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PHYSIOLOGICAL RESPONSE OF NIGERIAN LOCALLY ADAPTED CHICKENS WITH DIFFERENT HEAT SHOCK PROTEIN 70 GENOTYPES TO ACUTE HEAT STRESS

Rasheed Babatunde Fatai[1], Mabel Omolara Akinyemi[2], Osamede Henry Osaiyuwu[1], Kayode Muslim Ewuola[3], Adebowale Emmanuel Salako[1]

[1]Department of Animal Science, University of Ibadan, Ibadan, Nigeria
[2]Department of Biological Sciences, Fairleigh Dickson University, Florham Campus, Madison, United States of America
[3]Department of Animal Science, Faculty of Agriculture, University of Abuja, Abuja, Nigeria
Corresponding author: Rasheed Babatunde Fatai, fatairasheed36@gmail.com
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Abstract: Slow growth rate and acute heat stress are among the major constraints to indigenous chicken production in Nigeria. Characterization of heat-tolerance genes is a requisite for selective breeding of poultry for improved heat-tolerance and productivity. Therefore, this study investigated variation in HSP70 gene and its association with heat-tolerance traits of Nigerian locally adapted chickens. One-day old chicks comprising 118 Yoruba Ecotype Chicken-YEC and 138 FUNAAB Alpha Chicken-FAC were tagged and fed ad-libitum on starter (0 - 6 weeks) and grower (7-24 weeks) diets. At week 12, blood was sampled, DNA was extracted, amplified and electrophoresed following standard procedures. *HSP70* gene was genotyped using *Mme*I restriction endonuclease. At week 23, 36 chickens (six chickens per identified HSP70 genotypes) selected from each of YEC and FAC were exposed to 40±1°C for one hour. Cloaca temperature, respiratory rate (RR) and pulse rate (PR) were recorded and heat stress index (HSI) calculated. Data were analysed using descriptive statistics and ANOVA. Alleles A and B with genotypes: AA, AB, and BB were detected. After acute heat-stress, YEC with BB had higher RR value compared to those with AA and AB. The PR value of FAC with genotype BB was significantly higher ($p<0.05$) than those of AA, but similar to chickens with AB. Within FAC, HSI of BB-HSP70 was lower than AA-HSP70 but similar to AB-HSP70, while within YEC HSI of BB-HSP70 was similar to those of AA-HSP70 and AB-HSP70. The HSP70 gene was polymorphic in the studied chickens and genotype BB-HSP70 was associated with thermo-tolerance.

Key words: indigenous chickens, improved breed, heat stress, HSP70, heat stress index, PCR-RFLP

Introduction

The indigenous chickens are among the most adversely affected poultry species by the influence of high ambient temperatures and high relative humidity that characterize the tropical climate. This is because indigenous chickens are mainly reared under the extensive management system *(Ogundipe, 1990)*. Unlike the intensive commercial chicken management system where measures are always in place against adverse effects of heat stress, smallholder chicken producers usually provide temporary light shade and radiation shield which are often grossly inadequate to assuage heat stress effects *(Adedokun and Sonaiya, 2001)*. Recommended measures against the effects of heat stress are not only unaffordable to the smallholder farmers but also practically difficult to implement by smallholder chicken producers that dominate rural sub-Sahara Africa.

Physiological response to heat stress is a good indicator of measure of the degree of comfort or discomfort in farm animals. Pulse rate is widely considered as the simplest way to determine the physiological condition of an animal particularly under heat-stress. Increment in pulse rate builds blood spill out of the center to the surface and because of it more warmth is lost *(Marai et al., 2007)*. The rectal temperature is recognized as an ideal indicator for heat stress valuation in animals and a significant increase or decrease in body temperature beyond the normal range of 41^{o}C will alter the homeostasis and consequently affects the normal body functioning and productivity of the poultry *(Franco-Jimenez et al., 2007; Adedeji et al., 2015)*. Changes in respiratory rate occasioned by increase in ambient temperature of the animal above its thermo-neutral range denotes heat stress condition in animals *(Lemerle and Goddard, 1986)*. The loss of heat often occurs through the respiratory tract when animals are trying to maintain the thermal equilibrium. An increase in ambient temperature above the thermo-neutral zone of the animals stimulates heat shock proteins (HSPs) production *(Nascimento et al., 2012; Mazzi et al., 2003)*. The predominant and temperature sensitive HSPs are HSP70 and HSP90. Both of them have protective roles during heat stress in farm animals *(Liang et al., 2016)*. The HSPs are expressed during hyperthermic stress to aid the maintenance and prevention of protein degradation, regeneration of denatured proteins and contribute to the cell survival by eliminating the impaired polypeptides within cells *(Marai et al., 2007; Archana et al., 2017)*. Single nucleotide polymorphisms (SNPs) in chicken HSP70 gene have earlier been identified and its attendant effects on growth traits and egg production performance of acute heat stressed chickens have been evaluated *(Liang et al., 2016)*. Significant association have been reported between HSP70 genotypes and thermo-tolerance in chickens *(Mahmoud, 2000; Tamzil et al., 2014)*. However, information on the variation in HSP70 gene and their possible association with heat tolerance has been poorly documented in Yoruba and FUNAAB-Alpha chickens in Nigeria. Yoruba ecotype chickens are often found around Rainforest and Derived Savannah

