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Research Article

The Effects of Cyclical Higher Incubation Temperatures on Body and Organs Weights, Thyroid Hormones and HSP70 Gene Expression of Newly Hatched Broiler Chicks

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Abstract

The objective of present study was to evaluate the effects of cyclical higher incubation temperatures in different embryonic ages on liver HSP70 gene expression, plasma T3, T4 and triglyceride (TG) levels, hatchability, body, heart and liver weights of newly hatched chicks. For this purpose, 2.700 fertile eggs (average weight, 58±1 g) were obtained from Arian broiler breeder at 34 weeks age. In a completely randomized design, eggs were assigned to three incubation temperature treatments at 60% relative humidity with 6 replicate per each. The treatment groups were: 1) control group that eggs were incubated at 37.6°C during incubation period, 2) incubation temperature was increased to 39°C for 3 h daily at embryonic ages from 12 to 14 in treatment 1 (TT1) and 3) incubation temperature was increased to 39°C for 3 h daily at embryonic ages from 15 to 17 in treatment 2 (TT2). All above-mentioned parameters were assessed at the day of hatch. The results showed that cyclical higher incubation temperatures did not affect body and liver weights, plasma T3, T4 and TG levels (P>0.05), while heart weights of chicks in TT1 and TT2 were lower (P<0.05) than control group. Furthermore, TT1 significantly increased liver HSP70 gene expression compared to the control group and TT2. It was concluded that cyclical higher incubation temperatures at embryonic ages from 12 to 14 could increase liver HSP70 gene expression with no effect on body and liver weights, plasma T3, T4 and TG levels at day-old chicks.

Keywords: *Incubation temperatures, Embryonic ages, Gene expression, Newly hatched chicks*

Yumurtadan Yeni Çıkmış Broiler Civcivlerde Döngüsel Yüksek İnkübasyon (Kuluçka) Sıcaklıklarının Vücut ve Organ Ağırlıkları, Tiroit Hormonları ve HSP70 Gen Ekspresyonu Üzerine Etkileri

Özet

Bu çalışmanın amacı; farklı embriyonik yaşlardaki döngüsel yüksek inkübasyon sıcaklıklarının, yumurtadan yeni çıkmış civcivlerde karaciğer HSP70 gen ekspresyonu, plazma T3, T4 ve trigliserid (TG) düzeyleri ile çıkış gücü, vücut, kalp ve karaciğer ağırlıkları üzerine etkisini değerlendirmekti. Bu amaçla, 34 haftalık etçi Arian damızlıklardan (ortalama ağırlık, 58±1 g) 2.700 adet döllenmiş yumurta elde edildi. Tamamen rasgele bir dizaynla seçilen yumurtalar, her biri 6 tekrarlı tarzda %60 nispi nemde üç farklı kuluçka sıcaklık uygulaması için ayrıldı. Tedavi grupları şunlardı: 1) kontrol grubu, yumurtalar inkübasyon süresi içinde 37.6°C'de inkübe edildi, 2) Tedavi 1 (TT1), inkübasyon sıcaklığı 12-14 arası embriyonik yaşta günde 3 saat süreyle 39°C'ye yükseltildi ve 3) Tedavi 2 (TT2), inkübasyon sıcaklığı 15-17 arası embriyonik yaşta günde 3 saat süreyle 39°C'ye yükseltildi. Yukarıda belirtilen tüm parametreler, kuluçka çıkış günü değerlendirildi. Sonuçlar, döngüsel yüksek inkübasyon sıcaklıklarının TT1 ve TT2'deki civcivlerin vücut ve karaciğer ağırlıkları ile plazma T3, T4 ve TG düzeylerini etkilemezken (P>0.05), kalp ağırlıklarının ise kontrol grubuna göre daha düşük (P<0.05) olduğunu gösterdi. Ayrıca, TT1 karaciğer HSP70 gen ekspresyonunu kontrol grubu ve TT2'ye göre anlamlı düzeyde artırdı. Sonuç olarak, 12-14 arası embriyonik yaşta döngüsel yüksek inkübasyon sıcaklıklarının, günlük civcivlerin karaciğer HSP70 gen ekspresyonunu artırabilmesine karşın, vücut ve karaciğer ağırlıkları ile plazma T3, T4 ve TG seviyeleri üzerine bir etkisi olmadı.

Anahtar sözcükler: *İnkübasyon sıcaklıkları, Embriyonik yaş, Gen Ekspresyonu, Yumurtadan yeni çıkmış civciv*

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INTRODUCTION

When living organisms are exposed to thermal and non-thermal stressors, the synthesis of most proteins is retarded; however, a group of highly conserved proteins known as heat shock proteins (HSPs) are rapidly synthesized [1]. These proteins are essential for organisms living at the edge of their thermal range. It is well documented that one of the most important functions of HSPs is to protect organisms from the toxic effects of heating [1,2]. HSPs may play important roles in protein assembly and disassembly, protein folding and unfolding, protein translocation and the refolding of damaged proteins [2]. Of the many expresses HSPs, those with a molecular weight of approximately 70 kDa appear to be most closely associated with heat tolerance. Heat shock 70 kDa proteins (HSP70s) are ubiquitous molecular chaperones that function in a myriad of biological processes [2].

