A STUDY ON HEAT SHOCK PROTEIN SYNTHESIS IN CHICKEN EMBRYOS AFTER HEAT STRESS EXPOSURE DURING EMBRYONIC PERIOD IN RELATION TO HATCHING PERCENTAGE AND CHICKS WEIGHT

By
A. M. El Kaiaty¹; Fatma R. Mohamed¹; Eman M. Abou-Eita¹ and ²Abeer A. Eshera²
1- Department of Animal Production, Faculty of Agriculture, Cairo University.
2- Department of poultry Breeding, Animal Production Research Institute.

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ABSTRACT: The present study was aimed to investigate the effects of heat stress during embryonic period on heat shock protein 70 synthesis in chicken embryos, hatchability percentage and chick weight at hatch in a local chicken strain. The experimental work of the present study was carried out at El-Takamouly Poultry Project in Fayoum Governorate. The experimental eggs were taken from a 34-weeks old breeder flock (the poultry breeding research” Fayoum station”. Seven hundred and fifty Gold Montazah (GM) fertile eggs were equally divided into 5 experimental groups with three replicates of 50 eggs each. All eggs were incubated in automatic incubator as one batch. The first group (T1) served as a control group and incubated at 37.5 °C and 55% relative humidity (RH) throughout the incubation period (18 days). The second and third groups (T2, T3) were exposed to 39 °C and 40 °C with 55% RH for 3 hrs at day 15, respectively. The fourth and fifth groups (T4, T5) were exposed to 39 °C and 40 °C with 55% RH for 3 hrs at day 18, respectively. The thermal manipulations were carried out in another incubator. After TM treatments, the experimental eggs were quickly returned to the control incubator at day 19, eggs of all treatment groups were transferred to the hatcher with temperature of 37.0ºC and 65% RH for the remaining incubation period. At day 15 and 18, directly after heat stress exposure, 3 embryos from each thermal treatment and control group were taken and their livers and brains were removed for heat shock protein70 determination. After hatching, newly hatched chicks were counted and their weights were recorded. Also, hatchability percentage was calculated. The main results were as follows:

1- Regardless of age, exposing to 40 °C significantly increased HSP70 synthesis either in brain or liver. Meanwhile heat exposing age didn’t affect HSP70 synthesis.
2- The interaction between exposing temperature degree and age was significant, where, the highest amounts of HSP70 synthesis were exhibited by exposing eggs to 40 °C either at embryonic day 15 or 18 of incubation period.
3- Heat shock protein synthesis was higher in brain tissue than in liver tissue.
4- The highest hatchability percent was recorded for eggs exposed to 39 °C at day 18, while the highest chick weight at hatch was recorded for eggs exposed either to 40 °C or 39 °C at day 15.

From this study it could be concluded that exposing Golden Montazah fertile eggs to 40 °C for 3 hrs either at embryonic day 15 or 18 significantly increased HSP70 synthesis and chick weight at hatch, while the highest hatchability percentage was achieved by exposing eggs to 39 °C for 3 hrs at day 18 from incubation period.
INTRODUCTION

From the moment of fertilization of the ovum, embryogenesis and development are initiated and are affected by many factors in the microenvironment around the egg (Decuypere and Michels, 1992; Van Brecht et al., 2003). The rate of embryo development and growth appears to be narrowly regulated within a species and varies with respect to the age of breeders, duration and temperature of pre-incubation egg storage, and incubation temperature (Yalcin and Siegel, 2003).

Optimum incubation temperature for poultry embryos is typically 37.5 °C, and small deviations from this optimum can have a significant adverse impact on hatchability, length of the incubation period, chick quality, and later life performance in poultry (Loureens et al., 2005; Wilson, 1991). Temperature is the most pervasive environmental factor that influences poultry of all species and ages (Al-Saffar and Rose, 2002). When chicks and pouls hatch, they are considered to be poikilothermic, and low ambient temperature leads to hypothermia (Weytjens et al., 1999). On the other hand, heat stress causes hyperthermia in both the embryo and post-hatch bird and has a negative impact on both development and growth due to induced alterations in physiological, hormonal, and molecular status (Moraes et al., 2003).

