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Association of heat shock protein 70 gene polymorphisms with acute thermal tolerance, growth, and egg production traits of native chickens in Taiwan

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Abstract. Heat stress is among the most challenging environmental conditions affecting commercial poultry. It severely affects growth and egg production, particularly in tropical and subtropical regions. This study aimed to examine physiological responses – including triiodothyronine (T_3) levels, enzymatic activity of creatine kinase (CK) and lactate dehydrogenase (LDH), respiratory rates, and cloacal temperature – to acute heat stress associated with different genotypes of the *HSP70* gene and to evaluate the association of these polymorphisms with growth and egg production. Genotyping was performed by single-strand conformation polymorphism analysis. The polymorphisms identified were A258A, A258G, and G258G. Twenty 12-week old birds were randomly selected from each genotype and exposed to 40 °C ambient temperature for 1 h. Blood samples were collected at 0 and 1 h following heat stress. Respiratory rate and cloacal temperature were measured following 0, 30, and 60 min of exposure. After 1 h, the A258A genotype exhibited lower levels of CK activity and plasma T_3. Neither respiratory rate nor cloacal temperature displayed a significant association with the genotypes. Body weight gain differed among the genotypes for males ($F = 3.268$, $P = 0.041$) and females ($F = 14.029$, $P < 0.001$), and the A258A genotype exhibited the greatest weight gain at 0–16 weeks of age for both genders. There were no significant differences among genotypes regarding egg weight at first egg or the number of eggs laid until 40 weeks of age. The A258A genotype displayed higher heat tolerance with no negative effects on growth performance and egg production.

1 Introduction

As the global climate changes, chicken production is occurring under increasing ambient temperatures. High temperatures have a negative impact on productivity and produce stressful conditions for chickens, resulting in reduced performance, anorexia, heat stress, and mortality. In chickens, temperatures exceeding 35 °C can cause heat stress and reduce nutrient utilization in both broilers and egg-laying chickens (Yalçin et al., 2005; Khan et al., 2011). Physiological responses to cope with heat stress involve the functional integration of several organs to meet the metabolic needs of chickens attempting to dissipate heat and maintain homeostasis. Previous reports indicated that heat stress influenced muscle metabolism (Aksit et al., 2006; Lu et al., 2007; Gregory, 2010; Laudadio et al., 2012) and that acute heat stress caused skeletal muscle cell injury (Tang et al., 2013). The plasma activity of skeletal muscle enzymes, such as creatine kinase (CK) and lactate dehydrogenase (LDH), is affected by muscle cell injury. Exposure to high ambient temperatures significantly increased the plasma CK level in broilers after different periods of heat stress (Tang et al., 2013). Increased plasma CK levels are indicative of skeletal muscle damage and are a consequence of the disruption of the function and permeability of the muscle cell membrane (Brancaccio et al., 2007).

LDH is a key enzyme in cellular respiration, the process by which glucose from food is converted into usable energy for cells. Although LDH is abundant in tissue cells, levels in plasma are normally low. However, when tissues are damaged, additional LDH is released into the bloodstream, leading to increased plasma levels. Previous studies demonstrated that broilers had higher plasma LDH levels at a constant high temperature of 34 than at 23 °C (Zhang et al., 2012). Therefore, plasma CK and LDH levels can be considered parameters for assessing heat stress in chickens.

Thyroid hormones play an important role in the regulation of the metabolic rate. The level of triiodothyronine (T_3), one of the active forms of thyroid hormones, decreases to reduce metabolic heat production and alleviate heat stress (Reineke and Turner, 1945; Hahn et al., 1966; Bogin et al., 1996). Plasma T_3 variation is more accurate in determining the basal metabolic rate, and it has also been regarded as an indicator for assaying heat tolerance in chickens (Bowen and Washburn, 1985; Melesse et al., 2011). In chickens, there is an inverse relationship between the plasma concentration of T_3 and environmental temperature (May et al., 1986; Iqbal et al., 1990). The concentration of T_3 in serum decreases at high temperatures and increases at low temperatures (Huston and Carmon, 1962). Under high temperatures, broilers exhibited a significant increase in respiratory rate and cloacal temperature (da Silva et al., 2007). Respiratory rate and cloacal temperature are widely used and also considered the simplest ways to evaluate the physiological condition of an animal under thermal stress (Bianca and Kunz, 1978).

