Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

HSP70 And *HSP90β* Genes Polymorphism And Its Association With Thermotolerance In Fayoumi And Leghorn Chicken Breeds

Nagwa I. Sheraiba[1], Shabaan A. Hemeda[2], Hamada D.H. Mahboub[1], and Hanim S. Heikal[1].

(1) Department of Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

(2) Department of Animal Husbandry and Animal Wealth Development (Genetics and Genetic Engineering), Faculty of Veterinary Medicine, Alexandria University, Egypt.

*** Corresponding Author:** nagwa.abosheraiba@vet.usc.edu.eg ***Submitted:*** *16 Aug. 2019* ***Accepted:*** *7 Sept. 2019*.

ABSTRACT

This study was intended to studying polymorphisms of *HSP70* and *HSP90β* genes and its possible association with heat resistance in Fayoumi and Leghorn chickens. Chickens of each breed were classified phenotypically to heat susceptible and heat resistant groups based on chicken behavior during heat stress period. PCR-RFLP technique was used to genotype *HSP70* and *HSP90β* genes polymorphisms. Digestion of an amplified 360 bp of *HSP70* gene with *EaeI* restriction enzyme resulted in three genotypes, E1E1, E1E2 and E2E2. Results analysis indicated that there were no significant differences of different genotypes between heat susceptible and heat resistant Fayoumi chickens. In Leghorn chickens the genotype E2E2 was found in almost heat susceptible chickens with frequency of 0.75% and can be used as a marker for heat susceptibility while, E1E1 genotype occurs with frequency of 0.6 % in heat resistant chickens and can be used as heat resistance marker. Digestion of *HSP90β* gene amplified 498 bp with *MspI* restriction enzyme resulted in one genotype M1M1 in both heat susceptible and heat resistant Fayoumi chickens. Also, all leghorn resistant chickens showed M1M1 genotype. While, the genotype frequency in Leghorn susceptible chickens were 0.7 M1M1 and 0.3 M1M2. Accordingly, the *HSP90β* heterozygous M1M2 genotype can be used as marker assisted selection for culling of heat susceptible birds.

Key Words: *Chickens, HSP70, HSP90β, polymorphisms.*

INTRODUCTION

Heat stress represent great environmental challenges distressing poultry production and induce significant economic losses in the poultry production. During summer, events of extreme heat wave and modern poultry genotypes enhanced sensitivity of to heat stress have become major concerns in poultry industry. It leads to reduction in several physiological and metabolic factors in poultry such as feeding efficiency, growth rate, egg production, eggshell quality, fertility and survivability (Lin *et al.*, 2006; Kapakin *et al.*, 2012). Selection of heat resistant poultry lines can be used for relieving these problems. Molecular and physiological mechanisms elaborated in the stress response are manifested by a radical decrease in protein synthesis, except for the heat shock proteins (HSPs), the expression of HSPs is detectably increasing under stress conditions (Guerreiro *et al.*, 2004). In chicken, the *HSP70* and *HSP90* families are related to quick recovery of denatured proteins to their initial conditions and the acquisition of thermotolerance (Surai, 2015).

Polymorphisms of HSP70 and *HSP90β* genes were studied and markers for heat tolerance in chicken breeds were identified previously. Several studies indicated that, *HSP70* gene has

different genotypes that are accompanying with differing levels of chicken's heat tolerance. Zhen *et al.,* (2006) genotyped individuals for single nucleotide polymorphisms in chicken *HSP70* gene by single strand conformation polymorphism analysis. They found that, in the liver and leg muscle different genotypes at (C276G and A258G) sites were associated with mRNA expression of *HSP70* gene. Moreover, the heterozygous *HSP70* genotype is characterizes by significantly higher mRNA expression than other genotypes. The haplotype (GC) was favorable in heat resistance improving. Despite intense selection systems in broilers, polymorphisms of *HSP70* gene may participate in the defenses against heat stress (Abdolalizadeh *et al.,* 2015).