Embryogenesis comprises a series of temporally and spatially organized events in gene expression and cellular signaling processes in order to provide successful development under adverse changes in the microenvironment (Gabriel et al., 2002). Molecular mechanisms responsible for improved thermotolerance have not been fully elucidated (Givisiez et al., 2001). Epigenetic thermal adaptation is suggested to be the result of set-point changes of the thermoregulatory system during development of feedback mechanisms of the embryo (Arjona et al., 1988 and Yahav, 2009), which result in alterations in the thermoregulatory threshold response and increased adaptation to postnatal hot or cold environments. For instance, chicks exposed to high incubation temperatures during late embryogenesis can adapt better to high environmental temperatures post-hatch (Tzschentke, 2007 and 2008). When a cell experiences environmental stress, it stops, or at least slows down most of its original functions, such as transport processes, DNA, RNA and protein synthesis. However, there is a peculiar set of proteins called stress proteins also called Heat Shock proteins (HSPs), which are preferentially expressed under these restrictive conditions (King et al, 2002). Stress proteins are a group of proteins that are present in all cells and in all life forms, they are common constituents of all types of prokaryotic and eukaryotic cells, the function of Heat Shock proteins is similar in virtually all living organisms from bacteria to humans (Rene, 1996).

Heat Shock proteins is a one of group of proteins which increase their expression when the cells which contain them are exposed to different kinds of environmental stress conditions such as heat or cold stress, infection, inflammation, toxins, oxygen deprivation and heavy metal ions. The previous stressors rapidly induce the synthesis of heat shock proteins (HSP), which are also expressed constitutively in poultry species (Givisiez et al., 2001). Heat shock proteins are divided into several families based on their approximate molecular weight (Welch, 1993). Many hsp, especially the 70 kDa family (HSP70), are expressed in response to diverse stressors, and increased synthesis of these inducible proteins is involved in the protection of stressed cells and organisms (Gabriel et al., 2002).

Heat Shock proteins present in cells under perfectly normal condition, act like "chaperones", making sure that the cell's
proteins are in the right shape and in the right place at the right time. For example, HSPs help new or distorted proteins fold into shape, which is essential for their function (Lindquist and Craig, 1988). In their studies on the relationship between the synthesis of Hsp70 protein and the mechanism of heat tolerance in broilers embryos (Leandro et al, 2004 and Rafael, 2005). Reported significant increase of brain HSP70 levels in heat-stressed embryos (Ginivisiez et al 2001).

Rafael (2004) found that exposing turkey eggs at day 21 of incubation to 40 °C for 2 hrs didn’t affect hsp70 synthesis in the liver while exposing another group of eggs at the day 22 of incubation to 40 °C for 3 hrs significantly increased hsp70 synthesis in the liver compared with the control group. Also, Leandro et al. (2004). found that subjecting The fertile eggs at days 13, 16 and 19 of incubation to 40°C for 4–6 hrs increased Hsp70 synthase in heat stressed embryos, comparing with non – stressed embryos , the age of heating treatment didn’t affect HSP70 synthesis in chicken embryos .

Therefore, the objective of this study was to investigate the effect of heat stress during embryonic period on heat shock protein 70 synthesis in chicken embryos, hatchability percentage and chick weight at hatch in a local chicken strain.

**MATERIALS AND METHODS**

The experimental work of the present study was carried out at El-Takmouly Poultry Project, Fayoum Governorate, Egypt. Seven hundred and fifty Golden Montazah fertile eggs were obtained from a 34-weeks old breeder flock (the poultry breeding research" Fayoum station", Animal Production Research Institute) during January 2010. Eggs were randomly distributed into five experimental treatments with three replicates of 50 eggs each . the control group (T1) was incubated at 37.5 °C and 55% relative humidity (RH) throughout the incubation period until the 18 day . The second and third groups ( T2, T3 ) were exposed to 39 °C and 40 °C with 55% RH for 3 hrs at the 15th day of incubation, respectively . The fourth and fifth groups (T4, T5) were exposed to 39 °C and 40 °C with 55% RH for 3 hrs. at the 18th day of incubation respectively. The experimental groups from T2 - T5 were translocated to another incubator during thermal manipulation (TM) treatments (3 hrs.) and after that they soonly returned to the control incubator. At the 7th day from incubation, eggs were candled and infertile eggs and eggs containing dead embryos were removed. After the hatching period, the unhitched eggs were removed from the incubator, cracked open, and visually examined to determine the moment of embryonic mortality. Hatchability percentages of fertile eggs were calculated and the hatched chicks were individually weighted to the nearest 0.1 gm.