Breeding genetically heat-resistant chickens is necessary for raising poultry in a high ambient temperature environment. Several genes are associated with heat tolerance in chickens, such as the naked-neck gene (*Na*), the frizzle gene (*F*), the dwarf gene (*dw*), and the heat shock protein (*HSPs*) genes (Horst, 1989; Yunis and Cahaner, 1999; Gaviol et al., 2008). One of them, the *HSPs* gene, encodes proteins that fold other proteins to protect cells against heat stress, and it has been shown to affect the heat tolerance level of various breeds of chickens. Furthermore, polymorphisms of the *HSP70* gene result in different *HSP70* genotypes that are associated with differing heat tolerance levels in chickens (Tamzil et al., 2013). The association between polymorphisms of the *HSP70* gene and heat tolerance has been regarded as a marker for selecting heat-resistant breeds of chickens (Mazzi et al., 2003; Franco-Jimenez et al., 2007; Gaviol et al., 2008; Tamzil et al., 2013). However, there is no evidence that polymorphisms of the *HSP70* gene can be used effectively to select heat-tolerant chickens concomitant with desirable growth and egg production traits. Therefore, the present study evaluated the physiological responses of different genotypes of the *HSP70* gene to acute heat stress and focused on the association between the gene polymorphisms and economically desirable traits in indigenous Taiwanese chickens.

2 Materials and methods

2.1 Study animals

Native chickens bred by the Livestock Research Institute (LRI) were raised for meat and egg production (Lin et al., 2012, 2014). Taishu no. 9 is a native strain of the LRI chicken, which has a single comb and red feathers, and originated from the outcrossing of local red feather chickens. Taishu no. 9 does well in traditional management systems in Taiwan. Because of its ability to adapt to Taiwan summer weather, during which the average temperature reaches approximately 30 °C and relative humidity approximately 70 %, the LRI native Taishu no. 9 was chosen for the study.

2.2 Bird management and trait measurement

A total of 308 1-day-old LRI Taishu no. 9 chickens were used as experimental animals. All birds were raised under the same living conditions and received the same feed. The starter feed, eaten from 0 to 6 weeks of age, contained 18.2 % crude protein (CP) and 2947 kcal kg^{-1} of metabolizable energy (ME). The growth feed, which contained 15.5 % CP and 2855 kcal kg^{-1} of ME, was fed from 7 weeks of age until 5 % of the flock was producing eggs. Subsequently, the hen feed contained 15.5 % CP and 2751 kcal kg^{-1} of ME. For all ages, chickens were provided with ad libitum access to feed and water. The average daily temperature ranged from 25 to 32 °C, and the relative humidity ranged from 50 to 60 %. Nat-

ural lighting was provided after 6 weeks of age. When 5 % of the flock was producing eggs, light exposure was increased to up to 17 h day^{-1}. All females (170 chickens) were housed individually in laying cages beginning at 16 weeks of age, and their individual egg production from 18 to 40 weeks of age was recorded daily. Growth and egg production traits in this study included body weight at hatching (BW 0), body weight at 16 weeks of age (BW 16), egg weight at first egg (FEW), body weight at first egg (BWFE), age at first egg (AFE), and total number of eggs at 40 weeks of age (EN40). All animal experiments were conducted in accordance with Taiwan laws regarding animal protection, and all animal experimental procedures were approved by the Kaohsiung Animal Propagation Station, Livestock Research Institute, Council of Agriculture (Affidavit of Approval of Animal Use Protocol No. 102006).

