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Transcription Level of *HSP70, HSP90β, HSF1 and HSF3* Variation in Fayoumi and Leghorn Chicken's Breeds in Response to chronic Heat Stress.

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ABSTRACT

Climate is one of the most significant constraints on poultry production. Poultry, in particular, is highly sensitive to environmental challenges related to temperature, especially heat stress. High temperatures, particularly when combined with high humidity, place extreme stress on birds, which results in decreased performance. In general, organisms are stressed by changes in environmental temperature, which may lead to the evolution of genetic adaptations to withstand severe temperatures. The purpose of this study was to study the effect of chronic heat stress on the mRNA expression levels of the HSP70, HSP90β, HSF1, and HSF3 genes in the stress-sensitive organs of two chicken breeds LGH and FYM, additional we studied the modulatory effect of acetic acid on the genes expression level. Four groups were used in the experiment: a control or thermoneutral group (TN), a chronic heat stress group (CHS), a thermoneutral group supplemented with acetic acid (TN+AA), and a chronic heat stress group supplemented with acetic acid (CHS+AA). To determine the transcriptional mRNA expression levels of HSP70, HSP90β, HSF1, and HSF3, samples were taken from the liver and brain. The findings showed that Fayoumi chickens exhibited higher concentrations of HSP70 and HSP90β mRNA expression compared to Leghorn chickens under chronic heat stress, indicating greater heat resistance in Fayoumi chickens. This suggests that Fayoumi chickens have inherited thermotolerance traits. After chronic heat exposure, HSF1 mRNA decreased in Fayoumi chicken brain tissue but remained unchanged in liver tissue, while in Leghorn chickens, HSF1 mRNA increased in liver tissue. Additionally, HSF3 expression decreased in Fayoumi chicken brain tissue but increased in Leghorn chickens under chronic heat stress. Acetic acid supplementation provided greater protection against heat stress in Fayoumi chickens compared to Leghorns.

Keywords: HSP gene expression, Fayoumi chickens, Leghorn chickens, heat stress, acetic acid supplementation

INTRODUCTION

Extreme summer heat waves and modern poultry genotypes' heightened vulnerability to heat stress are major concerns for the poultry industry today. Numerous physiological and metabolic parameters, such as feeding efficiency, growth rate, egg production, eggshell quality, fertility, and survivorship, are reduced during heat stress. In order to overcome these problems, animal breeders have concentrated on choosing poultry lines that can withstand high temperatures (Lin et al., 2006; Kapakin et al., 2012). Because they drink more water and eat less food, birds kept in open-sided houses experience chronic heat stress. In broilers, growth rates, feed efficiency, and carcass quality are all adversely affected.

Prolonged periods of high ambient temperatures increase the time required for broilers to reach market weight and raise mortality rates. Heat stress shortens the shelf life of eggs and reduces egg production and quality in laying hens. During the summer, egg production might decrease to as low as 30%. High temperatures are thus a major factor in reducing the productive performance of layers. High humidity and ambient temperatures reduce fertility in broiler breeders, which leads to reduced hatchability. Furthermore, during heat stress, increased body temperature negatively affects gamete formation and the fertilization process (Bhadauria et al., 2014.)

Under typical physiological settings, most cells constitutively create a family of highly conserved proteins known as heat shock proteins (HSPs) (Kapakin et al., 2012). HSPs are categorized into six major groups based on size and function: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs (Lindquist, 1992). Four heat shock transcription factors (HSFs) are the main transcriptional regulators of HSP gene expression. HSFs interact with particular DNA sequences to modify the expression of the HSP promoter (Abdo et al., 2017). Chicken breeds with higher basal HSP70 expression at normal physiological temperatures exhibit better tolerance to high temperatures (Zhen et al., 2006). Heat shock factors (HSFs) induce gene expression related to defense mechanisms (Weake et al., 2010), and heat shock proteins (HSPs) are among the primary defenses against heat stress (Causton et al., 2001). Acetic acid supplementation offers an effective and cost-efficient method to mitigate the negative effects of heat stress, and its use should be considered during heat stress episodes to reduce economic losses (Ghazalah et al., 2011).

