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HSP70 gene polymorphism and association with growth performance and corticosterone in crossbred native chickens

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ABSTRACT

This study evaluated heat shock protein 70 polymorphism and its association with growth performance and circulating corticosterone concentration in Chai-Aree crossbred native chickens. A total of 150 birds were genotyped using PCR-RFLP, and circulating corticosterone concentration was determined by competitive ELISA. Three genotypes (CC, CG, and GG) were identified with frequencies of 0.40, 0.44, and 0.16, respectively, whereas allele frequencies of C and G were 0.62 and 0.38. Observed and expected heterozygosity were 0.44 and 0.47, respectively, indicating moderate genetic diversity. The population conformed to Hardy–Weinberg equilibrium ($\chi^2 = 0.66$; $p>0.05$). Growth performance and corticosterone concentration were analyzed using a general linear model. HSP70 genotype had no significant effects on body weight, average daily gain, feed conversion ratio, or circulating corticosterone concentration ($p>0.05$). These findings indicate that polymorphism at the HSP70 locus did not influence growth performance or systemic stress response under the experimental conditions. Although HSP70 contributes to genetic variation in crossbred native chickens, it has limited value as a standalone molecular marker. Future studies should investigate multiple candidate genes and genotype × environment interactions, particularly under heat stress conditions.

Keywords: corticosterone, crossbred native chicken, HSP70.

INTRODUCTION

Global warming and climatic instability have intensified heat stress as a major constraint in poultry production, particularly in tropical and subtropical regions (Rashamol *et al.*, 2018). Heat stress adversely affects feed intake, growth performance, immune competence, and survivability, thereby reducing production efficiency and compromising animal welfare. In tropical production systems, crossbred native chickens have attracted increasing attention because they combine the adaptability and desirable meat quality of indigenous chickens with the improved growth potential of commercial lines. However, genetic improvement for rapid growth may also increase metabolic heat production and reduce the ability of birds to cope with environmental stress, especially under hot and humid conditions (Nawab *et al.*, 2018; Nawaz *et al.*, 2021).

Among the molecular responses to thermal challenge, heat shock proteins (HSPs), particularly HSP70, play a critical role in cellular protection by stabilizing protein structure, assisting in the refolding of damaged proteins, and maintaining proteostasis under stress conditions. In poultry, HSP70 has been widely recognized as a heat-responsive gene and a promising biomarker of thermotolerance. Previous studies have demonstrated the presence of HSP70 polymor-

phisms, including the g.167C>G single nucleotide polymorphism (SNP) identified in the *Gallus gallus HSP70* gene (GenBank Accession No. AY143693.1), in chickens and suggested that variation at this locus may be associated with differences in heat tolerance and stress responsiveness among breeds and lines. This SNP can be genotyped by PCR-RFLP using the restriction enzyme *EaeI* to digest a 360-bp amplified fragment encompassing the polymorphic site. Therefore, the HSP70 g.167C>G polymorphism is considered a potential candidate marker for evaluating genetic adaptation to thermal stress in poultry (Mazzi *et al.*, 2003; Duangjinda *et al.*, 2017; Budi *et al.*, 2024).

Corticosterone, the principal glucocorticoid in birds, is widely used as an endocrine indicator of hypothalamic–pituitary–adrenal (HPA) axis activation and physiological stress responsiveness (Sapolsky *et al.*, 2000). Under heat stress conditions, activation of the HPA axis results in elevated circulating corticosterone, which is closely associated with alterations in feed intake, nutrient utilization, immune function, and growth performance in poultry (Lara and Rostagno, 2013). In addition, heat stress can modulate neuroendocrine regulation through the microbiota–gut–brain axis, thereby influencing HPA axis activity and corticosterone secretion (Cao *et al.*, 2021). These physiological and metabolic responses reflect the broader impact of environmental stress on systemic homeostasis and productivity, reinforcing the value of corticosterone as a reliable biomarker for assessing the biological consequences of thermal challenge (Oluwagbenga and Fraley, 2023). Furthermore, stress adaptation in poultry involves not only endocrine regulation but also cellular and molecular responses, particularly the expression of heat shock proteins such as HSP70, which play a critical role in maintaining protein integrity and cellular stability under stress conditions (Onagbesan *et al.*, 2023). Accordingly, integrating molecular analysis of HSP70 with corticosterone quantification provides a more comprehensive and mechanistic approach for evaluating stress responses and adaptive capacity in poultry.