2.3 PCR amplification of the chicken *HSP70* gene

Genomic DNA was extracted from the blood samples taken from all chickens at 12 weeks of age. The *HSP70* gene polymorphisms were investigated using polymerase chain reaction single-strand conformation polymorphism (PCR–SSCP) assays. The oligonucleotide primers *HSP70*–02F (TTTGATGCCAAGCGTCTCAT) and *HSP70*–02R (ATCTCCTCTGGGAAGAAGGT) were used for amplifying a 155 bp DNA fragment that spans the A258G substitution sites of the Gallus *HSP70* gene (GenBank accession numbers AY143693.1). PCR was performed in a 20 µL final reaction mixture containing $MgCl_2$ buffer at 3 mM, each dNTP at 100 µM, each primer at 10 pM, 1 U of SuperTherm DNA Taq polymerase (Roche, Mannheim, Germany), and 100 ng of genomic DNA. After an initial 7 min denaturation cycle at 94 °C, 40 cycles were performed at 94 °C for 60 s, 66 °C for 30 s, and 72 °C for 30 s, followed by a final 10 min extension at 72 °C. Electrophoresis of the PCR products for SSCP analysis was performed using Gene Gel Excel 12.5/24 kits (Bio-Science, Uppsala, Sweden). For staining, 5 µL of each PCR product was mixed with 5 µL of denaturing dye containing 0.05 % bromophenol blue and 0.05 % xylene cyanol. The mixtures were denatured at 95 °C for 12 min, rapidly cooled on ice, and then directly loaded onto 12.5 % acrylamide gels. Electrophoresis was performed using 600 V, 25 mA, and 15 W at 10 °C for 2 h to obtain the clearest separation of the different fragments. The gel was then stained with 0.1 % silver nitrate for further assessment. Amplimers representing each unique SSCP-banding pattern were purified, cloned, screened, and then sent for sequencing.

2.4 Heat stress exposure

Twenty 12-week old females were randomly selected from each genotype group and exposed to an acute heat stress test at 40 °C ambient temperature and a relative humidity of 60 % for 1.0 h in $40 \times 40 \times 80$ cm^3 chambers. The chambers contained a heater, thermostat, blower, digital thermometer, psychrometer, and a food and water area. Blood samples were collected at 0 and 1 h after heat stress.

2.5 Blood parameters

Blood samples were drawn from the brachial vein using blood tubes (Sarstedt, Nümbrecht, Germany) to measure T_3, CK, and LDH levels. After sampling, blood was centrifuged and the plasma was immediately separated. The activities of plasma CK and LDH were determined photometrically at 450 nm absorbance with the creatine kinase activity colorimetric assay kit (Biovision, CA, USA) and the lactate dehydrogenase activity assay kit (Biovision, CA, USA). Total plasma T_3 was determined by the colorimetric method with the T_3 Elisa kit (Cusabio Life Science, Wuhan, China).

2.6 Respiratory rate and cloacal temperature

Respiratory rate and cloacal temperature were measured for 10 randomly selected birds from each genotype group at 0, 30, and 60 min after heat exposure. The respiratory rate was measured by counting the panting breaths of the birds for 1 min. Cloacal temperature was obtained by introducing a digital thermometer into the cloaca of each bird until the reading stabilized (Nascimento et al., 2012).

2.7 Statistical analysis

Genotypic and allelic frequencies were statistically analysed using Microsoft Excel (Microsoft Inc., Redmond, WA, USA) following Nei and Roychoudhury (1974) and Nei and Li (1979). Following the formula below, the Hardy–Weinberg equilibrium was analysed using the chi-square test.

$$X^2 = \sum \frac{(O-E)^2}{E}$$

In the formula, O represents the observed number of each genotype and E represents the expected number of each genotype if the genotype distribution conformed to the Hardy–Weinberg equilibrium.

The data were analysed using IBM SPSS Statistics for Windows Version 19.0 (Armonk, NY, USA). The effects of genotypes on respiratory rate, cloacal temperature, enzyme activities, T_3 levels, BW 0, and BW 16 were analysed using an analysis of variance (ANOVA) with a general linear model.

The model used was $Y_{ij} = \mu + G_i + e$, where Y_{ij} is the observed traits, μ is the overall mean for the trait, G_i is the effect of the genotype, and the e is the residual error. All values were reported as least square means ± standard error of mean (SE).