This study investigated the effect acetic acid supplementation on the expression of the HSP70, HSP90β, HSF1, and HSF3 genes as well as the effects of chronic heat stress on the mRNA expression levels of these genes in Fayoumi (a local breed) and Leghorn (a commercial line) chickens.

MATERIAL AND METHODS

The experiment was carried out between March 2017 and May 2018. The University of Sadat City's Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee (IACUC) protocols were followed in all bird handling practices, sample collection, and disposal (Approval number: VUSC-001-4-16).

1. Bird Management

Sixty female chicks from two breeds, 30 Leghorn (LGH) and 30 Fayoumi (FYM) participated in the study. Every chick was healthy and devoid of any illnesses or clinical conditions. While

FYM chicks came from an integrated poultry project in El Azab, Fayoum Governorate, Egypt, LGH chicks came from an animal production research station in Burj El Arab, Alexandria Governorate, Egypt.

Birds were fed a commercial meal and reared under the same conditions. They have unlimited access to food and drink. According to Bhadauria et al. (2014), The relative humidity was from 50% to 60%, while the outside temperature was between 23°C and 25°C. A routine immunization program also protected the birds from disease.

2. Experimental Design

After the period of fourteen-day thermal adaptation and acetic acid supplementation, at the age of 56 days, Sixty female chicks from two breeds, 30 Leghorn (LGH) and 30 Fayoumi (FYM) (16 from acetic acid supplemented group, and 14 from no acetic acid supplemented group) were allocated into four groups to study the effect of chronic heat exposure (CHS) and acetic acid supplementation (AA) on the transcriptional expression level of *HSP* and *HSF* genes in these chickens.

CHS was induced by increasing environmental temperature from 25^{o}C to 37 ±1^{o}C for six hours daily (from 10:00 am to 4:00 pm) for five successive days (Duangjinda et al., 2017). Throughout the period of heat stress, chickens were given ad libitum feed and water.

The experimental groups in both Fayoumi and Leghorn chickens were designed as follows:

C1: Control group or thermoneutral group (TN), six chickens from each breed have been raised under thermoneutral environmental condition through all the experimental period.

C2: TN + AA, eight chickens from each breed have been supplemented by acetic acid in drinking water and raised under thermoneutral environmental condition.

C3: CHS, eight chickens from each breed have been exposed to high environmental temperature 37 ±1^{o}C for six hours daily for five successive days.

C4: CHS+AA, for five days in a row, eight hens of each breed were exposed to a high ambient temperature of 37 ±1oC for six hours per day, and their drinking water was supplemented with acetic acid.

3. Tissue samples

Five birds from each group were chosen based on their average weight at the end of the heat exposure session, liver and brain samples were taken. Prior to RNA extraction, the samples were kept at -80°C after being washed in phosphate-buffered saline (PBS) and snap-frozen in liquid nitrogen.

4. RNA Isolation and Reverse Transcription

TRI reagent (easy-REDTM, iNtRON Biotechnology) was used to extract total RNA from liver and brain tissues in accordance with the manufacturer's instructions. By seeing ribosomal RNA bands on an agarose gel stained with 2% ethidium bromide, RNA integrity was evaluated. A spectrophotometer was used to measure the concentration and purity of RNA; pure RNA was defined as having an A260/A280 ratio of approximately 2. The quality and quantity of RNA was determined using Colorimeters. The TOPscriptTM cDNA Synthesis Kit (enzynomics, Korea) was used to create the cDNA, which was then kept at -20°C.

5. Real-Time PCR

Gene expression was examined using primers for HSP70 (Amrutkar et al., 2014), HSP90β (Chen et al., 2013), and HSF1 and HSF3 (Xie et al., 2014). The chicken β-actin gene served as the internal regulator. Real-time PCR was carried out using a DTlite Real-Time PCR Detection System (DNA Technology, Russia) and SYBR Green PCR Master Mix (enzynomics, Korea). The relative amounts of mRNA expression were ascertained using the 2−ΔΔCt method (Livak and Schmittgen, 2001).

Table 1. Primers used for Real Time PCR and Gene expression.