Molecular marker approaches such as polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) are widely used to characterize genetic polymorphism and to evaluate associations with economically important traits. However, information on HSP70 polymorphism in relation to both growth performance and endocrine stress indicators, particularly circulating corticosterone concentration, remains limited in crossbred native chickens reared under tropical conditions. It was hypothesized that genetic variation in the HSP70 gene is associated with variation in growth performance and circulating corticosterone concentration in Chai-Aree crossbred native chickens. Therefore, the present study aimed to determine the genetic diversity of HSP70 and to evaluate its association with growth performance and circulating corticosterone concentration in Chai-Aree crossbred native chickens. The findings of this study may contribute to the development of molecular markers for selecting chickens with improved adaptation to heat stress in tropical production systems (Duangjinda *et al.*, 2017; Budi *et al.*, 2024).

MATERIALS AND METHODS

Experimental Birds and Management

A total of 150 unsexed day-old Chai-Aree crossbred native chickens (male and female) obtained from Farm Chai-Aree Co., Ltd. were used in this study. Birds were reared together in a single open-sided house (3 × 5 m) at a stocking density of 10 birds/m² for 70 d under tropical summer conditions, with ambient temperatures ranging from 37 to 40°C during the experimental period. All birds were individually identified with wing tags prior to data collection, and body weight was recorded individually throughout the experimental period. Feed and water were provided *ad libitum*. Birds were fed antibiotic-free commercial broiler diets according to age, containing not less than 21% crude protein during 0–3 weeks, 19% during 4–6 weeks, and 17% during 6–10 weeks. Feed intake was recorded collectively for the entire flock. Average feed intake per bird was estimated by dividing the total feed intake by the number of birds present during each measurement period. Feed conversion ratio (FCR) was then estimated for each bird by dividing the average feed intake per bird by its individual body weight gain. Natural lighting and routine vaccination programs were applied

throughout the experimental period.

All procedures involving animals were approved by the Animal Care and Use Ethics Committee of Kalasin University (approval no. KSU AE-054) and were conducted in accordance with institutional guidelines for animal care and welfare.

Growth Performance

Individual body weight was recorded at 2, 4, 6, 8, and 10 weeks of age. Feed intake was determined from daily records of feed offered and refusals and expressed as average feed intake per bird. Average daily gain (ADG) was calculated individually for each bird, and feed conversion ratio (FCR) was calculated as average feed intake per bird divided by individual body weight gain.

Corticosterone Determination

For corticosterone analysis, blood samples were collected from the wing vein between 06:00 and 08:00 h at 10 weeks of age using serum tubes. Samples were centrifuged at 3,000 rpm for 10 min to obtain serum. A 5-µL serum aliquot was mixed with 5 µL of dissociation reagent, incubated at room temperature for 5 min, and diluted with 490 µL of 1× assay buffer to obtain a 1:100 dilution. Serum corticosterone concentration was determined using a Corticosterone ELISA Assay Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Standard solutions (0, 625, 1,250, 2,500, 5,000, and 10,000 pg/mL) were used to generate the calibration curve. Standards and diluted samples were added to antibody-coated wells, followed by incubation and washing according to the assay protocol. Absorbance was measured at 450 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Sample concentrations were interpolated from a four-parameter logistic standard curve (R^2 = 0.997), corrected for the dilution factor, and expressed as ng/mL. Corticosterone concentration was used as an endocrine indicator of physiological stress.

DNA Extraction and PCR Amplification

Whole blood samples collected in EDTA tubes were used for genomic DNA extraction. Genomic DNA was isolated using a Genomic DNA Mini Kit for blood samples (Geneaid) according to the manufacturer's instructions. DNA concentration and purity were determined by measuring optical density at 260 and 280 nm. The HSP70 gene fragment was amplified using the forward primer 5′-AACCGCACCACACCCAGCTATG-3′ and reverse primer 5′-CGCTTACTTCAACGACTCCCAG-3′, as described by Mazzi *et al.* (2003). PCR was performed in a final volume of 20 µL consisting of 10 µL of PCR Master Mix (2×) (Thermo Scientific, USA), 0.5 µL of forward primer, 0.5 µL of reverse primer, 1 µL of genomic DNA template, and 8 µL of nuclease-free water. The thermal cycling conditions consisted of an initial denaturation at 95°C for 12 min; 30 cycles of denaturation at 95°C for 10 s, annealing at 64.5°C for 30 s, and extension at 72°C for 20 s; followed by a final extension at 72°C for 5 min. PCR amplification generated a 360-bp fragment encompassing the HSP70 g.167C>G polymorphic site.