In Lingshan and White Recessive Rock chickens G141A SNP in the *HSP90β* gene 5' promoter region was existing in chickens with a great heat tolerance (Chen *et al.,* 2013). Wan *et al.,* (2017) identified polymorphisms in the *HSP90AB1* gene, they explained that G allele single nucleotide polymorphism at G6798A occurring in exon 14 improved heat tolerance and displayed a longer survival in Huainan chickens during heat stress. These SNPs in *HSP70* and *HSP90* genes have ability to be used in breeding programs for improving chickens heat tolerance.

The objective of this study was to evaluate the presence of polymorphisms in the *HSP70* and *HSP90β* genes in Fayoumi and Leghorn chickens by using PCR-RFLP technique. Existence of polymorphisms in these genes opens the possibility that one of them might be associated with heat stress tolerance and help to identify potential genetic markers for heat tolerance in this chicken breeds.

MATERIALS AND METHODS

Experimental birds and samples collection

All birds handling procedures as well as samples collection and disposal were according to the regulations of Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City. A total of 160 8th week old female pure breed Fayoumi and Leghorn chickens (80 Fayoumi and 80 Leghorn) were used in this study. All chickens were apparently healthy and free from any clinical disorders or diseases. The Fayoumi chickens pure breed of 6th weeks old were obtained from integrated poultry project in El Azab - Fayoum Governorate-Egypt. While, the Leghorn chickens pure breed of 6th weeks old were obtained from animal production research station in Burj El Arab- Alexandria- Egypt. The birds were exposed to heat stress by raising environmental temperature to 37 ±1^{o}C for three successive hours. Birds were classified into 4 groups for breed and response to heat stress. This classification was based on chicken behavior during heat stress period. The heat susceptible chickens were suffered from panting, wings spreading and squatting on the ground otherwise, the heat resistant birds appeared normal (Nardone *et al.,* 2010). Blood samples were collected from wing vain from the 4 groups (20 chicken/group) in sterile vacutainer tubes containing K2-EDTA as an anticoagulant. These samples were transported to the laboratory in liquid nitrogen tank then stored at -20°C. Although only 80 sample is required, we started by 160 chicken to confirm heat susceptibility and heat resistance of chicken samples.

Isolation and evaluation of genomic DNA

Genomic DNA was extracted from the whole blood using PureLink® Genomic DNA Purification Kit (Invitrogen) following the manufacturer protocol.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP):

PCR primers of chickens *HSP70* gene and *HSP90β* gene is described in table (1). Forward and reverse primers of *HSP70* gene were used to amplify 360 bp from the beginning of the coding region and *EaeI* restriction enzyme was used for genotyping polymorphisms in this region as described by (Duangjinda *et al.,* 2017). On the other hand, *HSP90β* gene Primers were used to amplify 498 bp from the 5' flanking region and exon 2 of this gene, and polymorphisms in this region was genotyped by using *MspI* restriction enzyme according to (Chen *et al.,* 2013).

Table 1. Primer sequences and restriction enzymes used in PCR-RFLP technique

Gene	Primer sequence (5'-3')	TM	Size	RE
HSP70	F:5'-AACCGCACCACACCCAGCTATG-3' R:5'-CTGGGAGTCGTTGAAGTAAGCG-3'	64 ^{o}C	360bp	*EaeI*
HSP90β	F:5'-GGTCGCGTGGAACTCTCTGGAA-3' R:5'-GTCGGAGGCGTTGGAGATGAG-3'	59.7^{o}C	498 bp	*MspI*

The amplification was carried out using a preprogrammed thermal cycler version 2.09 (GENEAMP®, PCR system 2720, Applied Biosystems) and DreamTaq Green PCR Master Mix (Thermo Scientific, Fermentas). Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA template, 1 µl of each primer (10 pmol /µl) and some deionized water making up a final volume of 25 µl. Cycles applied were: initial denaturation at 94 ^{o}C for five minutes; following by 35 cycles of denaturation at 94 ^{o}C for 30 seconds, annealing at temperatures of 64 ^{o}C for *HSP70* gene and 59.7 ^{o}C for *HSP90β* gene for 45 seconds, and extension 72 ^{o}C for 45 seconds; then a final extension at 72 ^{o}C for five minutes.

