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The Hsp70 Expression Profiling in Fayoumi and Matrouh Chicken Subjected to Heat Stress

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Abstract

The present study was undertaken to test the effect of heat stress (39 °C) for 4 hours on hsp70 mRNA profiling in two chicken genotypes. Expression levels of hsp70 were used to assess the heat tolerance of two Egyptian local genotypes (Fayoumi and Matrouh). The expression level of the hsp70 gene is high in the Fayoumi type as opposed to the Matrouh type. In blood, after four hours of exposure, the hsp70 mRNA expression analysis revealed higher expression levels. These results showed that the acquired thermotolerance is positively linked to the stress memory. Within the two genotypes, Fayoumi type exhibited the highest means for hsp70 production, and therefore the heat stress is better tolerated, which indicates that Fayoumi mortality rate might be lower genetically under heat stress.

Keywords: mRNA; hsp70; Heat stress; Adult chicken.

1 Introduction

Poultry is one of the leading agricultural sectors that produce tones of meat and eggs annually. The best quality protein source is chicken meat and eggs; millions of people living in poverty use both. Chicken meat and eggs in addition to being nutritious, contain essential vitamins, minerals, and essential fatty acids (Anonymous 2002).

Prokaryotic and eukaryotic cells respond by synthesizing ubiquitous heat shock proteins at high temperatures, a protein family of 70-90,000 MW, and other smaller proteins of 20-30,000 MW (Schlesinger et al 1982). Other cellular effects of heat shock include altering the morphology of cells (Simard et al 1974; Thomas et al 1980), DNA synthesis inhibition disturbing the mitosis and cell cycle (Gerner; Russell 1977), generalized repression in RNA polymerase ll-dependent transcription (Spradling et al 1977; Finally; Pederson 1981), and the selective translation of the mRNAs of heat shock genes (Lindquist 1980; Lindquist et al 1982).

Heat stresses have negative implications for broilers and hens, from reduced growth and egg production to less poultry and eggs' safety. However, the detrimental effect of heat stress on poultry welfare should nevertheless be a significant concern (Lucas; Rostagno 2013). Birds respond by heat shock protein synthesis, which shields organs and cells from heat stress's adverse effects. (Amrutkar et al 2014). The heat shock response is a crucial component of the body's adaptation to elevated temperature resulting in various physiological changes. It is the primary agent that manages

the protein folding (Parsell; Lindquist 1993). This action is mostly controlled at the transcription level by heat shock factor 3 (HSF3) in birds and HSF1 in mammals (Akerfelt et al 2010; Fujimoto; Nakai 2010).

Chicks' thermal conditioning had a beneficial impact at an early age and enhanced the post-natal thermal tolerance potential, demonstrated by decreased expression of Heat Shock Proteins (HSP) genes and stress indicators in colored broiler chickens. The HSP and HSF genes participate in the primary cell defense mechanisms after exposure to high temperatures. Additionally, they play a crucial role in cell response to different stresses (Vinoth et al 2016; Cedraz et al 2017; Lin et al 2018). The expression of hsp70 gene in chickens varied between different tissue or organ and strains or races. Birds that have been better tolerated by heat generated higher hsp70 gene levels (Gan et al 2013; Liu et al 2014). Broiler chicks are more adapted to warm and warm climates; for example, Sinai's naked neck cocks were recommended as a male line of the commercial parent stock for their heat tolerance features. In contrast, the expression of hsp70 gene decreased at both the liver and spleen levels under cold exposure (Galal et al 2019).

Thermal manipulation (TM) reduced the rates and the body temperature but increased body weight. TM usually decreased hepatic expression, but cold stress did not alter the HSF3 splenic expression. In comparison, the expression of hsp70 both hepatically and splenetically decreased under cold exposure. The results of their analysis may dramatically indicate that TM affects the genetic reaction of broilers to cold treatment (Tarkhan et al 2020).

The current study aimed to evaluate the expression levels of the heat shock protein known as hsp70 using real-time PCR between Fayoumi and Matrouh chicken samples exposed to heat stress conditions.

2 Materials and Methods

2.1 Birds

This experiment was performed in the "El-Takamoly" chicken farm in El Fayoum Governorate, Egypt. A total of 12 Fayoumi and Matrouh fully mature chickens were divided into two groups. The control group where the two genotypes were reared at room temperature in a 55-60% humidity atmosphere. Whereas the treatment group was subjected to heat stress 39°C for 4 hours in a 55-60% humidity atmosphere. Six blood samples were collected randomly from each genotype to tubes containing l mg/ml EDTA.