The analysis of covariance (ANCOVA) statistical procedure was employed to analyse the association of a single polymorphism with body weight gain from hatching to 16

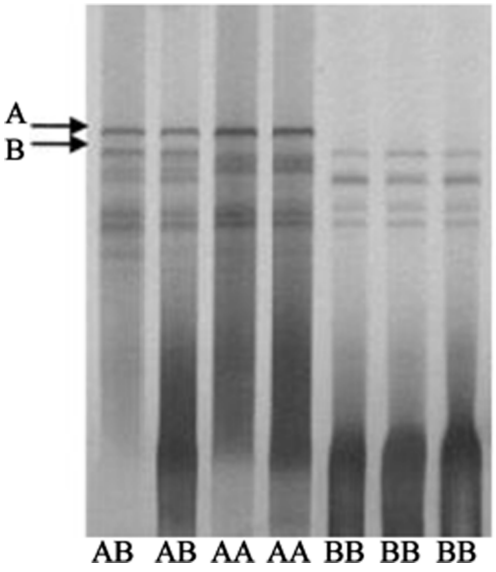

Figure 1. Non-denaturing polyacrylamide gel electrophoresis, showing different single-strand conformation polymorphism (SSCP) variants in the *HSP70* gene. AA (A258A), AB (A258G), and BB (G258G) genotypes were identified.

weeks of age (BWG 0–16), with BW 0 as a covariate. In this study, egg production data were not distributed normally and the Kruskal–Wallis H test, a nonparametric analysis, was employed to compare the effects of the three genotypes.

3 Results

3.1 Identified SNPs of the chicken *HSP70* gene

The PCR products of the *HSP70* gene were analysed using SSCP. The results showed that the PCR products exhibited polymorphisms and indicated the presence of two alleles, which were denoted *HSP70*–A and *HSP70*–B. Three genotypes, AA, AB, and BB, were identified (Fig. 1). Two genotypes (AA and BB) were cloned and sequenced, and one single-base mutation was found at the 258th bp of the *HSP70* gene from the start site; the mutation was an A → G non-synonymous nucleotide substitution. Of the 308 chickens, the frequencies of *HSP70* genotypes AA (A258A), AB (A258G), and BB (G258G) were 0.2403, 0.4416, and 0.3182, respectively. Genotypic and allelic frequency variation as determined using the chi-square test indicated that allele B predominated in the male, female, and entire populations, with frequencies of 0.5435, 0.5353, and 0.5390, respectively. Both SNP loci conformed to the Hardy–Weinberg equilibrium (male $\chi^2 = 0.5902$, $P = 0.4423 > 0.05$; female $\chi^2 = 3.7590$, $P = 0.0525 > 0.05$; whole population $\chi^2 = 3.8283$, $P = 0.0504 > 0.05$).

3.2 Enzyme activities and T_3 level in plasma under acute heat stress

As shown in Table 1, there were no significant differences in CK activity among all genotypes before the heat stress test. All genotypes exhibited increased CK activity after the acute heat stress. CK activities in AA, AB, and BB genotypes increased by 19.4, 46.1, and 81.7 % after acute heat stress, respectively. The activity of CK in the BB genotype showed the most significant increase, and the AA genotype had the lowest increase in activity ($P < 0.05$).

The AB genotype had significantly higher LDH activity and the AA genotype had the lowest activity before the heat stress test ($P < 0.05$). After exposure to 40 °C ambient temperature for 1 h, LDH activity was highest in the AB genotype and lowest in the AA genotype ($P < 0.05$). Similar to CK activity, all genotypes exhibited increased LDH activity after exposure to 40 °C ambient temperature for 1 h. However, the elevation in LDH activity did not differ significantly among the genotypes.

Before the heat stress test, the AB genotype had a significantly higher T_3 level and the AA genotype exhibited the lowest level ($P < 0.05$). After the acute heat stress, T_3 levels decreased in all genotypes. The AA genotype had the lowest T_3 level and the AB genotype the highest level, but these differences were not significant. Plasma levels of T_3 in AA, AB, and BB genotypes decreased by 37.2, 48.2, and 38.1 %, respectively, and the decline in the T_3 level in the AA genotype was significantly less than that of the AB genotype ($P < 0.05$).

3.3 Respiratory rate and cloacal temperature

The effect of *HSP70* genotypes on respiratory rate and cloacal temperature of the LRI native chickens at 40 °C ambient temperature is presented in Table 2. Neither respiratory rate nor cloacal temperature exhibited significant associations among the *HSP70* genotypes after 0, 30, and 60 min of thermal stress conditions ($P > 0.05$).

