with the primary site of this synthesis being the liver. Heat stress can induce increased lipolysis, the breakdown of triglycerides into fatty acids and glycerol, as a response to elevated energy demands during heat stress. In poultry the relationship between elevated fatty acids and cholesterol metabolism in chickens is intricate, involving various mechanisms (Liao et al. 2022).

In the current study, there was an observed increase in triglycerides and a decrease in cholesterol levels for all genotypes, except for GR. This may be attributed to the elevated output of triglycerides by the liver and increased fat deposition in the heat stress group due to chronic heat exposure (Lu et al. 2017, 2018). Our findings align with the results of

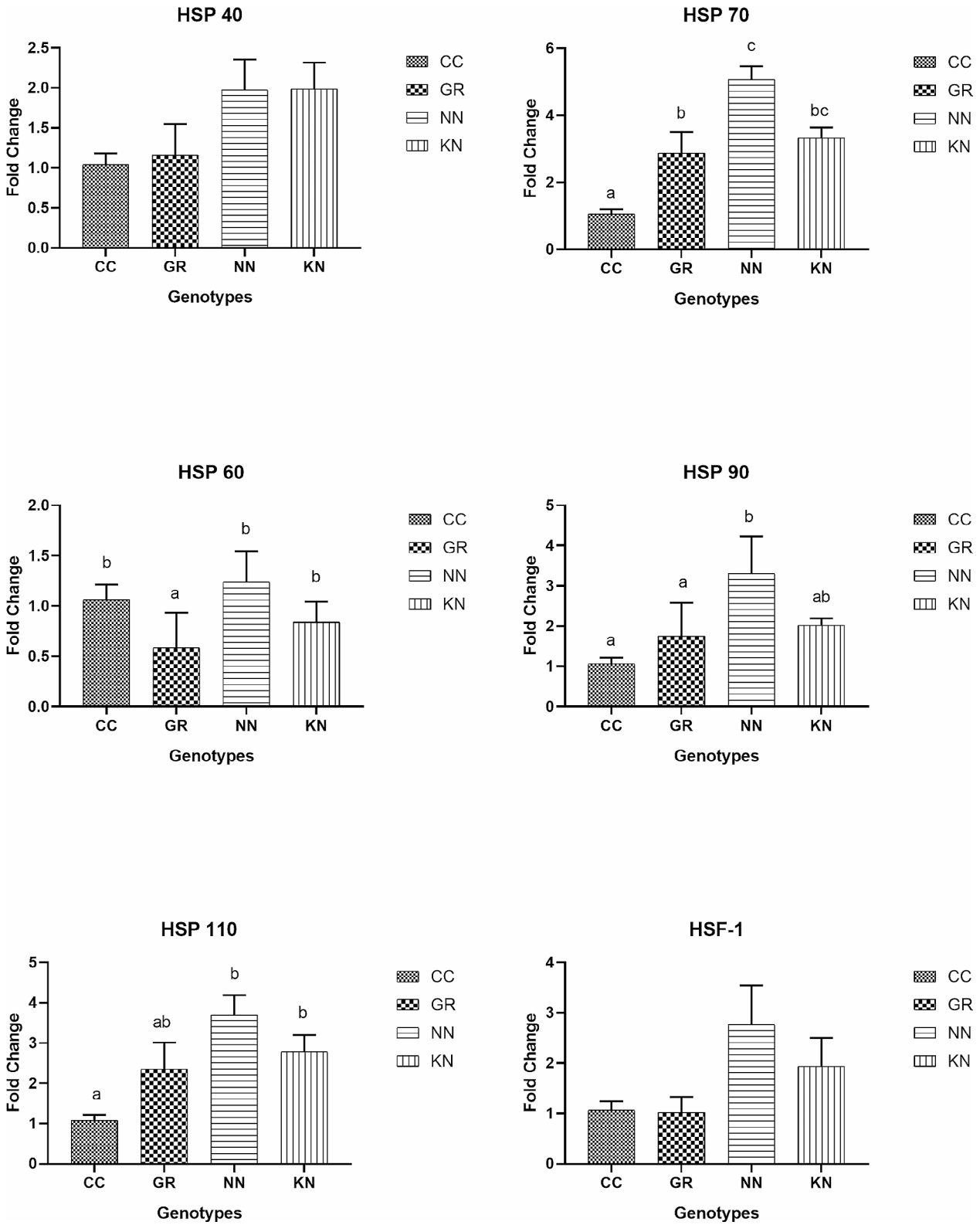

Fig. 2 Impact of heat stress on relative hepatic mRNA expressions in Giriraja, Country chicken, Naked neck, and Kadaknath birds
Values bearing different superscripts differ significantly from each other ($P < 0.05$)
GR Giriraja Chicken, *CC* Country Chicken, *NN* Naked Neck Chicken, *KN* Kadaknath Chicken
HSP70 Heat shock protein 70, *HSP40 Heat shock protein 40*, *HSP60 Heat shock protein 60*, *HSP90 Heat shock protein 90*, *HSP110 Heat shock protein 110*, *HSF-1 Heat shock factor-1*

