EFFECTS OF THERMAL CONDITIONING TREATMENTS ON BRAIN HSP70 LEVEL IN BROILERS UNDER HEAT STRESS

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Abstract

The aim of this study was to evaluate the effect of thermal conditioning treatments during incubation and at 5 d on brain Hsp70 levels of broilers heat stressed from 21 to 49 d and to determine its relationship with thermotolerance.

Eggs were divided into two incubation groups; control incubation (37.8ºC) and high incubation (eggs were heated at 39.6ºC for 6 h between 10 and 18 days of incubation). Chicks/incubation temperatures were assigned to 3 treatments groups: Control (C); standard brooding temperature was used in this group. Heat conditioned and heat stress (HC&HS); chicks were conditioned at 36 ºC for 24 hour on day 5 and then they were exposed to 34 ºC between 10.00-17.00 h daily from day 21 to 49. Heat stress (HS); broilers were exposed to 34 ºC between 10.00-17.00 h daily from day 21 to 49. Brain samples from four individuals were obtained from all groups on day 49 before slaughtering. Hsp70 was determined with Western Blotting method from brain samples and Hsp70 levels were calculated by BioOneD++ software.

There was an interaction between incubation temperature and treatments ($p<0.05$). HC & HS broilers incubated at control temperature had lower Hsp70 level than C and HS groups. However when broilers conditioned during incubation, HC & HS broilers had the highest Hsp70 level than the others.

As a result, it was concluded that thermal conditioning during incubation may have help to reduce the effects of heat stress.

Keywords: Hsp70, thermal conditioning, thermal stress, broilers, heat shock proteins.

Introduction

Temperature is one of the most important environmental factors that affect the physiological function of organisms. In broiler chickens, when the environmental temperature goes above the optimum temperature conditions, yield characteristics like growth rate, feed conversion and live weight gain affect negatively ((Etches *et al*., 1995, Yalçın *et al*., 1997). In order to decrease the effect of heat stress on broilers, methods such as increasing the ventilation rate, cooling, adding some supplemental to feeds, thermal conditioning are implemented (Mazzi, 2002, Yalçın *et al*., 1997).

Cells respond to changes in environmental conditions with a quick and simple reaction. When the environmental conditions change, cells increase the synthesis of a protein group which is called heat shock or stress proteins. *In vivo* or *in-vitro*, cells respond against the stress factors by decreasing the synthesis rate of almost all cellular proteins and increasing the synthesis of heat shock proteins (Rivera, 2004). It was found out that the cells, which lack heat shock protein genes, thus devoid of mentioned protein expression, show less tolerance to stress when compared to cells which express heat shock protein.

Also, it was determined that stimulated thermal tolerance degree is related with the expression of Hsp's (Krebs and Bettencourt 1999; Solomon *et al*., 1991).

Members of Hsp70 family are ubiquitously expressed heat shock proteins. Their expressions are highly induced by heat stress and play an important role in folding the proteins in cell and the protection of cell against the

[3] This study was summarized from a part of the pH.D thesis of first author.

deleterious effects of stress. In order to discover the relationship between the synthesis of Hsp70 protein and the mechanism of tolerance to heat, there are studies of broilers on Hsp70, tolerance to heat and the response to heat stress on cellular level. The relation of heat stress in cellular and molecular level and heat shock proteins are studied in many different tissues and ages in broilers under various heat stress conditions (Einat *et al*. 1996; Yahav *et al*. 1997; Givisiez *et al*, 1999; Leandro *et al*, 2004).

Gabriel *et al*. (1996) discovered that the Hsp70 mRNA expression in the liver tissues of broilers, which were subjected to acute heat stress at 35ºC for 5 hours, increased over time like in mammals. For animals which are subjected to mild postnatal heat stress, it was observed that they had more survival rate in high temperatures that might occur in their rearing period (Arjona *et al*., 1988; Yahav *et al*., 1997). Yahav *et al*., 1997 studied the relationship between heat shock proteins and thermal conditioning experiments. Acute heat stress was applied on thermal conditioned broilers and control cases. Hsp70 mRNA synthesis in their hearts and lungs were examined. They found out that survival rate at the end of heat stress was higher for thermal conditioned group and Hsp70 gene expression only increased when core temperature rose and hyperthermia occurred.