Gene	Primer set (5' -3')	Tm	Product size (bp)
HSP70	F:5'-GGCACCATCACTGGCTT-3' R: 5'-TCCAAGCCATAGGCAATAGCA- 3'	60 °C	74 bp
HSP90β	F: 5'-CGGAGATCGCGCAGCTCATGT-3' R: 5'-CGGGTTGGGGACGATGTCGA-3'	60 °C	182 bp
HSF1	F: 5'-CAGGGAAGCAGTTGGTTCACTACACG-3' R: 5'-CCTTGGGTTTGGGTTGCTCAGTC-3'	60 °C	192 bp
HSF3	F: 5'-TCCACCTCTCCTCTCGGAAG-3' R: 5'-CAACAGGACTGAGGAGCAGG-3'	60 °C	71 bp
β-actin	F: 5'- GGAAGTTACTCGCCTCTG-3' R: 5'- AAGACACTTGTTGGGTTAC- 3'	60 °C	114 bp

6. Statistical analysis

The mean ± SE was used to express the fold change values for the HSP70, HSP90β, HSF1, and HSF3 genes. ANOVA and the generalized linear model (GLM-procedures, SAS Institute, 2002) were used to statistically analyze differences in various tissues in order to evaluate the effects of treatments both within and between the two chicken breeds.

RESULTS

The purpose of this experiment was to determine how AA protected both FYM and LGH chickens from heat stress and to explain how the mRNA expression levels of the HSP70, HSP90β, HSF1, and HSF3 genes were affected at 56 days of age by CHS (37 ±1oC for six hours per day for five days in a row).

Table 2. HSP gene expression levels in brain tissues in several Fayoumi (FYM) and Leghorn (LGH) chicken groups.

Chronic experiment gene expression levels in brain tissue				
Genes / Treatments	TN	TN+AA	CHS	CHS+AA
HSP70 FYM	0.18 ± 0.02^{d}	1.43 ± 0.22^{c}	8.2 ± 0.19^{a}	5.1 ± 0.28^{b}
HSP70 LGH	0.36 ± 0.03^{d}	0.26 ± 0.01^{d}	0.25 ± 0.02^{d}	0.49 ± 0.01^{d}
HSP90 FYM	0.22 ± 0.03^{b}	0.31 ± 0.01^{b}	0.4 ± 0.02^{b}	2.55 ± 0.13^{a}
HSP90 LGH	0.36 ± 0.05^{b}	0.2 ± 0.03^{b}	0.12 ± 0.02^{b}	0.14 ± 0.02^{b}
HSF1 FYM	1.59 ± 0.16^{a}	0.93 ± 0.01^{b}	0.23 ± 0.01^{cd}	0.13 ± 0^{d}
HSF1 LGH	0.25 ± 0.02^{cd}	0.53 ± 0.04^{bcd}	0.42 ± 0.07^{cd}	0.64 ± 0.05^{bc}
HSF3 FYM	1.31 ± 0.02^{b}	0.89 ± 0.03^{c}	0.62 ± 0.02^{cd}	0.06 ± 0.01^{e}
HSF3 LGH	0.46 ± 0.06^{d}	1.42 ± 0.07^{b}	1.6 ± 0.12^{b}	2.76 ± 0.02^{a}

1. Heat shock protein 70 (HSP70) gene

Samples of brain tissue (table 2) showed that, LGH chicken breed not significantly differed in the *HSP70* mRNA expression level among different treatments. While, in FYM chickens CHS significantly up regulate the expression of *HSP70* gene (8.2±0.19) over all other groups. In comparison to the control TN group, TN+AA increases HSP70 mRNA expression by 1.43±0.22. Furthermore, in FYM hens, AA and CHS significantly upregulate the HSP70 gene compared to the control group.

When the two breeds were compared, it was found that, although the baseline expression of the HSP70 gene was the same, FYM chickens had higher relative mRNA expression than LGH chickens in the various treatments ($P < 0.05$).