Several investigations have demonstrated that heat shock response occurs in a variety of tissues [1,3]. Amongst all members of 70 kDa family, the one that has attracted most attention is HSP70. However, the mechanisms regulating HSP70 gene expression in broiler chicken have not been extensively studied. Being physiologically important tissue, the liver was selected in order to determine the changes in the gene expression levels of HSP70 after the process of cyclic thermal increase.

Embryonic temperature is considered to be the most important factor during incubation [3]. Since embryo is not capable of regulating its own temperature until hatching, the embryo temperature is regulated by the incubator air temperature [4]. Consequently, the temperature that the embryo encounters will depend on the incubator temperature, the ability of heat to transfer between the incubator and the embryo, and by its own metabolic heat production [5].

There are two parts involved in converting the yolk and albumen in a fertile egg into an embryo: 1. Differentiation and 2. Growth [6]. During the first half of incubation (embryonic ages from 1 to 10.5) chicken embryos go through a differentiation phase involving organ formation. They are capable of absorbing heat from the surrounding air, since they have a lower temperature than the incubator. The second half of incubation (embryonic ages from 10.5 to 21) is characterized by growth, and embryos must lose heat and metabolic rate and heat production increases, thereby increasing oxygen consumption and CO_2 levels [7].

The thermal conditions during embryonic period may have a great impact on development of physiological systems and maybe inducing epigenetic adaptations by having an effect on the chicks' development [8]. Thus, improving incubation conditions may have positive long-term effects on chicks. During the differentiation phase, the tissues are formed, and by embryonic ages 12 of incubation 90% of the organs are already present. Hyperthermia in developing tissues or cells has detrimental consequences for embryonic organogenesis. At a cellular level, hyperthermia may cause loss of protein function, which can be attributed to the normal misfolding of proteins with elevated temperature. A decrease in protein function, together with abnormal modifications that include changes in the phosphorylation state of eukaryote initiation factors and ribosomal proteins, will contribute to disruption of protein synthesis [9]. Thus, hyperthermia is a stress factor that, if present during embryonic development, will disrupt the plasma membrane and membrane protein function, altering cell structure and negatively impacting DNA, RNA, and protein synthesis [9]. Elevated temperatures during the avian embryo's early stage of development may have serious detrimental effects on its development and viability. For this reason, we used cyclical higher incubation temperatures in second half of incubation.

Some previous studies suggested that cyclical higher incubation temperatures can result in long-lasting modification to cellular and molecular neuronal mechanisms of temperature regulation [1,10,11]. Daily cyclical higher incubation temperatures, depending on the length of exposure and days of temperature modification, appear to improve tolerance of chicks to higher ambient temperatures [12]. Plasma T_3, which has an important role in the hatching process and thermoregulatory mechanisms [13], and plasma triglyceride (TG), which are the most abundant lipid present in birds, may be changed by thermal conditioning [10]; so, we evaluated these two factors.

The objective of this study was to evaluate the effect of daily higher incubation temperatures between embryonic ages from 12 to 17 on body, heart and liver weights and HSP70 relative gene expression in liver. Plasma T_3, T_4 and TG levels of chicks were also evaluated.

MATERIAL and METHODS

The study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch (approval date: 25.05.2013; no: 11096).

Eggs and Incubator Condition

A total of 2.700 fertile eggs (with average weight of 58±1 g) were obtained from a 34-wk old Arian broiler breeder flock. Eggs were incubated in single-stage incubators. From embryonic ages from days zero to 11, eggs were incubated under the same condition of 37.6°C and 60% relative humidity, while being turned once per h. At day 11 of incubation, dead embryos were identified by candling and removed from the experiment.

In a completely randomized design, eggs with live embryo (no: 2628) were assigned to three incubation temperature treatments at 60% relative humidity with 6

replicate and 146 eggs per each. The treatment groups were: 1) control group that eggs were incubated at 37.6°C during incubation period, 2) incubation temperature was increased to 39°C for 3 h per day at embryonic ages from 12 to 14 in treatment 1 (TT1) and 3) incubation temperature was increased to 39°C for 3 h per day at embryonic ages from 15 to 17 in treatment 2 (TT2). On the 18th day of incubation, the eggs were transferred to a hatcher. The hatchability was recorded for each treatment. Immediately after hatching completed (day 21 of incubation, with 8 h interval between the first and the latest hatched chick) male chicks were selected by vent sexing method in each treatment. All newly hatched male chicks were weighed and then three male chicks from each replicate (18 birds per treatment) were randomly selected. Blood and tissue sampling was started about two h and finished four h after hatching completed. Selection of chicks for sampling and sampling method was the same for all groups.