The amplified PCR product of *HSP70* and *HSP90β* genes were digested with the fast cut restriction enzyme *EaeI* (NEB®, New England, USA) and *MspI* (Enzynomics, Korea) respectively according to the manufacturer's instructions. After the end of digestion time, resulting products were separated by agarose gel electrophoresis and bands were visualized under gel documentation system for data analysis.

Statistical analysis:

Genotype frequencies were determined according to Falconer and Mackay equation (*Falconer and Mackay*, 1996).

$$Gi = \Sigma ni / N$$

where Gi is the i genotype frequency, ni is the number of animals with genotype i, and N is the total number of samples.

Chi-squared test is used to study the significant difference between different genotypes in different breeds used in this study and heat susceptibility and heat resistance. Fisher's exact test was performed at the Website of Gunma University, Japan (http://aoki2.si.gunma-u.ac.jp/exact/fisher/getpar.html) (accessed on 12 May 2019).

RESULTS

PCR-RFLP analysis and genotyping frequency of HSP70 gene

Chickens *HSP70* PCR oligonucleotide primers amplified products of 360 bp DNA fragment from all eighty DNA samples of Fayoumi and Leghorn heat susceptible and heat resistant chickens as shown in figure 1. This amplified DNA fragment contains a point mutation and the mutation created different restriction patterns with different genotypes related to heat tolerance in chickens. Chickens with genotype E1E1 have three DNA fragments with molecular weight 200 bp,110 bp and 50 bp. While, chickens with genotype E2E2 have two DNA fragments with molecular weight 250 bp and 110 bp. So that, individuals contain both bands at 200 bp and 250 bp considered as the heterozygote genotype (E1E2).

Genotyping frequency data of *HSP70* gene in Fayouni and Leghorn chickens is illustrated in table 2. In Fayoumi chicken the genotypes frequencies of heat susceptible birds were 0.3 E1E1, 0.5 E1E2 and 0.2 E2E2 while, in resistant birds the frequencies were 0.3 E1E1, 0.55 E1E2 and 0.15 E2E2 with Fisher's exact test; p =1. There were no significant differences between the three genotypes.

In Leghorn chicken breed the resistant chickens showed only two genotypes E1E1 and E1E2 while the susceptible chickens showed the three genotypes with different frequencies. The genotypes frequencies of heat susceptible birds were 0.1 E1E1, 0.15 E1E2 and 0.75 E2E2 while, in heat resistant birds were 0.6 E1E1, 0.4 E1E2 and 0.0 E2E2 with Fisher's exact test; $p < 0.001$. There were a highly significant differences between genotypes.

Table 2: Genotyping frequencies of HSP70 gene in Fayoumi and Leghorn chickens.

Breed	Phenotype	Number of birds carrying the genotype and their frequency			Total no	*p* value of (2 × 3 contingency table, Fisher's exact test)	*Chi square*		
		E1E1	E1E2	E2E2			Value	df	p-value
Fayoumi	Susceptible	6/20 = 0.3	10/20 = 0.5	4/20 = 0.2	40	1	0.19	2	0.91
	Resistant	6/20 =0.3	11/20 =0.55	3/20 =0.15					
Leghorn	Susceptible	2/20 = 0.1	3/20 = 0.15	15/20 = 0.75	40	**6.54**× 10^{-7} ***	24.42 ***	2	4.99× 10^{-6}
	Resistant	12/20 = 0.6	8/20 = 0.4	Zero					

*** indicate highly significant results

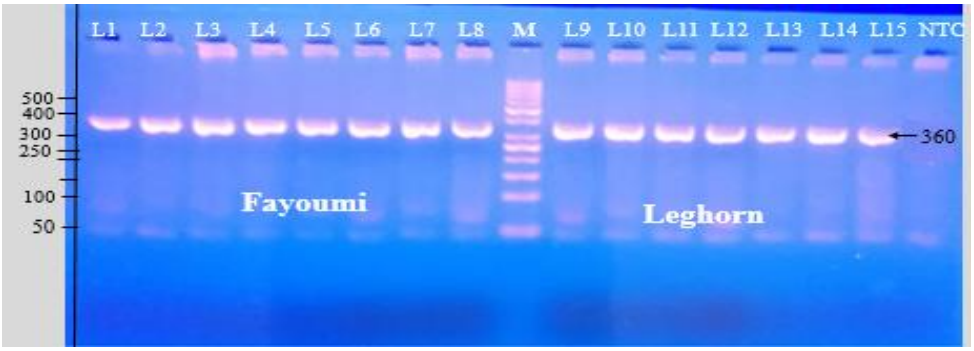

Fig.1: PCR amplicons of chickens *HSP70* gene (360 bp) on 2.5% agarose gel. M: 50 bp DNA molecular weight marker, NTC: no templet control, L1-L8: PCR amplicons of Fayoumi chickens, L9 -L15: PCR amplicons of Leghorn chickens.