Table 2. elucidated that, both FYM and LGH hepatic HSP90β mRNA expression is markedly elevated after CHS. Additionally, FYM hens treated with acetic acid (TN+AA) exhibit a substantial increase in HSP90β mRNA expression, whereas LGH chickens do not. In FYM and LGH chickens, respectively, CHS and AA markedly increased the HSP90β mRNA by 12.36±0.59 and 11.57±2.19. HSP90β mRNA is high significantly in the CHS+AA and CHS groups than in the TN+AA group in LGH chickens.

Comparison between the two breeds explained that the relative *HSP90β* mRNA expression level was significantly increased ($P < 0.05$) in FYM chickens supplemented with AA

during TN condition than LGH chickens of the same treatment.

II.4.2.3 Heat shock factor 1 (HSF1) gene

Table 2 explains the relative mRNA expression levels of the HSF1 gene of various treatments in brain tissue from LGH and FYM chickens. There are no discernible variations in the relative levels of HSF1 mRNA expression among the several groups of LGH chickens. Relative HSF1 mRNA expression data in FYM chickens showed that long-term heat exposure reduced gene expression to 0.23±0.01. Additionally, supplementing with AA under thermoneutral conditions drastically lowers the level of gene expression to 0.93±0.01. Furthermore, compared to the control group (TN), which has the greatest HSF1 relative mRNA expression level (1.59±0.16), the chronic heat exposure plus acetic acid supplementation (CHS+AA) significantly lowers the HSF1 gene expression level by 0.13±0.

Comparison between FYM and LGH chicken breeds explain that the basal *HSF1* mRNA expression level of the thermoneutral (TN) groups was increased significantly in FYM chicken breed than LGH chicken breed and there are no significant differences between other groups.

Table 2 explains the relative mRNA expression levels of the HSF1 gene of various treatments in brain tissue from LGH and FYM chickens. There are no discernible variations in the relative levels of HSF1 mRNA expression among the several groups of LGH chickens. Relative HSF1 mRNA expression data in FYM chickens showed that long-term heat exposure reduced gene expression to 0.23±0.01. Additionally, supplementing with AA under thermoneutral conditions drastically lowers the level of gene expression to 0.93±0.01. Furthermore, compared to the control group (TN), which has the greatest HSF1 relative mRNA expression level (1.59±0.16), the CHS plus AA administration significantly lowers the HSF1 gene expression level by 0.13±0. Comparison between Fayoumi and Leghorn breeds explain that, the basal HSF1 mRNA expression level of the TN groups was increased significantly in FYM than LGH and there are no significant differences between other groups.

II.4.2.4 Heat shock factor 3 (HSF3) gene

Table 2. demonstrate *HSF3* gene relative mRNA expression levels of different treatment in brain tissue from FYM and LGH chicken breeds. Comparing the chicken group exposed to CHS to the thermoneutral control group showed that the LGH chickens, but not the FYM chickens, had significantly higher HSF3 relative mRNA expression. In LGH hens, AA supplementation under thermoneutral conditions dramatically increased the relative HSF3 mRNA expression level compared to the control group, however in FYM chickens, it decreased. The LGH chicken group fed with AA and subjected to CHS had the highest significant expression level of HSF3, 2.76±0.02. On the other hand, FYM hens treated with AA and subjected to prolonged heat stress had the lowest levels of HSF3 mRNA (0.06±0.01).

Comparison between the two chicken breeds revealed a contrasting expression pattern of the *HSF3* gene in FYM and LGH chickens where FYM chickens significantly decrease the gene expression level by different treatments and LGH chickens

significantly increase it in compression with the thermoneutral group.

Table 3. HSP gene expression levels in liver tissues in several Fayoumi (FYM) and Leghorn (LGH) chicken groups.