Lu et al. (2019), where birds exposed to heat stress exhibited a significant increase in triglycerides. Similar observations were noted in pigs exposed to heat stress, where plasma triglyceride uptake and storage were facilitated in adipose tissues, leading to greater fatness (Kouba et al. 2001). This change in fat distribution in heat-exposed pigs is considered an adaptation to high ambient temperatures, promoting increased heat loss (Le Dividich et al. 1998; Kouba et al. 2001). Furthermore, heat-exposed chickens also displayed enhanced fat deposition (Geraert et al. 1996). Moreover, in line with the findings of Qu et al. (2015), poultry exposure to HS triggers an upregulation of lipoprotein lipase (LPL). This heightened LPL activity facilitates increased hydrolysis within serum lipoproteins, resulting in elevated levels of fatty acids and glycerol. Consequently, these substrates are re-esterified into triglycerides within adipocytes, ultimately leading to an augmentation of adipose tissue mass. This increase in adipose tissue mass, as previously discussed, may represent an adaptive mechanism employed by genotypes such as NN, KN, and CC, aiming to reduce metabolic heat generation during HS. In contrast, Moraes et al. (2003) and Shim et al. (2006) reported a decrease in triglyceride and an increase in cholesterol levels in birds exposed to higher temperatures.

Interestingly, there is a significant decrease in the cholesterol level of NN, indicating a lower proportion of fat compared to the rest of the experimental birds. Similar results were observed in NN by Rajkumar et al. (2010) and Patra et al. (2004). According to Patra et al. (2004), lower levels of cholesterol in NN might be because of the lower muscle cholesterol concentration. To conclude, during heat stress, poultry adjusts their protein utilization, contributing to the observed increase in total protein levels and an immune response mediated by elevated globulin. This study provides valuable insights into poultry responses to heat stress, highlighting the intricate relationship between fatty acids, cholesterol metabolism, and the varied metabolic adaptations in different genotypes.

In the context of endocrine responses to heat stress among various genotypes, it is noteworthy that a decline in serum thyroid hormone levels was observed across all genotypes, with the exception of the NN genotype when analyzed within its respective group. The significance of thyroid gland hormones in mitigating heat stress is rooted in their essential role in regulating the metabolic rate of birds throughout growth and egg production. This metabolic regulation is crucial for maintaining physiological functions under varying environmental conditions. However, prolonged exposure to heat stress has been observed to markedly impede the functionality of the thyrotrophic axis in layer hens, leading to a noticeable decline in plasma thyroid hormone concentrations (Decuypere and Kuhn, 1988). Further, the findings pertaining to diminished T_3 levels in our study align with previous research conducted by Melesse et al. (2011), and Gogoi (2016), all of whom reported a significant reduction in plasma T_3 levels in heat-exposed birds compared to their control counterparts. This disruption in thyroid hormone levels may have implications for the overall metabolic homeostasis of the birds, potentially impacting their ability to cope with environmental stressors.

Remarkably, in the case of the NN genotype, there is evidence suggesting its ability to sustain thyroid hormone levels even amidst heat stress conditions, in contrast to other genotypes. This observation underscores the superior adaptive capacity of NN to tropical climates, highlighting its resilience in maintaining metabolic activity without compromising thyroid hormone regulation. This unique trait may confer a distinct advantage to NN in navigating environmental challenges, contributing to its enhanced adaptability and survival in such conditions.

Further, the findings pertaining to diminished T_3 levels in GR align with previous research conducted by Melesse et al. (2011), and Gogoi (2016), all of whom reported a significant reduction in plasma T_3 levels in heat-exposed birds compared to their control counterparts.

Similarly, the present study corroborates the decreased T_4 levels observed by Bayraktar et al. (2021) in heat-stressed birds relative to control birds. Additionally, the study reveals that heat stress induces an elevation in corticosterone secretion, which, in turn, exerts a negative impact on circulating T_3 levels. This phenomenon subsequently triggers the release of corticosteroids, which act to suppress thyroid gland activity, which was evident in GR genotype. Nonetheless, research on the NN genotype has indicated its superior capacity for heat dissipation, enabling it to mitigate the impacts of heat stress. Further exploration into the specific characteristics of these breed may provide deeper insights into its adaptive mechanisms under heat stress conditions.