2.2 RNA extraction and cDNA synthesis

Pure total RNA was isolated from blood samples (under sterilization conditions) using total RNA purification Kit 50 preps (Jena Bioscience, Germany). Transcriptor Synthesis cDNA Kit was used for cDNA synthesis following the manufacturer instruction. Once this process was completed, cDNA was reserved at -20℃ until quantification.

2.3 Real-time quantitative PCR (RT-PCR)

In this step the obtained cDNA from the previous step was quantified using the specific hsp70 and rpl5 primers as listed in **Table 1**. Real-time quantitative PCR was performed using 20 µl master mix which consists of 10 µl SYBERGREEN(P3), 0.5µl primer (P1), 0.5 µl primer (P2), 1.2µl buffer, 6.6 µl H_2O, and 1.2 µl of cDNA template. The PCR cycle program was performed as follow: initial denaturation at 92 °C / 2 min, repeated 40 cycles of 92 °C / 5 sec for denaturation phase, 56 °C / 15 sec for annealing phase, and 72 °C / 26 sec for extension phase. Dissociation test was performed from 95 °C to 50 °C at 10 min interval to test for dimerization.

Table 1. The primers used for the different studied genes

Gene	Sequence (5′→3′)	Annealing Temp. °C
Heat shock protein (hsp70)	F- GAGTGGCGCAGCGTAGAAAG R- TGCCTTTATACACACCCAACAG	59
House-keeping gene (rpl5)	F - AATATAACGCCTGATGGGATGG R -CTTGACTTCTCTCTTGGGTTTCT	58

Cycle threshold (CT) was calculated based on default setting of sequence detection results of real-time software. The equation of the graph is used to calculate the number of cDNA molecules per microgram from mRNA-converted cDNA.

The gene expression in the form of relative quantification was estimated using Ct values. The fold change of target genes in comparison to control was calculated according to livak method $2^{-\Delta\Delta Ct}$ (Livak; Schmittgen 2001).

2.4 Statistical analysis

One-Way ANOVA was performed using SAS v8.2 using a general linear model followed by Duncan's multiple range tests. The statistical models used in this study were as follows: $Y_{ij} = \mu + S_i + e_{ij}$

where μ = Overall mean, Li = strain effect, eij = Experimental error.

3 Results and Discussion

Extraction of ribonucleic acid (RNA) was successfully performed for all samples of the experiment. RNA was converted into cDNA by reverse transcription using a reverse transcriptase enzyme. The obtained cDNA from the previous process was used to quantitatively analyze the gene expression (qPCR) of the heat shock gene (HSP) for all samples representing in the two breeds (Fayoumi - Matrouh) using the reaction primers rpl (HKG) as an indicator to know the extent of the change in the gene expression of the thermal tolerance gene (HSP). The quantitative analysis of gene expression showed the successful amplification of all samples used and their biological replicates, as shown in **(Figure 1a)**.

To ensure the quantitative amplification's success, a (Melting Curve) test was performed, which showed one clear peak, evidence of the presence of one gene, and only one band for the target gene (HSP). This result reflects the specialization of the used primers, and accordingly, the amplification curves were used to obtain values (Ct) The amount of change in gene expression as shown in **(Figure 1b)**.

By extracting the CT values for all samples and their replicates, it was found that the CT values ranged from (27-32) for the rpl gene (HKG; as an indicator) and from 30-33 for HSP. Concerning the HKG, the samples with the highest values were (B1 T R3 - B2 T R2) and the lowest value (B2 C R1). For the thermal tolerance gene (HSP), the values were higher (B1 C R2 - B1 T R3 - B2 C R3) and lower values (B1 T R2 - B2 C R2). By subtracting the CT values of the thermal tolerance gene (HSP) from the CT values of the gene (HKG) to obtain (ΔCT), the (Δ CT) values ranged from (-4 : 0) and by calculating the rate of change in the gene expression (fold change) for each genotype. The mean for the first type (5.3) and the second type (4) as shown in **(Figure and Table 2).**