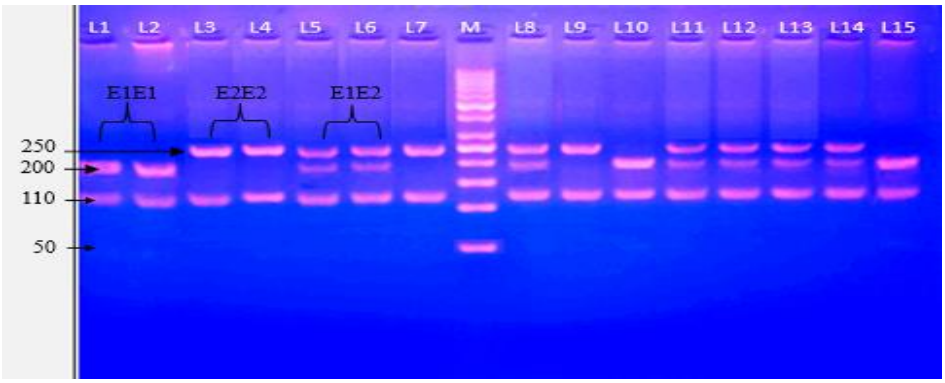

Fig.2: Restriction digestion of *HSP70* gene PCR products of Fayoumi chickens by *EaeI* restriction enzyme on 3% agarose gel. M: 50bp DNA molecular weight marker, L1-L15: different restriction pattern of *HSP70* gene PCR products of Fayoumi susceptible and resistant chickens. Lan1 and lan2 represent E1E1genotype; lan3 and lan4 represent E2E2 genotype; and lan5 and lan6 represent E1E2 genotype.

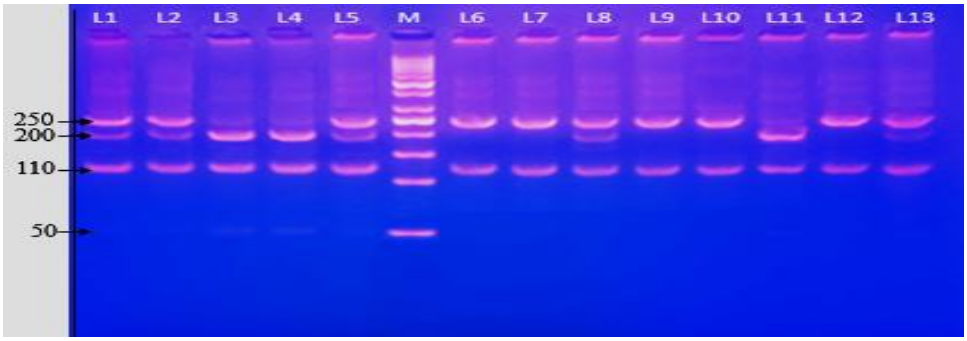

Fig.3: Restriction digestion of *HSP70* gene PCR products of Leghorn chickens by *EaeI* restriction enzyme on 3% agarose gel. M: 50bp DNA molecular weight marker, L1-L13: different restriction pattern of HSP70 gene PCR products of Leghorn susceptible and resistant chickens.

PCR-RFLP analysis and genotyping frequency of HSP90β gene

The effect of *MspI* restriction enzyme on *HSP90β* gene (498 bp) in the Fayoumi chicken breed resulted in one uncut 498 bp DNA fragment and only M1M1genotype is presented in both heat susceptible and heat resistant birds (figure 4). In Leghorn chickens the restriction patterns of *MspI* restriction enzyme differed in heat susceptible and heat resistant birds. All heat resistant Leghorn chickens revealed uncut 498 bp DNA fragments representing the M1M1 genotype, while the heat susceptible birds showed two different restriction patterns of one uncut band at 498 bp (M1M1 genotype) and three bands at 498, 250 and 248 bp of (M1M2 genotype) as shown in figure (5).