Regarding corticosterone production, it is noteworthy that corticosterone serves as the primary glucocorticoid hormone in poultry, playing a crucial role in the metabolism of adipose tissue, the regulation of appetite, and the modulation of body thermogenesis (Luijten et al. 2019). Notably, GR exhibited the highest corticosterone levels, while NN displayed significantly the lowest. Remarkably, NN exhibited a capacity to regulate serum corticosterone levels compared to other genotypes, implying superior energy metabolism even when exposed to heat stress. This suggests a potential adaptive advantage in managing the physiological responses to stress within the NN genotype.

Elevated concentrations of glucocorticoids, as evidenced by increased serum corticosterone levels, contribute to the aggregation of brown adipose tissue and heightened

lipogenesis in white adipose tissue, leading to impaired metabolism (Infante et al. 2017). Furthermore, following the GR genotype, the KN breed showed the highest corticosterone levels. This observation may be attributed to the dark phenotypic pigmentation characteristic of the KN genotype, potentially exacerbating heat load in birds and consequently triggering the release of stress hormone. Nejad and Lee (2023) reported analogous findings, noting elevated cortisol levels in cows with black coat color relative to those with white coat color. The pigmentation of the coat influences the absorption and reflection of radiant heat load from the environment (Daramola and Adeloye 2009). In our study, it is conceivable that KN, characterized by darker pigmentation, absorbed more heat load from the environment compared to NN and CC, potentially contributing to the observed higher cortisol levels.

The current findings are consistent with previous studies conducted by Rimoldi et al. (2015), Xu et al. (2018), and Olfati et al. (2018), who reported heightened corticosterone levels in birds exposed to heat compared to their non-exposed counterparts. Corticosterone, a glucocorticoid hormone, exerts inhibitory effects on glucose uptake by muscle and adipose tissues, stimulating the breakdown of muscle protein and adipose tissue. This process promotes gluconeogenesis, facilitating the provision of energy necessary to cope with stress. Activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis in response to heat stress likely underlies these observed effects (Oluwagbenga and Fraley 2023).

Within the framework of the GR genotype, which may exhibit susceptibility to heat stress, the observed increase in corticosterone levels could potentially signify its physiological reaction to environmental stressors. GR genotype, characterized by their lower heat tolerance compared to other genotypes, may exhibit a more pronounced stress response under heat stress conditions, leading to higher corticosterone levels. This decrease in heat tolerance in GR is already established in our previous studies, where livability was reduced due to heat stress compared to other genotypes, namely NN, CC, and KN (Hemanth et al. 2024). Further, this increase in corticosterone might be due to increase in the additional energy, necessary for the activities like respiration rate, heart rate and peripheral blood circulation (Tamzil et al. 2013). Similarly, Tamzil et al. (2013) observed an increase in corticosterone levels in commercial birds when compared with kampong genotype and Arabic chickens, indicating susceptibility of commercial genotypes to stress.

Glucocorticoids like corticosterone can exert both positive and negative effects on the body, depending on the duration and intensity of the stressor. Acute stressors may lead to beneficial outcomes, such as heightened alertness, vigilance, and improved immune responses. Conversely, chronic stress can result in adverse effects, including impaired growth, reproduction, and immune function (Romero et al. 2015). Thus, understanding the interplay between stressors and the hormonal response in different genotypes is crucial for elucidating their adaptive mechanisms and potential implications for overall health and welfare in poultry farming.

Heat shock proteins (*HSP*s) play a pivotal defensive role against heat stress, facilitating protein folding and refolding, protecting proteins from degradation, and maintaining cellular structural integrity (Cedraz et al. 2017). Cellular responses to environmental stressors involve the upregulation of *HSPs* synthesis, enhancing tolerance against diverse stress conditions (Surai 2015). This upregulation of *HSPs* protects cellular proteins from stress induced damage (Murugesan et al., 2017). In the present study, heat stress upregulated all *HSP*s and *HSF-1* when compared with CC (control), with significant breed differences observed in *HSP60*, *HSP70*, *HSP90*, and *HSP110* in the liver cells across four genotypes. This upregulation of *HSP*s in these genotypes might serve as a cytoprotective mechanism, promoting cellular survival.

In our study, hepatic expression of *HSP70* and *HSP90* exhibited upregulation even during chronic heat stress especially with highest fold change in the NN genotype, contrary to some previous findings (Al-Zghoul 2018; Tang et al. 2018), but aligning with others indicating upregulation of these *HSPs* during chronic heat stress (Xie et al. 2014). On a cursory look, NN and KN (indigenous) genotype expressed significantly higher *HSPs* and *HSF-1* mRNA than GR (commercial) genotype. This phenomenon may be attributed to the increased susceptibility of commercial layers to heightened production demands during heat stress (Murugesan et al., 2017), potentially leading to compromised homeostasis. Additionally, the heightened expression of *HSPs* also indicates a protective mechanism against damage from free radicals to liver tissues (Ogi et al. 1999) in indigenous genotypes.