PCR-RFLP Analysis of HSP70 Gene Polymorphism

The PCR amplicons were digested with the restriction enzyme EaeI (New England Biolabs, USA) which recognizes the sequence 5′-Y/GGCCR-3′, to detect the HSP70 g.167C>G polymorphism. Enzymatic digestion was performed according to the manufacturer's instructions in a total reaction volume of 10 µL containing 8 µL of PCR product, 1.0 µL of 10× rCutSmart buffer, 0.2 µL of EaeI restriction enzyme, and nuclease-free water to the final volume. The reaction mixture was incubated at 37°C for 60 min, followed by heat inactivation at 65°C for 20 min. Restriction fragments were separated by electrophoresis on a 2% agarose gel, and genotypes (CC, CG, and GG) were identified based on the resulting banding patterns.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 27.0 (SPSS Inc., Chicago, IL, USA). Data are presented as least square means (LSM) with their standard errors (SE). Genotype and allele frequencies were calculated based on PCR-RFLP results and expressed as proportions within the population. Allele frequencies were estimated using the fol-

lowing equations:

$$p = \frac{2N_{CC} + N_{CG}}{2N}, q = \frac{2N_{GG} + N_{CG}}{2N}$$

where p and q represent the frequencies of alleles C and G, respectively; N_{CC}, N_{CG}, and N_{GG} are the numbers of CC, CG, and GG genotypes, respectively; and N is the total number of individuals. Observed heterozygosity (Ho) and expected heterozygosity (He) were calculated as follows:

$$H_o = \frac{\text{Number of heterozygous individuals}}{N}, H_e = 1 - \sum p_i^2$$

where p_i represents the frequency of the i-th allele in the population. Deviation from Hardy–Weinberg equilibrium was tested using a chi-square (χ^2) test implemented in GenAlEx version 6.0 (Peakall and Smouse, 2006; Falconer and Mackay, 1996).

The effects of HSP70 genotype on growth performance traits (body weight, average daily gain, feed intake, feed conversion ratio, and serum corticosterone concentration) were analyzed using a general linear model (GLM) according to the following model:

$$Y_i = \mu + G_i + e_i$$

where Y_i is the observed value, μ is the overall mean, G_i is the fixed effect of HSP70 genotype, and e_i is the residual error. Differences among means were analyzed using the GLM procedure, and Duncan's multiple range test was applied when significant effects were detected. Differences were considered significant at $p<0.05$.

RESULTS

PCR Amplification and Genotype Identification

Genomic DNA extracted from 150 blood samples was of sufficient quality for downstream molecular analysis. PCR amplification of the HSP70 gene yielded a single, clear product of approximately 360 bp in all samples (Figure 1)

Restriction digestion with EaeI produced distinct and reproducible banding patterns that enabled classification of individuals into three genotypes (Figure 2). The GG genotype was characterized by a single undigested fragment of approximately 360 bp. The CC genotype exhibited two restriction fragments of approximately 229 and 109 bp, indicating complete digestion. The heterozygous CG genotype displayed all three bands (360, 229, and 109 bp), confirming the presence of both alleles.

Genotype and Allele Frequencies of HSP70

The HSP70 locus showed moderate genetic variation in the studied population. Genotype frequencies of CC, CG, and GG were 0.40, 0.44, and 0.16, respectively, whereas allele frequencies of C and G were 0.62 and 0.38, respectively (Table 1). The observed heterozygosity (Ho) was 0.44, whereas the expected heterozygosity (He) was 0.47, indicating a moderate level of genetic diversity at the HSP70 locus. The chi-square value was $\chi^2 = 0.66$, indicating no significant deviation from Hardy–Weinberg equilibrium ($p>0.05$).

Association of HSP70 Genotypes with Growth Performance and Corticosterone Concentration

Body weight at 2, 4, 6, 8, and 10 wk of age was not affected ($p>0.05$) by HSP70 genotype (Table 2). Similarly, average daily gain (ADG) at all evaluation periods and feed conversion ratio (FCR) during 0–10 wk were not affected ($p>0.05$) by genotype (Table 2). However, birds with the CC genotype tended to have greater body weight and ADG, along with a lower FCR, than those with the CG and GG genotypes.

Corticosterone concentration was not affected ($p>0.05$) by genotype and ranged from 0.69 to 0.76 ng/mL (Table 2). Overall, HSP70 polymorphism at the studied locus was not associated ($p>0.05$) with growth performance, feed efficiency, or circulating corticosterone concentration.