zones of Nigeria while, FUNAAB-Alpha chickens are crossbred between Nigerian indigenous chickens and an exotic breed of chicken *(Ajayi, 2010; Ilori et al., 2016).*

Material and Methods

Experimental birds

A total of 256 apparently healthy one-day-old chicks comprising 138 FUNAAB-Alpha and 118 Yoruba ecotype chicks were used for this study. The chicks were tagged at a one-day-old and managed on deep litter system for 24 weeks. They were fed chick starter (0-6 weeks) and grower (7-24 weeks) diets ad-libitum with unrestricted access to fresh clean water.

Blood sampling

At week 12, blood samples were collected via the jugular vein into sterilized tubes with EDTA as anticoagulant, kept immediately in icebox and transported to the laboratory for DNA extraction.

DNA extraction, PCR amplification and restriction digestion

Genomic DNA was extracted with the Zymo® Quick-DNA™ Mini Prep kit by following the manufacturer's protocols. The PCR was performed using primers designed by *Akaboot et al. (2012)* F: 5'-AACCGCACCACACCCAGCTATG-3' and R: 3'-CTGGGAGTCGTTGAAGTAAGCG-5' in a 50 µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. After an initial 5-minute denaturation at 94°C, 35 cycles denaturation for 30 s were performed at 94°C, annealed for 60s at 60 °C with 90 s extension at 72°C then, followed by a final 5-minute extension at 72℃. The amplified DNA were subsequently digested using *Mme*I restriction enzyme with the following reaction mixtures: 9 µL nuclease free water, 1 µL 10 x assay buffer, 0.5 µL restriction enzyme, 5 µL PCR product. The mixture was incubated at 37℃ for 15 minutes. The bands were separated using 1.5% agarose gel electrophoresis for 45 minutes at 100 V and then viewed with BIO-RAD gel documentation system (USA). The reaction protocols were as described by *Akaboot et al. (2012)* with slight modifications.

Acute heat stress exposure and data collection

At week 23, a total of 36 chickens (6 each of the identified HSP70 genotype) were purposefully selected from the flock and exposed to an acute heat stress at 40±1°C for 1.0 hour *(Tamzil et al., 2014)*. At 0 and 1 hour after heat stress the following physiological parameters were measured as follow: cloaca temperature (CT): inserting a clean clinical thermometer into the vent for one minute after which the readings were taken; respiration rate (RR): counting the number of movements of abdominal region or vent of each bird for a minute using a stopwatch; pulse rate (PR): placing the stethoscope under the wing vein and counting the number of beats per minute. Heat stress index (HSI): The heat stress index was calculated as described by *Isidahomen et al. (2012)* as follows:

$$\text{HSI} = \frac{\text{Average respiratory rate value}}{\text{Average pulse value}} \text{ x } \frac{\text{Normal pulse rate value}}{\text{Normal respiratory rate}}$$

Statistical analysis

Allele and genotype frequencies, test for Hardy-Weinberg equilibrium and Heterozygosity were obtained using POPGENE 1.32 software package *(Yeh, 1999)*. Data on physiological parameters were analysed using the generalized linear model (GLM) of SAS (2010). The following linear model was employed:

$Yij = \mu + Gi + Bj + (GB)ij + eij$

Where: Yij: is observed physiological, μ: is the overall mean, Gi: is the fixed effect of ith genotype, Bj: is the fixed effect of jth breed, (GB) ij is the interaction effect of ith genotype and jth breed, eij: is random error associated with each record.

Results and Discussion

The PCR amplification of HSP70 gene resulted in a single and specific band in all the studied chicken populations (360 bp), while restriction fragment analysis using *Mme*I restriction endonuclease resulted in three genotypes: AA (uncut 360 bp), BB (229 bp) and AB (360 bp and 229 bp) (Plate 1).