Further, the elevated mRNA expression levels of *HSP60*, *HSP70*, *HSP90*, and *HSP110* in NN genotypes indicate superior thermoregulatory capabilities compared to other genotypes. This superiority is attributed to the presence of the "Na" gene in NN genotypes, which reduces feather coverage, thereby enhancing heat dissipation through increased surface area for evaporative cooling. Consequently, NN genotypes exhibit better adaptation to chronic heat stress, resulting in heightened cellular response and up-regulation of *HSPs* in the liver. Further, *HSP40* is a eukaryotic homologue of bacterial DNAJ protein, which co-operates with HSP70 to facilitate protein folding as reported previously. In our study, *HSP40* and *HSP70* were upregulated in both NN and KN. This suggests that *HSP40* may function

as a co-chaperone during early development, while *HSP70* becomes the primary chaperone as cells age (Bastaki et al. 2023).

HSP90 serves as a critical molecular chaperone vital for preserving protein equilibrium and facilitating cellular responses to stressors, thereby playing an indispensable role in diverse cellular processes and signaling cascades. Differential upregulation of *HSP90* was observed in NN, followed by KN, with the least regulation evident in GR. Previous research (Xie et al. 2014; Wang et al. 2015) corroborates the upregulation of *HSP90* during acute stress but notes its stability following prolonged HS exposure. This later observation is not surprising and indicates that the birds become acclimated to chronic HS which in turn results in no further increase in cellular *HSPs* (Givisiez et al. 1999). It's noteworthy that GR exhibited impaired regulation of *HSP90* mRNA expression, potentially compromising essential protein homeostasis. Conversely, NN and KN demonstrated more effective modulation of *HSP90* expression, suggesting a more robust cellular response to thermal stress.

HSF-1 acts as a transcriptional regulator, inducing the expression of *HSPs* in reaction to cellular stress, thereby fostering cellular survival and the maintenance of proteostasis. Notably, research by Xie et al. (2014) indicates that while *HSF* genes display upregulation following acute heat stress, their expression remains unchanged during prolonged stress, suggesting the critical role of *HSFs*, particularly in the swift transcriptional activation of *HSPs* under acute heat stress conditions. In our current investigation, a notable finding is the significantly lower regulation of GR *HSF-1* compared to NN and KN, a pattern that correlates with the expression levels of other *HSPs*, suggesting potential differences in heat stress response mechanisms among genotypes. Heat shock factors *HSF1* and *HSF3*, which are considered avian specific (Kawazoe et al. 1999) are crucial in heat shock response in chickens. Although previous studies suggested *HSF3*'s role in long-term heat stress (Tanabe et al. 1997; Pardue et al. 1992), its specific contribution remains unexplored in our results. Indigenous poultry, particularly NN, displayed significantly higher expression of *HSP*s and *HSF1* compared to commercial poultry, emphasizing their superior adaptability to heat stress. Understanding these molecular intricacies is essential for devising strategies to protect proteins and cells in the face of climate-induced challenges.

Conclusion

This study investigating the adaptive responses of distinct indigenous poultry breeds exposed to severe heat stress provides a comprehensive understanding of the underlying physiological mechanisms. The intricate interplay of biochemical markers, endocrine dynamics, and molecular expressions underscores the breed-specific strategies employed in the face of climatic challenges. Notably, the Naked Neck breed stands out as an exemplar of resilience, showcasing a unique capacity to maintain equilibrium under heat stress conditions. This delineation of breed-specific attributes not only enriches our understanding of avian adaptability but also lays the foundation for future strategies in poultry husbandry. As we formalize these findings, they serve as a roadmap for the development of robust and resilient poultry stocks capable of navigating the complexities of a changing climate. The genetic and physiological insights gleaned from this study propel us toward a future where sustainable poultry farming harmonizes with environmental exigencies. In essence, this conclusion resonates as a preamble to future research trajectories and pragmatic interventions, fostering a continuum of adaptability in poultry production amidst an evolving climatic landscape.

Acknowledgements The author extends gratitude to The Dean, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India, the Director, ICAR National Institute of Animal Nutrition and Physiology (NIANP), Bangalore, India, and the National Innovations in Climate Resilient Agriculture, Indian Council of Agricultural Research (NICRA-ICAR), New Delhi, for their financial support and provision of necessary facilities for this research. Their contributions were instrumental in the successful completion of the study.

Declarations

Conflict of interest The authors declare no competing interests.

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