DISCUSSION

The HSP70 locus exhibited moderate genetic diversity in the studied crossbred native chicken population and conformed to Hardy–Weinberg equilibrium, indicating that this locus is not under strong directional selection and that allelic variation is stably maintained (Falconer and Mackay, 1996). Similar patterns have been reported in indigenous chicken populations, where polymorphisms within the HSP70 gene contribute to genetic diversity and may facilitate adaptive responses under environmental chal-

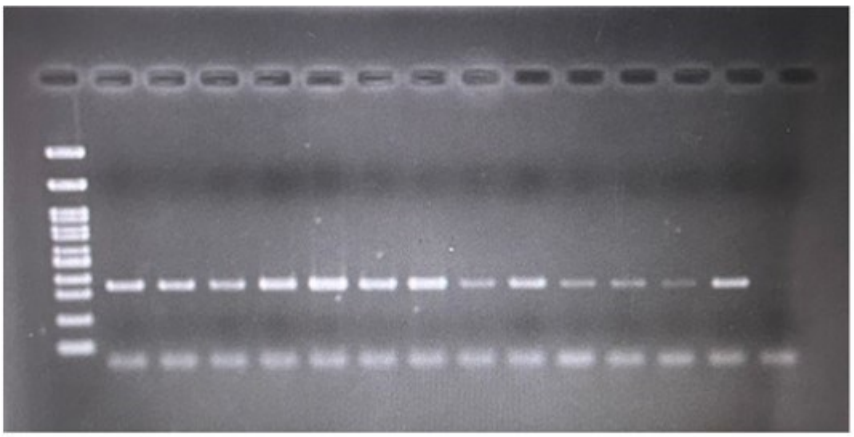

Figure 1. PCR amplification of the HSP70 fragment in crossbred native chickens. The expected amplicon size was 360 bp.

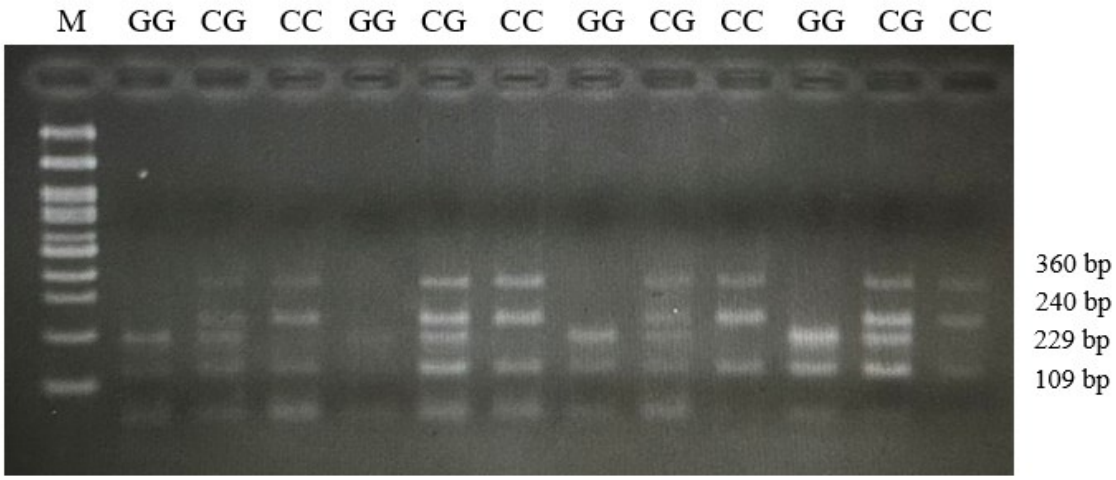

Figure 2. PCR-RFLP banding patterns of HSP70 after digestion with EaeI, showing the CC, CG, and GG genotypes

lenges (Aryani *et al.*, 2019). Such diversity may provide a genetic basis for resilience under fluctuating environmental conditions (Nawab *et al.*, 2018). Despite the presence of polymorphism, no significant associations were detected between HSP70 genotype and growth performance traits or corticosterone concentration. This finding suggests that variation at this locus alone did not exert a measurable phenotypic effect under the conditions evaluated. Growth performance and physiological stress responses are complex traits controlled by multiple genes together with gene–environment interactions (Oluwagbenga and Fraley, 2023). Consequently, the influence of a single candidate gene such as HSP70 is expected to be modest and highly dependent on environmental context. HSP70 is an inducible molecular chaperone whose biological function becomes particularly important during cellular stress, including heat stress, oxidative stress, and other physiological challenges. Its primary role is to maintain protein homeostasis by preventing protein misfolding, promoting refolding of damaged proteins, and protecting cells against stress-induced injury (Nawaz *et al.*, 2021). Therefore, functional consequences of HSP70 polymorphisms are more likely to become evident under conditions that strongly activate the cellular stress response rather than under normal production conditions. Although birds in the present study were reared during the tropical summer season, with ambient temperatures ranging from 37 to 40°C, no defined heat-stress treatment was experimentally imposed and HSP70 gene expression or protein abundance was not determined. Consequently, the present study could evaluate