3.4 Association of *HSP70* polymorphism with growth and egg production traits

The effect of *HSP70* genotypes on growth traits in the LRI chickens is presented in Table 3. The body weight at hatching of the AA genotype was lowest and the BB genotype was heaviest for both sexes ($P < 0.05$). Because body weight at hatching differed among *HSP70* genotypes, we used the ANCOVA to test the effect of *HSP70* genotypes on BWG 0–16, with BW 0 as a covariate to exclude the effects of initial weight. The results showed that during 16 weeks, body weight gain differed among the *HSP70* genotypes both in males ($F = 3.268$, $P = 0.041$) and females ($F = 14.029$, $P < 0.001$). The BB genotype had a lower body weight gain, and the AA genotype was heaviest in both genders.

Table 1. The effect of *HSP70* genotypes on enzyme activities and T_3 levels in the plasma of the LRI native chickens at 40 °C ambient temperature.

Traits	*HSP70* genotype		
	AA	AB	BB
Number	$N = 19$	$N = 20$	$N = 20$
CK (IU L^{-1})			
0 h	1349.0 ± 163.3	1847.0 ± 147.3	1524.0 ± 158.3
1 h	1611.0 ± 173.7^{a}	2698.0 ± 103.7^{b}	2769.0 ± 257.6^{b}
Activity increase	262.0 ± 31.8^{a} (19.4%)	851.0 ± 129.2^{b} (46.1%)	1245.0 ± 207.8^{c} (81.7%)
LDH (IU L^{-1})			
0 h	482.0 ± 19.5^{a}	611.0 ± 36.4^{b}	582.0 ± 40.7^{b}
1 h	552.0 ± 29.3^{a}	705.0 ± 40.7^{b}	693.0 ± 47.0^{b}
Activity increase	70.0 ± 22.7 (14.5%)	94.0 ± 29.7 (15.4%)	111.0 ± 23.4 (19.1%)
T_3 (nmol L^{-1})			
0 h	94.9 ± 10.3^{a}	122.7 ± 10.9^{b}	104.9 ± 5.7^{ab}
1 h	59.1 ± 5.5	67.7 ± 4.6	64.9 ± 7.4
Drop level	35.3 ± 6.3^{a} (37.2%)	59.1 ± 7.5^{b} (48.2%)	40.0 ± 6.4^{ab} (38.1%)

Mean ± SE. ^{a,b,c} Means within a row lacking a common superscript letter differ significantly ($P < 0.05$).

Table 2. The effect of *HSP70* genotypes on respiratory rate and cloacal temperature of the LRI native chickens at 40 °C ambient temperature.

Traits	*HSP70* genotype		
	AA	AB	BB
Number	$N = 10$	$N = 10$	$N = 10$
Respiratory rate (panting min^{-1})			
0 min	33.6 ± 3.4	34.4 ± 1.5	35.2 ± 1.5
30 min	46.0 ± 2.3	44.2 ± 1.9	42.3 ± 3.4
60 min	48.0 ± 3.2	46.5 ± 1.6	45.0 ± 1.9
Cloacal temperature (°C)			
0 min	42.0 ± 0.5	41.8 ± 0.2	41.7 ± 0.1
30 min	42.1 ± 0.2	42.0 ± 0.1	41.9 ± 0.2
60 min	42.1 ± 0.2	42.1 ± 0.1	42.0 ± 0.2

Mean ± SE

We used the Kolmogorov–Smirnov test to determine the normality of the egg production data. The results indicated that the egg production data were not distributed normally. Therefore, we employed the nonparametric Kruskal–Wallis H test to compare the effects of the three genotypes on the egg production traits. The association between the *HSP70* genotypes and egg production in the LRI native chickens is shown in Table 4. No significant association was observed between the FEW and EN40 with the *HSP70* genotypes ($P = 0.718$ and $P = 0.246$, respectively). Both BWFE and AFE differed among the *HSP70* genotypes ($P < 0.001$ and $P = 0.023$, respectively). The associations observed revealed that the AA genotype had significantly heavier BWFE, the AB genotype had a significantly earlier onset of egg production, and the BB genotype had a lower performance for both traits.