The genotyping frequency analysis of *HSP90β* 498 bp PCR product shown in Table 3. In Fayoumi chickens, M1M1 genotype has frequency of 100% in both heat susceptible and heat resistant chickens. In Leghorn chickens the genotypes frequencies of heat susceptible birds were 0.7 for M1M1 genotype and 0.3 for M1M2 genotype while, the leghorn resistant birds all showed the undigested M1M1 genotype (Fisher's exact test; $p < 0.05$). There were a significant difference between heat susceptible and heat resistant Leghorn birds.

Table 3: Genotyping frequencies of *HSP90β* gene in Fayoumi and Leghorn chickens.

Breed	Phenotype	Number of birds carrying the genotype and their frequency		Total no	*p* value (2 × 2 contingency table, Fisher's exact test)	*Chi square*		
		M1M1	M1M2			value	Df	p-value
Fayoumi	**Susceptible**	20/20 =1	0	40	1	0	0	1
	Resistant	20/20 =1	0					
Leghorn	**Susceptible**	14/20 =0.7	6/20 =0.3	40	**0.0202** *	7.059*	1	0.0089
	Resistant	20/20 =1	0					

* indicate significant results

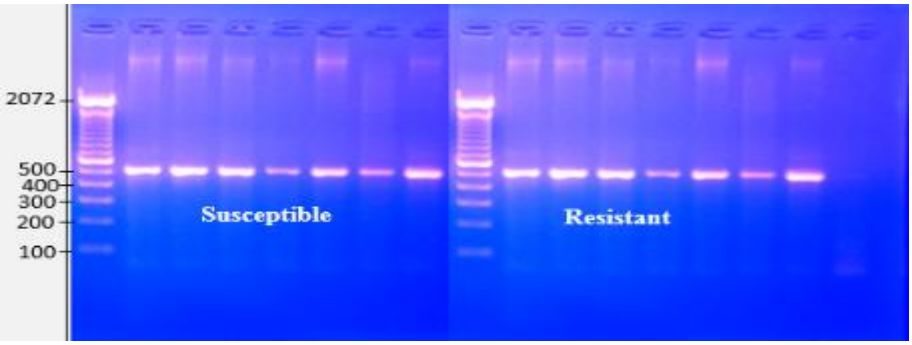

Fig.4: Restriction digestion of HSP90β gene PCR products of Fayoumi (susceptible and resistant) chickens by MspI restriction enzyme on 3% agarose gel showing all un digested DNA fragments. M: 100bp DNA molecular weight marker, L1-L7: digestion products of Fayoumi susceptible chickens, L8 -L14: digestion products of Fayoumi resistant chickens.

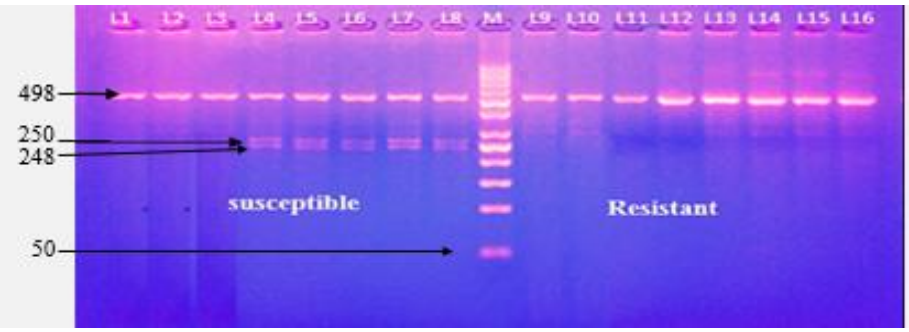

Fig.5: Restriction digestion of *HSP90β* gene PCR products of Leghorn (susceptible and resistant) chickens by *MspI* restriction enzyme on 4% agarose gel. M: 50 bp DNA molecular weight marker, L1-L3 un digested PCR products L4-L8: digested PCR products of leghorn susceptible chickens, and L19 -L16: un digested PCR products of leghorn resistant chickens.