**Determination of heat shock protein 70 (HSP70):**

3 embryos were randomly selected from each treatment group, directly after the end of heat stress exposure, their brains and livers were removed , placed in liquid nitrogen and immediately stored at -80 °C until estimating heat shock protein which estimated by HSP70 EIA kit according to Eddy (1999).

**Statistical analysis:**

Data for hatchability percentage and body weight at hatch were subjected to one way analysis of variance using the general liner model ( GLM ) procedure of SAS (2004) to determine the effect of thermal manipulation. Differences between treatment means were tested using Duncan's Multiple Range Test (Duncan, 1955). One way analysis model:

$$ Y_{ij} = \mu + T_i + e_{ij} $$

Where: $\mu= $ the overall mean
T_i = the effect of the thermal treatment (i=1, 2, .....5 )
e i j = Experimental error.

Data for heat shock protein in brain and liver were subjected to two way analysis of variance using GLM procedure of SAS (2004) to determine the effect of temperature degree and exposing age. The fixed model used as following:

$$Y_{ij} = \mu + T_i + A_j + (T^*A)_{ij} + e_{ijk}$$

where : $\mu = \text{The overall mean}$

$T_i = \text{temperature degree (1,2)}$

$A_j = \text{exposing age (1,2)}$

$(T^*A)_{ij} = \text{Interaction between temperature degree and exposing age}$

$e_{ijk} = \text{Experimental error.}$

RESULTS AND DISCUSSION

1- Heat shock protein 70 synthesis

The results of heat shock protein synthesis in chicken embryos as influenced by heat stress at two different ages are shown in Table.1. Concerning the effect of heat stress, it could be observed that amounts of HSP70 synthesis either in brain or in liver significantly (p≤ 0.5) increased by thermal manipulation (TM). Where, the degree of 40 °C was exhibited the highest level of HP70 (10.85 ng/mg) in brain, followed by 39 °C degree (9.88 ng / mg). On the other hand, in liver there were no significant differences in the amounts of HSP70 synthesized between 39 and 40 °C degrees. However, the lowest amounts of HSP70 synthesized were recorded for 37.5 °C in both brain and liver tissues. These results are agreement with the findings of (Givisiez et al., 2001) who reported a significant increase in brain HSP70 levels in chicken heat stressed embryos, while its levels were not significantly different in the liver. Also, Rafael (2004) found a significant increase in HSP70 levels in the liver of turkey embryos exposed to 40 °C for 3hrs at day 22 of incubation.

Regardless of heat stress treatment, Table (1) shows that there were no significant differences in HSP70. Levels in both brain and liver due to exposing age, which is consistent with Leandro et al. (2004) who found that the age of heating treatment didn’t affect HSP70 synthesis in chicken embryos.

Concerning the interaction between TM and embryo’s age, Data in Table (1) indicates that brain HSP70 levels were significantly ( p ≤ 0.5 ) higher in all TM treatments comparing with (37.5 °C) control group at the two exposing ages. However, it was observed that there was no significant difference in brain HSP70 levels within the same heat-stress temperature degree at the two ages studied, except for the control group. Additionally, the highest levels of brain HSP70 were recorded for embryos exposed to 40 °C comparing with those exposed either to 39 °C or 37.5 °C at exposing ages. Meanwhile, different trend was appeared regarding liver, HSP70. Where, there was no any significant difference between any of TM treatments (39 and 40) at both studied ages. However, the embryo of control group (37.5 °C) had recorded the lowest levels of brain and liver had recorded the lowest levels of brain and liver HSP70. Either at days 15 or 18 of incubation period. Generally, the level of HSP70 in embryos brain was higher than its level in their livers in all experimental treatments (Fig. 1). Generally the brain tissue has shown the highest hsp70 levels when compared to other tissues in rats, (Beck et al., 1995) and in rabbits (Macari et al., 1997 and Manzerra et al., 1997). In this respect, Flanagan et al. (1995) suggested that the different accumulative profiles of HSP70 in tissues are submitted. The same authors added that the organism might respond to heat stress rapidly in tissues that are more important for the normal function of the body.