4 Discussion

In broiler chickens, CK is released into the circulation system following changes in the permeability of the sarcolemma in response to various pathologies and exposure to environmental stress (Mitchell and Carlisle, 1992; Mitchell and Sandercock, 1995). Recent studies showed that the CK activity of broilers was significantly increased by exposure to acute heat stress and daily cyclic heat treatment, reflecting heat-stress-induced myopathy (Sandercock et al., 2001; Yalçin et al., 2009). Melesse et al. (2011) observed that CK activity might be associated with the recovery of tissues over time, suggesting some chicken genotypes could confer adaptation to long-term exposure to high environmental temperatures. Similarly, Chen et al. (2014) reported that CK was elevated at the beginning of cold stress and then decreased with an increase in the duration of hypothermia. These studies indicated that CK activity increased during short-term stress but decreased during long-term stress. Ward and Peterson (1973) reported that the increase in enzyme activity might be partly attributed to cellular damage as a direct consequence of heat stress. In the present study, CK activity was not significantly different among genotypes before acute heat stress treatment. How-

Table 3. The effect of *HSP70* genotypes on growth traits in the LRI native chickens.

Traits	*HSP70* genotype		
	AA	AB	BB
Male			
Number	$N = 31$	$N = 64$	$N = 43$
BW 0 (g)	28.9 ± 2.4^{b}	27.8 ± 1.0^{a}	30.4 ± 1.3^{c}
BW 16 (g)	1993.8 ± 28.3	1916.0 ± 27.6	1910.3 ± 21.6
*BWG 0–16 (g)	1965.4 ± 23.6^{b}	1891.7 ± 35.8^{ab}	1876.6 ± 30.8^{a}
Female			
Number	$N = 43$	$N = 72$	$N = 55$
BW 0 (g)	29.1 ± 2.9^{b}	27.4 ± 2.4^{a}	29.6 ± 2.3^{b}
BW 16 (g)	1389.6 ± 17.8^{b}	1337.9 ± 23.8^{ab}	1244.6 ± 22.0^{a}
*BWG 0–16 (g)	1359.6 ± 18.5^{b}	1314.4 ± 25.2^{ab}	1210.2 ± 21.7^{a}

Mean ± SE. BW 0: body weight at hatching; BW 16: body weight at 16 weeks of age; BWG 0–16: body weight gain from 0 to 16 weeks of age. ^{a,b,c} Means within a row lacking a common superscript letter differ significantly ($P < 0.05$) according to the Scheffe test. * BWG 0–16 was analysed with BW 0 as a covariate.

Table 4. The effect of *HSP70* genotypes on egg production traits in the LRI native chickens.

Traits	*HSP70* genotype				
	AA	AB	BB	Chi-square	*P* value
Number	$N = 43$	$N = 72$	$N = 55$		
FEW (g)	30.4 ± 7.1	30.6 ± 7.3	28.9 ± 4.6	0.690	0.718
BWFE (g)	1745.1 ± 451.5^{b}	1678.0 ± 171.1^{b}	1546.7 ± 175.9^{a}	20.949	< 0.001
AFE (day)	144.0 ± 8.0^{ab}	142.3 ± 9.4^{a}	147.8 ± 10.2^{b}	7.555	0.023
EN40 (egg)	77.2 ± 4.8	73.2 ± 21.5	74.7 ± 9.2	2.805	0.246

Mean ± SE. FEW: egg weight at first egg; BWFE: body weight at first egg; AFE: age at first egg; EN40: number of eggs laid up to 40 weeks of age. ^{a,b,c} Means within a row lacking a common superscript letter differ significantly ($P < 0.05$) according to the Kruskal–Wallis H test.

ever, following 40 °C ambient temperature for 1 h, CK activity was significantly higher than before heat stress treatment in all genotypes. This indicated that exposure to 40 °C ambient temperature for 1 h might cause acute heat stress in the LRI native chickens with resulting cell injury, and the BB genotype with highest CK and LDH activities implied a lower ability to endure heat stress at 40 °C ambient temperature.