Table 1. Genotype and allele frequencies of the HSP70 locus in crossbred native chickens

Population	Genotype			Allele		χ^2	He / Ho
	CC	CG	GG	C	G		
Chai-Aree crossbred native chicken	0.40 (60)	0.44 (66)	0.16 (24)	0.62	0.38	0.66	0.47 / 0.4

N = Number of samples ; He = expected heterozygosity; Ho = observed heterozygosity.

Table 2. Least square means (± SE) of growth performance and corticosterone concentration according to HSP70 genotype in crossbred native chickens.

Traits	Age or period (week)	Genotype			P-value
		CC	CG	GG	
Body weight (g)					
	2	102.63 ± 2.95	97.36 ± 2.83	100.00 ± 4.75	0.44
	4	301.79 ± 7.63	284.60 ± 7.31	290.36 ± 12.28	0.27
	6	579.53 ± 9.78	551.07 ± 9.38	561.09 ± 15.75	0.11
	8	821.13 ± 14.37	795.22 ± 13.64	784.95 ± 23.05	0.29
	10	1,160.88 ± 21.23	1,111.39 ± 20.35	1,087.14 ± 34.17	0.11
ADG (g/day)					
	0-4	9.48 ± 0.27	8.87 ± 0.26	9.07 ± 0.44	0.27
	4-6	19.84 ± 0.40	19.03 ± 0.38	19.34 ± 0.64	0.34
	6-8	18.05 ± 0.59	18.03 ± 0.57	16.84 ± 0.95	0.51
	8-10	23.48 ± 0.74	21.99 ± 0.71	20.73 ± 1.19	0.11
	0-10	16.07 ± 0.30	15.36 ± 0.29	15.01 ± 0.49	0.11
FCR		3.55 ± 0.07	3.72 ± 0.07	3.79 ± 0.11	0.12
Corticosterone (ng/mL)	10	0.76 ± 0.03	0.69 ± 0.03	0.74 ± 0.04	0.16

Values are least square means ± SE. ADG = average daily gain; FCR = feed conversion ratio; SE = standard error.

only the association between HSP70 genotype and phenotypic traits, but could not directly assess whether the identified polymorphism altered HSP70 functional activity. This limitation may partly explain the absence of significant associations between genotype and growth performance or circulating corticosterone concentration. Moreover, circulating corticosterone represents an integrated endocrine response influenced by numerous physiological pathways rather than by a single gene. Likewise, growth performance is regulated through complex interactions among endocrine regulation, metabolism, immune function, and multiple genetic pathways (Oluwagbenga and Fraley, 2023; Onagbesan *et al.*, 2023). Therefore, the absence of association observed in this study may also reflect the limited explanatory power of single-locus analysis for complex biological traits. Stress tolerance and production efficiency are regulated by integrated genetic networks involving heat shock proteins, antioxidant defense systems, immune regulation, and endocrine signaling (Nawab *et al.*, 2018; Abare *et al.*, 2023). Future studies should combine HSP70 genotyping with controlled environmental stress challenges, particularly standardized heat-stress models, together with measurements of HSP70 gene expression and protein abundance. Such approaches would provide a more biologically relevant framework for determining whether HSP70 polymorphisms influence stress adaptation and their subsequent effects on growth performance and physiological stress responses in poultry.

CONCLUSION

The HSP70 locus in Chai-Aree crossbred native chickens exhibited moderate genetic diversity and conformed to Hardy–Weinberg equilibrium but was not associated with growth performance or circulating corticosterone concentration under the evaluated conditions. These findings suggest that the investigated HSP70 polymorphism alone has limited predictive value for these complex traits in this population. Given that HSP70 functions as a stress-inducible molecular chaperone, its phenotypic effects are likely to depend on the specific polymorphism and environmental conditions. Future studies incorporating controlled heat-stress models, HSP70 expression analyses, and multi-locus approaches are warranted to clarify its functional significance in poultry.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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