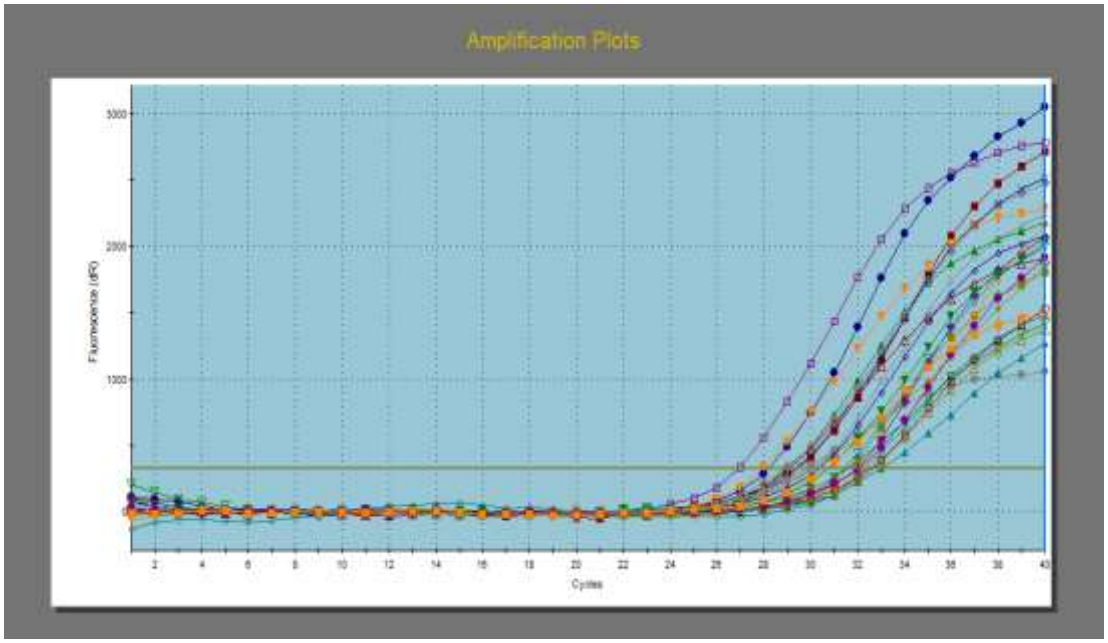

Fig 1a. Amplification curve of 12 samples from Fayoumi and Matrouh breeds.

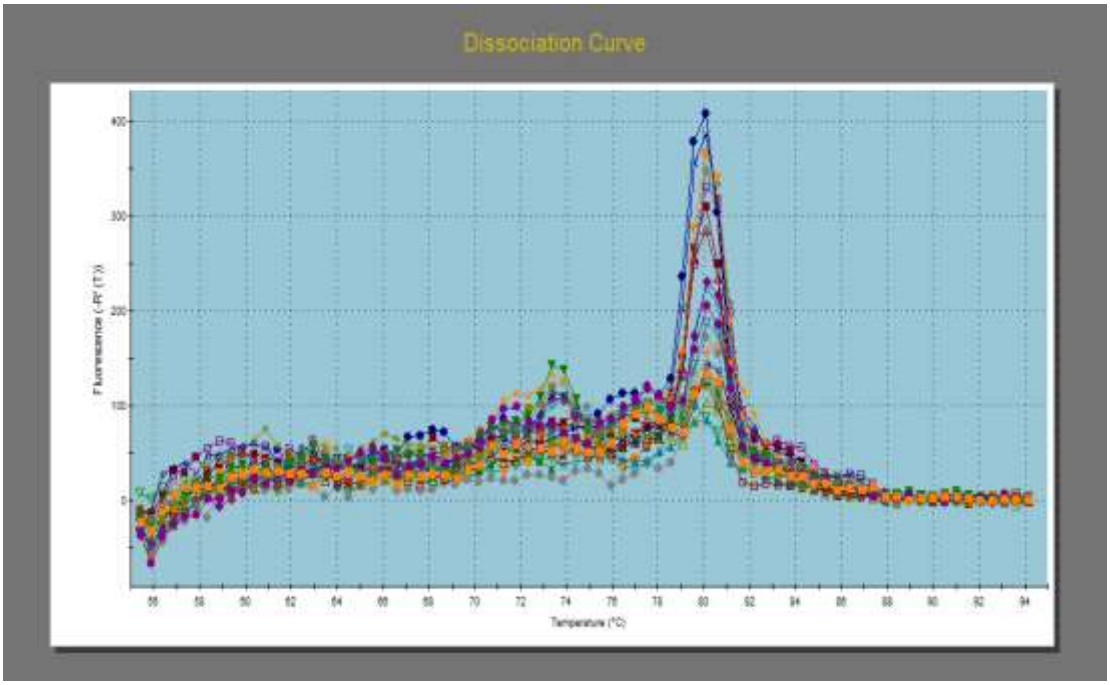

Fig 1b. Melt peak chart (Dissociation curve) of hsp70F and RPL5 gene.