Chronic experiment gene expression levels in liver tissue					
Genes / Groups		TN	TN+AA	CHS	CHS+AA
HSP70	FYM	1.57±0.18^{bc}	5.01±0.62^{b}	3.47±0.24^{bc}	14.38±1.31^{a}
	LGH	0.5±0.04^{c}	1.33±0.06^{c}	1.62±0.13^{bc}	2.44±0.09^{bc}
HSP90	FYM	3.02±0.12^{b}	12.49±1.07^{a}	12.93±0.37^{a}	12.36±0.59^{a}
	LGH	2.7±0.17^{b}	4.72±0.69^{b}	12.69±0.77^{a}	11.57±2.19^{a}
HSF1	FYM	2.49±0.35^{a}	2.86±0.12^{a}	2.62±0.19^{a}	2.76±0.22^{a}
	LGH	0.56±0.06^{c}	1.36±0.09^{b}	2.39±0.09^{a}	2.33±0.3^{a}
HSF3	FYM	1.59±0.19^{b}	1.74±0.19^{b}	3.48±0.39^{a}	3.47±0.39^{a}
	LGH	0.35±0.05^{d}	0.73±0.01^{d}	1.27±0.03^{bc}	1.33±0.06^{bc}

II.4.2.1 Heat shock protein 70 (HSP70) gene

LGH chickens' relative HSP70 mRNA expression level did not significantly change across treatments, according to liver tissue (table 3); however, the CHS and CHS+AA groups' relative HSP70 mRNA expression levels increased non-significantly by 1.62±0.13 and 2.44±0.09, respectively; FYM chickens' relative HSP70 mRNA expression level increased non-significantly by 3.47±0.24 in the chickens exposed to CHS; acetic acid supplementation under thermoneutral environmental conditions non-significantly increased the HSP70 mRNA expression level by 5.01±0.62, while CHS plus AA supplementation significantly increased FYM chickens' HSP70 mRNA expression level by 14.38±1.31.

When comparing the two breeds, it was found that FYM chickens had a higher level of HSP70 gene mRNA expression than Leghorn chickens. The groups that were exposed to chronic heat stress and supplemented with acetic acid (CHS+AA group) and those that were supplemented with acetic acid under thermoneutral conditions (TN+AA group) showed the most notable differences, with a P value < 0.05.

II.4.2.2 Heat shock protein 90β (HSP90β) gene

CHS was observed to dramatically enhance the hepatic HSP90β mRNA expression in both LGH and FYM hens

(Table 3). Furthermore, HSP90β mRNA expression was significantly upregulated in FYM hens fed with AA (TN+AA) but not in LGH chickens. The HSP90β mRNA in LGH hens was significantly elevated by CHS and AA supplementation by 12.36±0.59 and 11.57±2.19, respectively. In contrast, the TN+AA group demonstrated a significant increase in HSP90β mRNA in CHS+AA and CHS over TN+AA.

Comparison between the two breeds explained that the relative *HSP90β* mRNA expression level was significantly increased ($P < 0.05$) in FYM chickens supplemented with acetic acid during thermoneutral condition (TN+AA) than LGH chickens of the same treatment.

II.4.2.3 Heat shock factor 1 (HSF1) gene

The results of *HSF1* relative mRNA expression levels in liver tissue from FYM and LGH experimental chickens are shown in table 3. No Significant differences were demonstrated in the hepatic *HSF1* relative mRNA expression levels in FYM chickens. While, in LGH chickens, exposure to chronic heat stress either alone or in combination with acetic acid supplementation induce an observable increase by 2.39±0.09 and 2.33±0.3 respectively in comparison to control group.

When comparing the two breeds, it was found that FYM chickens had higher relative mRNA expression of the HSF1 gene than LGH hens, and this difference was particularly apparent in the TN and TN+AA groups.

II.4.2.4 Heat shock factor 3 (HSF3) gene

Table 3 shows that following exposure to CHS group, the HSF3 relative mRNA was significantly upregulated in liver tissue by 3.48±0.39 in FYM chickens and 1.27±0.03 in LGH chickens. Additionally, compared to the control group, the HSF3 mRNA in both FYM and LGH chickens was markedly elevated by CHS and AA supplementation. In FYM and LGH chickens, AA supplementation under TN circumstances had no discernible impact on the expression of HSF3 mRNA.

Comparing the two breeds explained that FYM chickens have higher *HSF3* relative mRNA expression than LGH chickens in all groups.