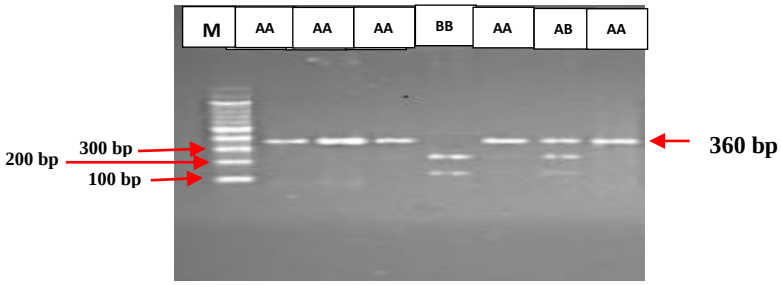

Plate 1. RFLP gel image of HSP70 gene in Nigerian Chickens
M: 100 bp DNA marker, Genotypes: AA, BB and AB.

The AA genotype (360 bp) had the highest frequency while BB genotype (229 bp) was the least frequent in the studied chicken populations (Table 1). Thus, using *Mme*I restriction enzyme; HSP70 gene can be safely concluded to be polymorphic in the studied Nigerian chickens. *Mahmoud (2000)* obtained three distinct allelic fragments from restriction digestion of chicken HSP70 gene with *Pst*I enzyme. Two polymorphic sites (A258G and C276G) and three genotypes (AA, AB and BB) were also reported in broiler and Taiwan native chickens, respectively *(Mazzi et al., 2003; Liang et al., 2016)*. Chi-square analysis of the observed and expected genotype frequencies showed deviation from the Hardy-Weinberg equilibrium (Table 1). This implies that, HSP70 locus in the studied birds has not been significantly affected by factors such non-random mating, mutation, genetic drift and or selection.

Table 1. Genotype frequencies of HSP70 gene in Nigerian chickens

		Genotype frequency			Heterozygosity		
Ecotype	N	AA	AB	BB	Observed	Expected	HWE (χ^2)
Yoruba	67	0.62	0.24	0.15	0.24	0.39	10.3*
FUNAAB Alpha	72	0.65	0.19	0.15	0.22	0.38	16.7*

Rectal temperature (RT), pulse-rate (PR) and respiratory rate (RR) are among the most important measure of physiological response of poultry to the heat stress. Following acute heat stress exposure, the observed average respiratory rate (RR) from this study ranged between 43.7±3.93 and 58.2±6.09 beat/min (Tables 2 and 3). This is closer to the earlier reported average RR value (44.6 and 51.07 beat/min) by *Adedeji et al. (2015)* in Nigerian chickens under heat stress. The higher RR value in FUNAAB-Alpha compare with the Yoruba chickens agreed with the reports of *Yalcin et al. (1997)* that body size of chicken influences the RR and pulse rate.

Table 2. Physiological responses of acute heat-stressed Yoruba chickens

Parameter	Duration of heat	AA	AB	BB
RR (beat/min)	0 hour	35.8±1.37^{b}	38.5±1.38^{ab}	39.7±1.37^{a}
	1 hour	43.7±3.93	46.8±3.54	45.7±4.03
CT (^{O}C)	0 hour	40.3±0.82^{b}	40.0±0.27^{b}	41.1±0.82^{a}
	1 hour	40.8±0.45	40.2±0.86	41.6±0.79
PR (breath/min)	0 hour	239.0±47.75	295.5±30.96	281.8±21.68
	1 hour	295.5±30.96	302.7±46.09	299.2±46.23
HSI	0 hour	1.30±0.19	1.13±0.12	1.16±0.11
	1 hour	1.44±0.51	1.37±0.26	1.42±0.17

CT: cloaca temperature, RR: respiration rate, PR: pulse rate, HSI: heat stress index.
Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

Body size affects tolerance to heat stress and the exotic chickens are less tolerant to heat stress than the tropically adapted chickens. More so, crossbreeding might have led to reduced thermo-tolerance of the FUNAAB-Alpha chickens. In addition, *Defra (2003)* submitted that body weight, species and breed affected the heat production by poultry, thus, increase in ambient temperature led to increase panting rate consequently increases the respiratory rates. More so, the observed changes in physiological parameters by the acute heat stressed birds could indicate an attempt to maintain thermal equilibrium.