Table 2. The CT values, delta-CT, ratio of relative expression for each the experimented genotypes (G)

ID	CT	HKG	HSP	Delta CT	Delta Delta CT	Fold Change	hsp70
Control	R1	29	32	-3	-2	4	5.3
G1C	R2	30	33	-3	-3	8	
	R3	29	32	-3	-2	4	
Treatment	R1	31	32	-1			
G1T	R2	30	30	0			
	R3	32	33	-1			
Control	R1	27	31	-4	-2	4	4
G2C	R2	28	30	-2	-2	4	
	R3	29	33	-3	-2	4	
Treatment	R1	29	31	-2			
G2T	R2	32	32	0			
	R3	30	31	-1			

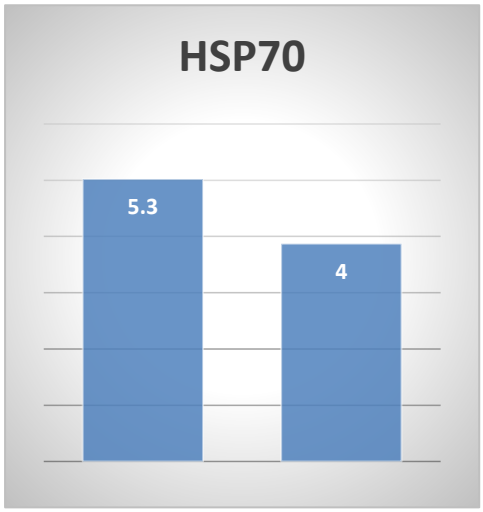

Fig 2. Fold change histogram Non-significant $p<0.05$

The hsp70 expression level of the gene for Fayoumi was higher than that Matrouh type. The previous results may indicate that the Fayoumi type was more heat tolerance than Matrouh type **(Fig 2)**. In blood, after four hours of exposure, the hsp70 expression analysis revealed higher expression levels. These results showed that the acquired thermotolerance is positively linked with stress. The obtained results agreed with Gan et al (2013); Amrutkar et al (2014); Liu et al (2014 & 2018).
The small number of samples (three samples per type only) to estimate the gene expression led to increase in the variance and standard error, which affected the accuracy and sensitivity of the statistical test between those two genotypes Fayoumi and Matrouh chickens.

4 Conclusion

In conclusion, the results suggest that the Fayoumi type responds to heat stress by increasing the synthesis of heat shock proteins (HSP). It better-tolerated heat stress produced greater quantities of hsp70 that play a crucial role in cell response to various stresses.

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ملامح التعبير الجيني لجين بروتين الصدمة الحرارية لطرازي الدجاج (فيومي – مطروح) تحت ظروف الاجهاد الحراري

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الموجـــــــز

تم إجراء هذه الدراسة لاختبار تأثير الإجهاد الحراري (39 درجة مئوية) لمدة 4 ساعات على تحديد نمط التعبير الجينى للحمض النووي الريبوزي الرسول لجين بروتين الصدمة الحرارية 70 في طرازين جينيين من الدجاج. تم استخدام مستويات التعبير عن جين بروتين الصدمة الحرارية 70 لتقييم مدى تحمل الحرارة لطرازين جينين مصريين (فيومى ومطروح). إن مستوى تعبير جين بروتين الصدمة الحرارية 70 مرتفع في نوع "فيومي" مقارنة بنوع "مطروح ."فى الدم، بعد أربع ساعات من التعرض، اظهر تحليل التعبير الجيني للحمض النووي الريبوزي الرسول لجين بروتين الصدمة الحرارية 70 عن مستويات أعلى للتعبير .أظهرت هذه النتائج أن المظهر الذي تم الحصول عليه مرتبط للغاية مع ذاكرة الإجهاد .وفى إطار الطرازين الوراثيين للدجاج، اظهر نوع الدجاج الفيومى أعلى مستويات التعبير الجينى لجين بروتين الصدمة الحرارية 70 وبالتالى فإنه يكون اكثر تحملا للإجهاد الحراري ، مما يشير إلى أن معدل وفيات الطرز الفيومي قد يكون أقل جينيا تحت ضغط حراري.