DISCUSSION

Heat tolerance in poultry production was attained great attention due to the need for genetic lines that can withstand climate changes (Felver-Gant *et al.,* 2012). *HSP70* and *HSP90* are the predominant genes having protective role during heat stress in farm animals (Pawar *et al.,* 2016).

Results of *HSP70* genotyping showed in table (2) explained that, the E1E2 and E2E2 genotypes presented with frequency of E1E2 (0.5, 0.55) and E2E2 (0.2, 0.15) in susceptible and resistant Fayoumi chickens respectively. Equal frequency of 0.3 was observed for E1E1 genotype in Fayoumi susceptible and resistant chicken. The near genotypes and alleles frequencies in Fayoumi breed in this result may be due to Fayoumi is highly conserved breed and didn't undergo selection as in other commercial breeds. On the other hand, in Leghorn chickens the genotype frequency in susceptible and resistant chickens were E1E1 (0.1, 0.6), E1E2 (0.15, 0.4) and E2E2 (0.75, zero) respectively. So, we could say that, in the Leghorn chicken breed the E1 allele is more tolerant to heat stress but, the E2 allele is more vulnerable to heat stress. This mutation in the *HSP70* gene can be used for MAS for heat tolerance within Leghorn chicken breed but not in Fayoumi chicken breed. We could apply the genotyping results of *HSP70* gene in breeding and selection programs aimed at improving the thermotolerance of Leghorn chickens where the E1E1 and E1E2 are heat tolerant chicken while, the E2E2 is heat susceptible chicken.

These results are in agreement with Various studies which have shown that the polymorphisms in chicken *HSP70* gene in different breeds has been found to be related to different heat stress resistance. Zhang *et al.,* (2002) genotyped individuals from different genetic backgrounds for two SNP in HSP*70* gene. They found that, G allele frequencies of A258G mutation and C allele frequencies of C276G mutation were higher in the F2 populations (result from crossing between Xinghua and Taihe Silkies chickens) than in Yangshan chickens. Duangduen, (2008) results were also in accordance with our observation as they reported that, chickens containing C1C2/ M2M2 genotype of *HSP70* gene after digestion with *CfrI* and *MmeI* restriction enzyme exhibited better heat tolerance than chickens with other genotypes. However, chickens presented the genotype of C2C2/ M1M2 was sensitive to heat stress. Tamzil *et al.,* (2013) analyzed *HSP70* gene polymorphisms using PCR-SSCP and genotyping results showed that there were three polymorphic sites found in amplified 360 bp from the beginning of *HSP7*0 gene coding region and this polymorphism accrue more heat tolerance in chickens. While**,** Moraa *et al.,* (2016) characterized unique *HSP70* heterozygotes and homozygotes that exist in indigenous chickens of Kenya which could be an adaptation to enable chickens to survive in various climatic conditions especially in regions with heat stress. So that they recommended that *HSP70* can be used as a marker in molecular breeding for drought/heat tolerance. Chen *et al.,* (2016) suggested that, the GG genotype of *HSP70* gene 5'-flanking region is advantageous for the prevention of thermal stress. Genotyping and allelic frequency variation of *HSP70* gene by Liang *et al.,* (2016) indicated that AA genotype has greater heat tolerance after acute heat stress in the indigenous Taiwanese chickens. Results of *HSP70* gene genotyping by Duangjinda *et al.,* (2017) using *CfrI* restriction enzyme also are indicated that C2C2 chickens were less tolerant to heat stress compared to other genotypes. Also, Phongkaew and Khumpeerawat, (2017) indicated that the C1C2 and M2M2 genotypes of the *HSP70* gene are associated with thermotolerance in chickens.