2- Hatchability% and chick weight at hatch.

Data of hatchability percent of fertile eggs and chick weight at hatch are presented in Table (2). It’s clear that using TM either at 39 or 40 °C at both ages tested significantly (p ≤ 0.05) increased
hatchability percent over than the control group (T1). However, the superiority hatching percent was achieved by eggs treated with 39 °C at the day 18 of incubation period (T4) followed by those exposed to 40 or 39 °C at the day 15 (T3 and T2), respectively. Meanwhile the lowest percentage was recorded for those of the control group (T1). Our findings are confirmed by those of Yahav et al. (2004) who stated that hatchability percent was significantly higher in late embryonic stage heat-treated than that early heat-treated and control (normal incubation temperatures). In contrary, Walstra et al. (2010) reported that high TM during day 14 to 18 embryonic period, didn’t affect hatchability percentage.

Chick weight at hatching significantly affected by TM treatments. Where, chicks of T3 (40 °C at d 15) were significantly (p ≤ 0.05) heavier in live body weight at hatch than each of T2- T4 as well as control group (T1). However, with the expection of T3, there were no significant differences in chick weight at hatch between TM treatments each other's and control group. These results were on line with those of Elsayed et al. (2009) who reported that increasing incubation temperature to 40.7°C or 39.5°C for 3 hrs daily, resulted in increased chick weight at hatch. Also, similar findings were obtained by Hulet et al. (2007) when they used different TM programs during incubation period. On the other hand, our results are disagreement with those of Walstra et al. (2010) who found that increasing incubation temperature didn’t affect chick body weight at hatch compared with normal incubation temperature. The confictions between the results of the embryonic TM studies may be due to the different of TM programs used as well as some other factors. In this study the increase of chick body weight at hatch as affected by egg TM treatments may be due to the higher incubation temperature accelerates embryonic growth and development as reported by (Yalçın and Siegel, 2003).

Finally, it could be concluded that exposing Golden Montazah fertile eggs to 40 °C for 3 hrs either at embryonic day 15 or 18 significantly increased HSP70 synthesis, hatchability percent and chick weight at hatch.
Table (1): Means ± S.E. of heat shock protein 70 as affected by heat stress at two different ages of embryonic period.

<table>
<thead>
<tr>
<th>Main effects</th>
<th>Treat</th>
<th>Heat shock protein (ng/ mg total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Brain</td>
</tr>
<tr>
<td>Temperature (° C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.5 ° C</td>
<td>37.5</td>
<td>6.48</td>
</tr>
<tr>
<td>39.0 ° C</td>
<td>39.0</td>
<td>9.88</td>
</tr>
<tr>
<td>40.0 ° C</td>
<td>40.0</td>
<td>10.85</td>
</tr>
<tr>
<td>Pooled S. E. M</td>
<td></td>
<td>± 0.152</td>
</tr>
<tr>
<td>Exposing age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 15</td>
<td>Day 15</td>
<td>8.89</td>
</tr>
<tr>
<td>Day 18</td>
<td>Day 18</td>
<td>8.91</td>
</tr>
<tr>
<td>Pooled S. E. M</td>
<td></td>
<td>± 0.127</td>
</tr>
</tbody>
</table>

Temperature * Exposing age

<table>
<thead>
<tr>
<th>Temperature ° C</th>
<th>Exposing age</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.5 ° C</td>
<td>Day 15</td>
<td>6.10</td>
<td>5.72</td>
</tr>
<tr>
<td>37.5 ° C</td>
<td>Day 18</td>
<td>5.85</td>
<td>5.43</td>
</tr>
<tr>
<td>40.0 ° C</td>
<td>Day 15</td>
<td>10.74</td>
<td>7.81</td>
</tr>
<tr>
<td>40.0 ° C</td>
<td>Day 18</td>
<td>9.92</td>
<td>7.62</td>
</tr>
<tr>
<td>Pooled S. E. M</td>
<td></td>
<td>± 0.224</td>
<td>± 0.383</td>
</tr>
</tbody>
</table>

a, b, ... means with different superscripts for temperature degree, exposing age or temperature degree * exposing age are significantly different (P < 0.05).