Table 3. Physiological responses of acute heat-stressed FUNAAB-Alpha chickens

Parameter	Duration of heat	AA	AB	BB
RR (beat/min)	0 hour	41.8±1.17	41.7±1.37	41.0±1.55
	1 hour	58.2±6.09	53.6±2.93	53.8±1.49
CT (^{O}C)	0 hour	40.0±0.72	40.5±0.58	40.3±0.26
	1 hour	40.4±0.64	41.7±1.29	40.6±0.53
PR (breath/min)	0 hour	284.6±8.66	296.9±23.22	312.4±34.27
	1 hour	316.9±24.81^{b}	319.8±23.84^{ab}	344.5±8.55^{a}
HSI	0 hour	1.23±0.03	1.22±0.08	1.14±0.11
	1 hour	1.59±0.23^{a}	1.46±0.14^{ab}	1.35±0.10^{b}

CT: cloaca temperature, RR (beat/min): respiration rate, PR (breath/min): pulse rate, HSI: heat stress index. Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

The heat stress index of UNAAB-Alpha chickens with AB and BB genotypes were similar ($p>0.05$) but significantly different from their counterparts with AA genotype (Table 3), indicating that individuals having B allele as potential heat resistant candidate; since higher heat stress index indicates higher severity of

the heat stress *(Isidahomen et al., 2012)*. In contrast with this observation, *Tamzil et al. (2014)* reported that chickens with heterozygote (AD) and homozygote (BB) genotypes of HSP70 as the most heat-tolerant and the least heat-tolerant, respectively. The discrepancies between the observed values from this study and reports of *Tamzil et al. (2014)* could be due to differences in chicken breeds studied (Nigerian indigenous chickens vs Indonesian native chickens) or varied laboratory protocols, as the authors utilised Polymerase Chain Reaction (PCR)-Single Stranded Conformation Polymorphism-(SSCP) as against the PCR-RFLP that was used in this study. In PCR-RFLP, a PCR amplicon is treated by a certain restriction enzyme that cleaves the DNA via the restriction sites to generate DNA fragments (thus, involves screening limited amplicons) while PCR-SSCP screens almost all amplicon sequences thereby capable of detecting more genotype or SNPs.

Conclusion

The RFLP analysis of HSP70 gene was polymorphic in the studied Nigerian chickens using *Mme*I endonuclease. The B allele was associated with lower heat stress index indicating that; chickens with B allele exhibited higher heat-tolerance. However, further investigations under long-term heat stress would be necessary to validate this observations.

Fiziološki odgovor nigerijskih lokalnih pilića različitih genotipova HSP70 na akutni toplotni stres

Rasheed Babatunde Fatai, Mabel Omolara Akinyemi, Osamede Henry Osaiyuwu, Kayode Muslim Ewuola, Adebowale Emmanuel Salako

Rezime

Spora brzina porasta i akutni toplotni stres su među glavnim ograničenjima za proizvodnju autohtonih pilića u Nigeriji. Karakterizacija gena otpornosti na toplotu je preduslov za selektivni uzgoj živine radi poboljšanja toplotne tolerancije i produktivnosti. Stoga je predmet ovog istraživanja bilo ispitivanje varijacije u genu HSP70 i njegove povezanosti sa osobinama otpornosti na toplotu nigerijskih lokalno prilagođenih pilića. Pilići uzrasta od jednog dana, i to - 118 pilića ekotipa Yoruba (Yoruba Ecotype Chicken – YEC) i 138 pilića FUNAAB Alpha - FAC su obeleženi i hranjeni, *ad-libitum* starter (0 - 6 nedelja) i grover (7-24 nedelje) obrocima. U 12. nedelji, uzorkovana je krv, ekstrahovana DNK, amplifikovana i podvrgnuta elektroforezi prema standardnim procedurama. Gen HSP70 je

genotipizovan korišćenjem *Mme*I restrikcione endonukleaze. U 23. nedelji, 36 pilića (šest pilića po identifikovanim HSP70 genotipovima) odabranih iz svakog od YEC i FAC je izloženo temperaturi od 40±1°C tokom jednog sata. Temperatura kloake, brzina disanja (RR) i puls (PR) su zabeleženi i izračunat je indeks toplotnog stresa (Heat Stress Index - HSI). Podaci su analizirani korišćenjem deskriptivne statistike i ANOVA. Detektovani su aleli A i B sa genotipovima: AA, AB i BB. Nakon akutnog toplotnog stresa, YEC sa BB je imao veću RR vrednost u poređenju sa onima sa AA i AB. PR vrednost za genotip FAC sa genotipom BB bila je značajno viša ($p<0,05$) od vrednosti AA, ali slična kao kod pilića sa AB. U okviru FAC, indeks toplotnog stresa za BB-HSP70 bio je niži od AA-HSP70, ali sličan AB-HSP70, dok je unutar YEC, indeks toplotnog stresa za BB-HSP70 bio sličan onima za AA-HSP70 i AB-HSP70. Gen HSP70 je bio polimorfan kod proučavanih pilića, a genotip BB-HSP70 je bio povezan sa termo-tolerancijom.

Ključne reči: autohtona živina, poboljšana rasa, toplotni stres, HSP70, indeks toplotnog stresa, PCR-RFLP

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