Einat *et al*. (1996) applied 37.2 ° C high environmental temperatures on broilers for four hours a day on day 42. After heat stress, Hsp70, Hsp90 and Hsp27 mRNA synthesis on lung and heart was examined, and it was discovered that synthesis increased and the highest level was achieved during the fourth hour of stress exposure.

Yalçın *et al*. (2005) examined the effects of conditioning during and after incubation on thermal resistance, live weight, physiological response and proportional asymmetry in young and old broiler, and they stated that conditioning might help in overcoming heat stress during rearing and parent age is an important factor in this mechanism.

The aim of this study was to evaluate the effect of thermal conditioning treatments during incubation and at 5 d on brain Hsp70 levels of broilers heat stressed from 21 to 49 d and to determine its relationship with thermotolerance.

Material and Method

Animal Material

Broiler chickens, which were from 28 wk old breeders and hatched from the eggs that were incubated at control (37.8°C) or high incubation temperatures (39.6°C for 6 hours between 10 and 18 days of incubation) On d hatch, chicks were wing banded and placed into floor pens. Chicks/incubation temperatures were assigned to 3 treatments groups: Control (C); standard brooding and rearing temperatures were used in this group. Heat conditioned and heat stress (HC&HS); chicks were conditioned at 36 ºC for 24 hour on day 5 and then they were exposed to 34 ºC between 10.00-17.00 h daily from day 21 to 49. Heat stress (HS); broilers were exposed to 34 ºC between 10.00-17.00 h daily from day 21 to 49.

Feed and water were provided *ad libitum,* and they were fed a 23.5 % crude protein and 3120 ME kcal/kg starter diet between 0^{th} and 10^{th} days, a 22.5 % crude protein and 3130 ME kcal/kg pelleted grower diet between 11^{th} and 21^{st} days and a 20.5% crude protein and 3240 ME kcal/kg pelleted finisher diet between 22^{nd} and 49^{th} days. Lighting schedule was 23 hours light: 1 hour dark. Table 1 shows that information about the material, rearing and incubation conditions.

Brain samples were obtained from all groups on day 49, only from male samples. The samples were immediately frozen in liquid nitrogen and stored at -80 ºC deep freeze until the laboratory studies.

Homogenization of Samples

Samples were homogenized by the method of Givisiez *et al*. (1999). Homogenization buffer (20 mM Tris, pH: 7.5; 9 g/l NaCl; 20 mM 2-Merkaptoethanol) was added 10 ml for 1g of sample (1:10). Samples were homogenized at 10.000 rpm for 5 times (30 seconds each) with ice bath intervals for 30 seconds. Homogenized samples were centrifuged at 10.000 rpm for 30 minutes at + 4º C. Aliquots were separated for total protein determination and Western Blotting analysis. The total protein amount of the homogenized brain samples was determined according to Lowry *et al*. (1951). A calibration curve was created by using 0.1 %, 0.08 %, 0.06 %, 0.04 %, 0.02 % and 0.01 % concentration of Bovine Serum Albumine as a standard. Spectrophotometric analysis of both standards and samples were carried out with two replications.

Gel Electrophoresis and Western Blotting Analysis

After determination of total protein amounts of homogenates, 15 micrograms total proteins were loaded and separated 10% polycrylamide gel in under denaturating conditions (Laemmli, 1970) at constant 200 V. Prestained protein marker (BioRad) was loaded in first lane of each gel.

Proteins were transferred from SDS-polyacrylamide gel to nitrocellulose membranes by using method of Towbin *et al* (1979) (66 V, +4 ° C, overnight). Membranes were washed four times for 5 min with 1 X TBST (10 mM Tris-HCl, pH: 8.0; 150 mM NaCl; 0.5 g/l Tween-20) after transfer step. Non specific sites were blocked using 10 ml 1 X TBST and non fat dry milk. The membranes were incubated with 2 μl monoclonal anti Hsp-70 antibody

(H-5147, SIGMA; dilution 1:5000) for one hour with constant shaking. Then, membranes were washed with cold 1 X TBST (5 min, 4 times). After rinsing of membranes, the membranes were incubated 0.67 μl secondary anti-mouse antibody conjugated to alkaline phosphatase (A-3562, SIGMA) diluted in 20 ml 1 X TBST, non fat dry milk powder (1:30.000 dilution) for 1 h. The membranes were washed in cold 1 X TBST (5 min, 4 times) and dyed with 10 ml pre-mixed BCIP/NBT (SIGMA) solution. Color reaction was stopped with 10 % of Tri Chloro Acetic Acid. Membranes were dried and scanned and pictures were recorded to determine later.