Tables 2 and 3 provide a summary of the gene expression in the brain and liver tissue of several FYM and LGH chicken breeds. CHS dramatically increased the expression of the HSF3 gene in LGH chickens and the HSP70 gene in FYM chickens, according to the results of gene expression in brain tissue. In the brain tissue of FYM chickens, AA supplementation under TN conditions markedly enhanced the expression of the HSP70 gene while decreasing that of the HSF1 and HSF3 genes.

Following AA administration under TN conditions, the HSF3 mRNA in the brain tissues of LGH hens increased, but HSP70, HSP90β, and HSF1 remained unaltered. HSP70 and HSP90β are markedly up-regulated in FYM chickens under CHS and AA supplementation; in contrast, HSF1 and HSF3 genes are down-regulated compared to the control group. Supplementing LGH hens with AA under heat stress circumstances increases the HSF3 gene while having little effect on the HSP70, HSP90β, or HSF1 genes.

CHS dramatically raised the expression of the HSP90β and HSF3 genes in the

liver tissue of FYM hens, whereas Leghorn chickens showed an increase in HSP90β, HSF1, and HSF3, and HSP70 showed no discernible changes following exposure to CHS. The levels of hepatic HSP90β expression in FYM chickens and HSF1 in LGH chickens were also markedly elevated by AA supplementation under TN conditions. Additionally, AA supplementation and CHS raise the expression of the genes for HSP70, HSP90β, and HSF3 in FYM chickens and HSP90β, HSF1, and HSF3 in LGH chickens.

DISCUSSION

Heat stress is a major problem for the poultry industry during high temperature conditions, and the need for genetic lines that can withstand climate changes led to a great deal of attention being paid to heat tolerance in poultry production. The genetic background of flocks is one of the factors that can help manage the negative effects of heat stress, but the genetic control of heat tolerance is complex and has low heritability, so the selection of heat-resistant birds is essential (Felver-Gant et al., 2012).

The *HSP70* relative mRNA expression level in FYM chickens brain tissue after CHS showed a significant up regulation *HSP70* relative mRNA expression level and there were no significant changes observed LGH chickens. In CHS exposure experiment the FYM chickens had higher concentrations of *HSP70* than LGH regardless of treatment suggesting that FYM had inherited this thermotolerance characters. The low responsiveness of LGH chickens after exposure to heat stress might be attributed to oxidative tissue damage during heat challenges as explained by (Sun et al., 2007; Yu, 2009; Xie et al., 2014). Felver-Gant et al., (2012) showed that laying hens exposed to heat stress had higher concentrations of *HSP70* mRNA expression in the liver after seven days, but this change did not occur after two weeks of heat stress. Furthermore, despite treatment, the concentrations of HSP70 were higher in kind gentle hens (a line of group-selected hens for high productivity and survivability) than in DeKalb XL hens (a commercial line of individually selected hens for high egg production), indicating that the thermotolerance traits were inherited by the kind gentle hens.

Additionally, the current study's findings showed that AA supplementation significantly raised the expression of HSP70 mRNA in liver in the CHS+AA group compared to the CHS group following five days of chronic heat exposure and acetic acid supplementation in FYM chickens. This suggests that FYM chickens are more resistant to heat stress than LGH when supplemented with acetic acid.

This result was consistent with previous observations that used other different supplements such as acetyl salicylic acid, vitamins (like co-enzyme Q10 and vitamin C) and rosemary extract to provide protection against deleterious effects of heat stress through inducing expression of heat shock protein genes. All these supplementations showed much better behavior when chickens were subjected to different durations of heat stress. Xu et al., (2017b) indicated that pretreatment either by aspirin or co-enzyme Q10 accelerated the induction of *HSP70* when chicken myocardial cells were exposed to hyperthermia.