In addition, our present study results of *HSP90β* genotyping by PCR-RFLP technique using *MspI* restriction enzyme explained that Fayoumi chickens have one uncut 498 bp DNA fragment in both heat susceptible and heat resistant birds. This result indicating that the *HSP90β* gene is monomorphic and can't be used for differentiation between heat susceptible and heat resistant Fayoumi chickens. We could explain that by the high resistance of Fayoumi chickens and that the breed is highly conserved. While, in Leghorn chicken's results indicated the presence of two alleles, which were denoted as *HSP90–M1* and *HSP90–M2* and two genotypes, M1M1 and M1M2 were identified. The frequency of *HSP90* M1M1 and M1M2 genotypes in heat

susceptible birds were 0.7 and 0.3, respectively, while in Leghorn resistant chickens all individuals were monomorphic showing the genotype M1M1. These results indicated that the presence of M1M1 genotype is indicator for thermotolerance while, the heterozygous M1M2 genotype indicate susceptibility to heat stress. So that, the M1M2 genotype can be used as marker assisted selection for culling heat susceptible chickens. The effects of *HSP90β* genotyping on heat tolerance have been investigated in several studies. Chen *et al.,* (2013) identified a mutation at G141A in the 5' flanking region of *HSP90* gene which was associated with heat tolerance in Lingshan and White Recessive Rock chickens. The authors are identifying GG as the genotype with high heat tolerance. Sigei *et al.,* (2016) multiple sequence alignment outputs revealed high sequence similarity across all the selected chicken species for *HSP90* candidate gene which, is suggestive of high evolutionary conservation. Our results are also consistent with these finding since our results revealed that Fayoumi chickens has only the wild genotype of *HSP90β* gene in both susceptible and resistant birds. Wan *et al.,* (2017) identified G 6798A SNP in *HSP90B1* gene that produced three genotypes. The chickens with GG genotype have longer survival time than individuals with the other two genotypes, indicating that allele G was favorable for heat resistance and has potentially to be used in improving heat resistance.

CONCLUSION

In respect to the results of PCR-RFLP and chicken behavior after exposure to acute heat stress, we can concluded that restriction digestion of *HSP70* gene by *EaeI* restriction enzyme can be used as a marker in Leghorn chickens for selecting heat resistant and culling heat susceptible chickens where E1E1 and E1E2 genotypes are resistant to heat stress and the E2E2 is heat susceptible genotype. In Fayoumi chickens' polymorphisms in *HSP70* gene can't be used to differentiate between heat susceptible and heat resistant birds due to near genotype and allele frequencies. With respect to HSP90β gene polymorphisms after digestion by *MspI* restriction enzyme, Fayoumi is a monomorphic chicken breed showing one genotype in both heat susceptible and resistant groups while, in Leghorn chickens *HSP90β* gene can be used for marker assisted selection in culling heat susceptible birds and increasing heat resistance of the breed.

REFERENCES

Abdolalizadeh, N., Noshary, A., and Eila, N. 2015. Identification of single nucleotide polymorphisms of Hsp70 gene in a commercial broiler strain. Research Opinions in Animal and Veterinary Sciences, 5(6): 265-269.

Chen, Z. Y., Gan, J. K., Xiao, X., Jiang, L. Y., Zhang, X. Q., and Luo, Q. B. 2013. The association of SNPs in Hsp90β gene 5′ flanking region with thermo tolerance traits and tissue mRNA expression in two chicken breeds. Molecular biology reports, 40(9): 5295-5306.

Chen, Z. Y., Zhang, W. W., Gan, J. K., Kong, L. N., Zhang, X. Q., Zhang, D. X., and Luo, Q. B. (2016). Genetic effect of an A/G polymorphism in the HSP70 gene on thermotolerance in chicken. Genetics and Molecular Research, 15(2).

Duangduen, C. 2008. Effect of HSP 70 genotypes on thermotolerance in Thai native chicken. In: Master's Degree Thesis, Khon Kean. University, Thailand. 60 p.

Duangjinda, M., Tunim, S., Duangdaen, C., and Boonkum, W. 2017. Hsp70 genotypes and heat tolerance of commercial and native chickens reared in hot and humid conditions. Revista Brasileira de Ciência Avícola, 19(1): 7-18.

Falconer, D. S., and Mackay, T. F. C. (1996). Introduction to quantitative genetics. 1996. Harlow, Essex, UK: Longmans Green, 3.