The two active forms of thyroid hormones are T_3 and thyroxine (T_4), and the inactive form is reverse triiodothyronine (r–T_3). The selective peripheral conversion of T_4 to T_3 or r–T_3 is believed to play an important role in thermoregulation in domestic fowl (Rudas and Pethes, 1984); thyroid hormone (TH) receptors preferentially bind T_3. Therefore, the metabolism of T_4 in peripheral tissues, resulting in the production and degradation of receptor-active T_3, plays a major role in thyroid function (Darras et al., 2000). A previous report indicated that T_3 is the main thyroid hormone regulating oxygen consumption, particularly in young chickens (Bobek et al., 1977). It has also been suggested that thyroid activity is affected by environmental temperature (McNabb and King, 1993; Yahav et al., 1997). Under heat stress, an organism must reduce metabolic heat output, as the process of acclimatization is mainly associated with a low basal metabolic rate at high ambient temperatures. Previous studies found that the T_3 concentration decreased in chickens exposed either to acute or long-term heat stress, but the concentration increased at low temperatures (Huston and Carmon, 1962; Iqbal et al., 1990; Melesse et al., 2011). Thyroid activity and, subsequently, metabolic rate might also decrease at high temperatures (Jonier and Huston, 1957). Thyroid hormone administration stimulates heat production through an increase in metabolic rate, resulting in reduced thermotolerance (Bowen and Washburn, 1985). In the present study, plasma T_3 levels were reduced in all genotypes of the LRI native chickens following acute heat stress, in agreement with previous studies. The lowest level of T_3 concentration was observed in the AA genotype, and this suggests im-

proved adaptability to acute heat exposure, which contributes to lower basal heat production as compared with other genotypes.

No significant association was observed between respiratory rate and cloacal temperature in the *HSP70* genotypes. In the present study, even 30 and 60 min led to physiological changes, representing an attempt to maintain thermal equilibrium. The results were in accordance with the observation by Nascimento et al. (2012) that treatments of 35–38 °C stress conditions for 90 min did not influence the respiratory rate or cloacal temperature of birds.

Heat stress induced a decrease in metabolic activity by minimizing excess heat production. As chickens reduced their feed consumption, body weight, which is essential for maintaining body temperature during heat exposure, also decreased (McNaughton and Reece, 1984; Cooper and Washburn, 1998). The lower feed intake, together with a decrease in circulating thyroid hormone levels, resulted in lower metabolic and thermogenic rates, which explains the decrease in animal productivity during exposure to stressful heat conditions (Melesse et al., 2011). In the present study, we found that BB genotype chickens had greater BW 0 than both sexes of the other genotypes. However, lower BW16 and BWFE were detected in the BB genotype than in other genotypes, although we employed an ANCOVA to account for the effect of initial weight. This implies that the BB genotype may be relatively less capable of enduring heat, resulting in greater energy use to deal with heat stress. Additionally, the AA genotype had a greater body weight gain during 16 weeks in the female, which might be attributed to maintaining a lower plasma T_3 level under normal physical conditions and also contributed to the conservation of energy for growth.

Egg production is also an important economic parameter in the poultry industry. Endocrine and many environmental factors potentially influence growth and egg production. However, the genetic makeup of a species ultimately has a fundamental influence on egg production. In the present study, the results revealed no significant associations between FEW and EN40. Significant differences in BWFE and AFE were detected among genotypes, and the AB genotype had a significantly earlier onset of laying, followed by the AA and BB genotypes. Melesse et al. (2013) found that in chickens under long-term heat stress conditions, body weight was significantly reduced and the AFE was earlier than average. Lien and Siopes (1993) noted maximum T_3 levels in turkeys during the early onset of laying. Melesse et al. (2013) also referred to the phenomenon of higher plasma T_3 concentrations coupled with an earlier age at first egg, which might be related to the adaptation of birds to changes in metabolic demands caused by physiological stress and increased metabolic activity related to an earlier onset of egg production. The AB genotype had a significantly earlier onset of laying, which might be attributed to higher plasma T_3 levels that increased metabolic activity under normal physical conditions.

5 Conclusions

In this study, the AA genotype had no negative associations with growth performance or egg production traits, which might be attributed to their maintenance of lower plasma T_3 levels, which increases energy use effectiveness. Lower levels of CK and LDH activity were also detected in the AA genotype after acute heat stress, indicating that the AA genotype has greater heat tolerance. However, further investigations are necessary to clarify the physiological responses, growth, and egg production of the *HSP70* genotypes under long-term heat stress.

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