A seemingly small subset of proteins depends on the HSP90 for folding, assembly, and stability. In addition to being crucial for immunity, overexpression of HSP90 would

prevent cells from dying (Wandinger et al., 2008; Abdian et al., 2011). Furthermore, there was strong evidence linking the HSP90 to the development of thermotolerance and the quicker restoration of heat-induced denatured proteins to their original condition (Baqchi et al., 2001). Chronic heat stress induced only hepatic *HSP90β* mRNA by 12.93±0.37 and 12.69±0.77 in Fayoumi and Leghorn chickens respectively. Moreover, FYM chickens are more resistant to heat stress than LGH, as evidenced by increased HSP90β mRNA expression in the former group. It is anticipated that an up-regulated expression of HSP90β will shield organisms against the harmful consequences of heat stress. According to Lei et al. (2009), severe tissue damage may be the cause of the reduced expression level of HSP90β in LGH chicken brain tissue following prolonged heat exposure.

Likewise, in FYM and LGH chickens, acetic acid supplementation under thermoneutral conditions increased the level of hepatic HSP90β mRNA expression, giving the cells greater protection. This implies that HSP90β expression can be pre-induced by acetic acid prior to the advent of stress. FYM hens treated with acetic acid and stress had higher levels of HSP90β transcription in both the liver and the brain, whereas LGH chickens only had liver-induced HSP90β mRNA.

The primary stress-induced transcription factor that controls HSPs in vertebrates is called HSF1. According to the earlier work, HSF3 is strongly associated with HSP expression and is mostly triggered by exposure to extremely high temperatures (Zhang et al., 2014). Even though HSF1 is expressed in avian cells, HSF3 and HSF1 are co-expressed and co-activated by different stressors, and cells without HSF3 are significantly less able to induce the heat shock response (Roesslein et al., 2015). It has been demonstrated that *HSF1* perform a subordinate role in the induction of *HSP70*, and *HSF3* is the primary heat shock factor responsible for *HSP70* induction in chicken. Both *HSF1* and *HSF3* are translocated into the nucleus after heat stress to activate the *HSP70* expression (Xu et al., 2017a). After chronic heat exposure *HSF1* mRNA decreased in FYM chickens brain tissue and unchanged in liver tissue while, in LGH chicken the *HSF1* mRNA increased in liver tissue after chronic heat exposure. and after long duration of heat stress the level of *HSP* genes will be increased and no requirement for expression of the *HSFs* genes. Furthermore, sustainable increased level of HSF1 transcription in LGH chickens after long duration of heat stress as in chronic experiment indicates requirement for more HSPs to overcome the side effects of heat stress and this indicated sensitivity of LGH chickens to heat stress. By increasing duration of heat stress in chronic experiment FYM chickens have no further requirements of *HSF1* mRNA and its level significantly decreased in brain tissue and unchanged in liver tissue. Chronic heat stress, pretreatment with acetic acid led to a numerical increase in groups supplemented with AA and exposed to heat stress. While, in liver tissue acetic acid supplementation induced the expression of HSF1 in Leghorn chickens and maintaining it at a higher-level during exposure to chronic heat stress. Agreeing with our observation that acetic acid supplementation induced the expression of HSF1 after exposer to heat stress, Zhang et al., (2018) explained that aspirin administration induced the expression of HSF1 and its binding to DNA during thermoneutral condition and

induced significant upregulation of its level after heat exposure.

Following exposure to heat stress or heat stress with acetic acid supplementation, HSF3 rose in liver tissue but reduced in FYM chicken brain tissue in the long-term trial. Exposure to CHS stress, AA supplementation, and CHS+AA all increased the expression of brain HSF3 genes in LGH hens. in LGH chickens hepatic HSF3 expression was increased by CHS and CHS+AA. HSF3 is the primary regulator of heat shock proteins in chickens, as evidenced by the fact that overexpressing mouse HSF3 in the same cells did not change the amounts of heat shock protein genes. A previous study's findings demonstrated that during heat stress, overexpression of chicken HSF3 in HSF1-null mouse embryonic fibroblast cells triggered the production of heat shock genes (Fujimoto et al., 2010).

Acetyl salicylic acid (ASA) treatment increased the expression of both HSF1 and HSF3 during heat stress, but Q10 treatment only increased the expression of HSF1, according to Xu et al. (2017a), who demonstrated that both HSF1 and HSF3 expression levels were elevated under heat stress. Additionally, they hypothesized that Q10 and ASA would increase Hsp70 levels under heat stress through distinct mechanisms.

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