Figure (1): Heat Shock Protein 70 levels in brain and liver of heat-stressed Golden Montazah chicken embryos
**Table (2):** Means ± S.E. of Hatchability % of fertile eggs and chick body weight at hatch as affected heat stress treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hatchability of fertile eggs (%)</th>
<th>chick weight at hatching (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>85.11 ± 0.615</td>
<td>32.49 ± 0.45</td>
</tr>
<tr>
<td>T2</td>
<td>90.34 ± 0.615</td>
<td>32.61 ± 0.45</td>
</tr>
<tr>
<td>T3</td>
<td>92.19 ± 0.615</td>
<td>34.11 ± 0.45</td>
</tr>
<tr>
<td>T4</td>
<td>92.81 ± 0.615</td>
<td>32.20 ± 0.45</td>
</tr>
<tr>
<td>T5</td>
<td>87.89 ± 0.615</td>
<td>33.16 ± 0.45</td>
</tr>
</tbody>
</table>

a, b, …..Means with different superscripts within the same column are significantly different (P < 0.05).

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**REFERENCES**


دراسة تحليل بروتيني للصدمة الحرارية في اجنة الدجاج بعد تعرضها للأجهاد الحراري خلال فترة

النو الجنيني وعلاقتها بالبكتيريا ومجموعة الهضم

الابحاث: 

1- قسم الابحاث الهضم: كلية الزراعة - جامعة القاهرة

2- قسم تربية الدجاج – معهد بحوث الابحاث الزراعية

تم إجراء هذا البحث لدراسة تأثير الأجهاد الحراري لاجنة الكتكوت وعمر التعريض الحراري على تحليل بروتين القدرة الحرارية ونسبة الفضي للبيض المخبص وكذلك وزن الكتكوت عند الصرف.

تم إجراء التجربة العملية في معالج تطوير شرائح الكتكوت بالقياسات الحيوية لتدريب لبداية الصرف. وقد تم اخذ بيض التجربة من متحلة حديثاً باليوم من 24 ـ 24 يوم عمره بعد 750 بيضة مخصصة من سلالات المنتزة الذهبية على 5 مجتمع تجريبي بالتساوي. اشتملت كل معاملة على ثماني مكررات لكل مكرر 50 بيضة،

وكانت موزعين المعاملات كما يلي:

- المعاملة الأولى كنترول (T1) تم فيها تعريض البيض على درجات الحرارة 40°C و4°C لمدة 3
- المعاملة الثانية والثالثة (T2, T3) تم تعريض البيض في مفرخ آخر لدرجات حرارة 39 و40 مدة 3
- المعاملة الرابعة والخامسة (T4, T5) تم تعريض البيض في مفرخ آخر لدرجات حرارة 39 و40 مدة 3
- المعاملة السابعة والثامنة (T6, T7) تم تعريض البيض في مفرخ آخر لدرجات حرارة 39 و40 مدة 3
- المعاملة التاسعة (T8) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة العاشرة (T9) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الحادية عشرة (T10) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الثانية عشرة (T11) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الثالثة عشرة (T12) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الرابعة عشرة (T13) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الخامسة عشرة (T14) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة السادسة عشرة (T15) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة السابعة عشرة (T16) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الثامنة عشرة (T17) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة التاسعة عشرة (T18) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة العاشرة عشرة (T19) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الحادية عشرة عشرة (T20) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الثانية عشرة عشرة (T21) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الثالثة عشرة عشرة (T22) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الرابعة عشرة عشرة (T23) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الخامسة عشرة عشرة (T24) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة السادسة عشرة عشرة (T25) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة السابعة عشرة عشرة (T26) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الثامنة عشرة عشرة (T27) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة التاسعة عشرة عشرة (T28) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة العاشرة عشرة عشرة (T29) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3
- المعاملة الحادية عشرة عشرة (T30) تم تعريض البيض في مفرخ آخر لدرجات حرارة 40°C و4°C لمدة 3

ويمكن القول أن نتائج البحث تشير إلى أن تأثيرات الأجهاد الحراري على QC والجودة الحرارية ونسبة الفضي للبيض عند كامليه.}

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