Felver-Gant, J. N., Mack, L. A., Dennis, R. L., Eicher, S. D., and Cheng, H. W. 2012. Genetic variations alter physiological responses following heat stress in 2 strains of laying hens. Poultry science, 91(7): 1542-1551.

Gan, J. K., Jiang, L. Y., Kong, L. N., Zhang, X. Q., and Luo, Q. B. 2015. Analysis of genetic diversity of the heat shock protein 70 gene on the basis of abundant sequence polymorphisms in chicken breeds. Genetics and Molecular Research, 14(1): 1538-1545.

Guerreiro, E. N., Giachetto, P. F., Givisiez, P. E. N., Ferro, J. A., Ferro, M. I. T., Gabriel, J. E., et al., 2004. Brain and hepatic Hsp70 protein levels in heat-acclimated broiler chickens during heat stress. Brazilian Journal of Poultry Science, 6(4): 201-206.

Kapakin, K. A. T., GÜMÜŞ, R., Imik, H., Kapakin, S., and SAĞLAM, Y. S. 2012. Effects of ascorbic and α-lipoic acid on secretion of HSP-70 and apoptosis in liver and kidneys of broilers exposed to heat stress. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 59(4): 279-287.

Liang, H. M., Lin, D. Y., Hsuuw, Y. D., Huang, T. P., Chang, H. L., Lin, C. Y., et al., 2016. Association of heat shock protein 70 gene polymorphisms with acute thermal tolerance, growth, and egg production traits of native chickens in Taiwan. Archives Animal Breeding, 59(2): 173-181.

Lin, H., Decuypere, E., and Buyse, J. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 144(1): 11-17.

Moraa, G. K., Oyier, P. A., Maina, S. G., Makanda, M., Ndiema, E. K., Alakonya, A. E., et al., (2016, May). Genetic background and hsp70 gene polymorphisms for heat tolerance in indigenous chickens of kenya. In Scientific Conference Proceedings (No. 1).

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M. S., and Bernabucci, U. 2010. Effects of climate changes on animal production and sustainability of livestock systems. Livestock Science, 130(1-3): 57-69.

Pawar, S. S., Sajjanar, B., Lonkar, V. D., Kurade, N. P., Kadam, A. S., Nirmal, A. V., et al., 2016. Assessing and mitigating the impact of heat stress on poultry. Advanced Animals and Veterinary Sciences, 4(6): 332-341.

Phongkaew, P., and Khumpeerawat, P. 2017. Heat shock protein 70 gene polymorphism in KU–Phuphan black–bone chicken. Genomics and Genetics, 10(1&2): 7-12.

Sigei, C., Kariuki, D., Ndiema, E., Wainaina, E., Maina, S., Makanda, M., ... & Ommeh, S. (2016, May). In silico detection of signatures for adaptive evolution at select innate immune and heat stress genes in indigenous poultry. In Scientific Conference Proceedings (No. 1).

Surai, P. F. 2015. Antioxidant systems in poultry biology: heat shock proteins. Journal of Science, 5(12): 1188-1222.

Tamzil, M. H., Noor, R. R., Hardjosworo, P. S., Manalu, W., and Sumantri, C. 2013. Polymorphisms of the heat shock protein 70 gene in Kampong, Arabic, and commercial chickens. Jurnal Veteriner, 14(3): 317-326.

Wan, Y., Ma, C., Wei, P., Fang, Q., Guo, X., Zhou, B., and Jiang, R. 2017. Dynamic expression of HSP90B1 mRNA in the hypothalamus of two Chinese chicken breeds under heat stress and association analysis with a SNP in Huainan chickens. Czech Journal of Animal Science, 62(2): 82-87.

Zhang, X., Du, H., Li, J., 2002. Single nucleotide polymorphism of chicken heat shock 604 protein (HSP70) gene. 7thWorld congress on genetics applied to livestock production, 605 France, Montpellier.

Zhen, F. S., Du, H. L., Xu, H. P., Luo, Q. B., and Zhang, X. Q. 2006. Tissue and allelic-specific expression of hsp70 gene in chickens: basal and heat-stress-induced mRNA level quantified with real-time reverse transcriptase polymerase chain reaction. British poultry science, 47(4): 449-455.