Determination of Hsp70 amount

Pure Hsp 70 protein (SPP-758, Stressgen, 0.85 mg/ml) was used to detect Hsp 70 amount in brain samples. Pure Hsp70 concentration was separated into 100, 200, 800, 1000 and 1200 ng/μl dilutions with 4 X sample loading buffer (final concentration 100 nanograms per μl). Western Blotting Analysis was performed after the electrophoresis as described before. Hsp70 amounts were calculated that will be ng/μg total protein by BioOne D++ (Vilber Lourmat, France) from this curve. Statistical analysis of the obtained data was performed in JMP (Ver 6.0, JMP User's Guide, 2005) package program. For the comparisons of day 49, Tukey test was applied ($p< 0.05$).

Results and Discussion

The influence of incubation and rearing temperature on Hsp70 level was not significant, however, the interaction between incubation and rearing temperature on the 49th day was found to be significant ($p< 0.05$). Table 2. shows the results of variance analysis for the effects of incubation and rearing temperature on Hsp70 levels in all groups. HC&HS group from control incubation resulted in a decrease in the Hsp70 level compared to C and HS groups. This result may imply that high ambient temperature during rearing period in heat conditioned individuals was not perceived as a stress factor on cellular level due to exposure to thermal stress in early ages and that there was not an increase in Hsp70 quantity in connection thereto. Yahav *et al*. (1997) studied the relation of thermal conditioning applications on the 5th day (36º C, 24 hours) with acute thermal stress on the 42nd day. They reported that thermal conditioned broilers, showed less Hsp70 mRNA level than the control group in heart and lung tissues within the acute thermal stress (42nd day).

Zulkifli (2002) with reference to Craig (1985), argued that neonatal stress may have induced Hsp70 mRNA transcription, however, RNA may have been "sequestered" and not translated until exposure to thermal stress later in life. In the present study, Hsp70 levels showed similar quantities in C and HS groups from high incubation, HC&HS broilers had the highest Hsp70 level than the others (Table 2). It was construed that heat conditioning on the 5th day after high incubation may have induced Hsp70 mRNA synthesis gradually for HC&HS groups.

Yalçın *et al*. (2005) reported that exposing broilers from young breeders to high temperature during incubation increased resistance to higher ambient temperatures and had a positive effect on live weight. It may be considered that there may be a relation between Hsp70 and resistance to higher temperatures as a result of the increase in Hsp70 quantity in HC&HS group from high incubation temperature.

Similar levels of Hsp70 for C and HS groups from high incubation suggested that broilers gained stress experience in advance by being exposed to high temperature in incubation and no increase was observed in Hsp70 level when they exposed to heat stress later in life.

As a result, it was concluded that thermal conditioning during incubation may have help to reduce the effects of heat stress.

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Table 1**.** Information about the material, rearing and incubation conditions

Incubation Temperature[1]	**Rearing Temperature**[2]
Control	Control (C)
Control	Heat conditioned & Heat stress (HC&HS)
Control	Heat stress (HS)
High	Control (C)
High	Heat conditioned & Heat stress (HC&HS)
High	Heat stress (HS)

[1] Incubation temperature; control (37,8°C), high (eggs were heated at 39.6ºC for 6 h between 10 and 18 days of incubation).
[2] Rearing temperature; C (standard rearing temperature), HC&HS (36 ºC for 24 hour on day 5 and then exposed 34 ºC between 10.00-17.00 h daily from day 21 to 49), HS (34 ºC between 10.00-17.00 h daily from day 21 to 49)

Table 2. Results of variance analysis for the effects of incubation temperature and breeding temperature on Hsp70 levels (ng/ μg total protein) in all groups of 49th day samples

	Hsp70 ($\overline{X} \pm s_{\overline{x}}$)	
Rearing Temperature	Incubation Temperature	
	Control	High
C	70,17 ± 5,61[a]	63,85 ± 5,61[b]
HC&HS	50,18 ± 5,61[b]	76,93 ± 5,61[a]
HS	83,15 ± 5,61[a]	61,25 ± 5,61[b]
	Significance Levels (p<0.05)	
Rearing Temperature	0,916	
Incubation Temperature	0,324	
Rearing Temp. X Incubation Temp.	0,001*	