Sampling and Measurements

The blood sample of selected chicks was collected in EDTA-gel containing vacuum tubes directly from heart. Plasma was separated after centrifugation at 2.000 × *g* for 10 min and stored at -20°C for subsequent measurements of T_3, T_4 and TG.

The concentration of plasma T_3 and T_4 were determined using commercially available ELISA kits according to manufacturer recommendations (Biocheck Inc., Foster City, CA, USA). Plasma total TG level was measured enzymatically using photometric method by autoanalyser (BS-120 model, Minbray Co., USA) and commercial kits (Pars Azmon Co., Tehran, Iran).

Immediately after blood sampling, three chicks from each replicate were randomly chosen and sacrificed by cervical dislocation, then liver and heart were removed and weighed. Liver sample was taken, then frozen quickly in liquid nitrogen and stored at -70°C until further analysis for gene expression of HSP70.

Liver HSP70 Relative Gene Expression

Total RNA was extracted using Accuzol reagent (10 ml/g of tissue) from the ground liver segments according to the manufacturer instructions (Bioneer, Cat. No. K-2102). Total RNA purity was determined by calculating the ratio of the absorbance readings at 260 and 280 nm. Additionally, the quality of RNA was assessed by visualization of distinct 28S and 18S rRNA bands after gel electrophoresis with ethidium bromide staining. A quantity of 1 ug of each RNA sample was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcriptase kit (Bioneer Co., Seoul, South Korea). The 20 µL cDNA synthesis reaction contained in addition to the RNA template, 2 µL of 10x RT buffer, 0.8 µL of 25X dNTP mix, 2 µL 10x RT Random Primers and 1 µL MulitScribe Reverse Transcriptase. A volume of 10 µL of nuclease-free water was added bring the reaction up to final volume. The resulting cDNA was stored at -20°C prior to use.

Quantitative PCR was performed with a specific primer pairs (*Gallus gallus*, AY; 372 bp-763790; forward: 5′-AGC GTAACACCACCATTCC-3′; reverse: 5′-ACGCTCCTGCAAGAT AGTGAT-3′) using Quanti Fast SYBER Green PCR kit (QIAGEN, Cat. No. 204052). GAPDH (M-32599; 230 bp; forward: 5′-TGAAAGTCGGAGTCAACGGAT-3′, reverse: 5′-ACGCTCCTG GAAGATAGTGAT-3′) was chosen as a reference gene. Amplification of the day-old chicks liver HSP70 gene was performed for 45 cycles, which consisted of an initial activations step (95°C, 5 min), denaturation cycle (95°C, 10s) and combined annealing and extension (60°C, 30s). The GAPDH reference gene was amplified at 45 cycles under the same conditions in a different tube. After each run, preparation of standard curve was performed by serial dilution of pooled cDNA from samples. The relative expression ratio of HSP70 as a target gene was normalized to GAPDH gene using $2^{-\Delta\Delta ct}$ method as previously described by Livak and Schmittgen [14]. Quantification for each treatment group was performed in triplicates.

Statistical Analysis

Statistical analysis was performed using SAS software (version 9.1; SAS Institute, Cary, NC, USA [15]). Statistical analysis were performed using the one-way ANOVA to determine the effects of treatments on HSP70 gene expression, body and organs weights, hatchability, plasma T_3, T_4 and TG levels on newly hatched male broiler chicks. Mean comparison was done using the Duncan's multiple range test. Probability values of less than 0.05 ($P<0.05$) were considered significant.

RESULTS

The effects of cyclical higher incubation temperatures on body, liver and heart weights and hatchability are presented in *Table 1*. The results showed that cyclical higher incubation temperatures did not significantly affect body and liver weights ($P>0.05$), while heart weight of chicks in TT1 and TT2 were significantly lower than control group ($P<0.05$). Also, there was no significant difference in hatchability among treatments ($P>0.05$).

The effects of thermal manipulation during incubation on plasma TG, T_3, T_4 levels are presented in *Table 2*. TG, T_3 and T_4 levels at the day of hatch were not influence by thermal treatments ($P>0.05$).

The effects of different cyclical thermal manipulation during incubation period on HSP70 gene expression are shown in *Fig. 1*. The expression of liver HSP70 gene were quantified by qPCR assay and expressed relative to expression of the GAPDH gene. TT1 significantly increased gene expression compared to TT2 and control group

Table 1. *Effect of cyclical higher incubation temperatures on body, liver and heart weights and hatchability on newly hatched male broiler chicks*

Tablo 1. *Yumurtadan yeni çıkmış erkek etlik civcivlerde döngüsel yüksek inkübasyon sıcaklıklarının vücut, karaciğer ve kalp ağırlıkları ve kuluçka randımanı üzerine etkisi*

Parameter	Experimental Treatments			SEM	P-Value
	C	TT1	TT2		
Body weight (g)	40.33	40.20	40.48	0.24	0.8309
Liver weight (g)	1.255	1.194	1.178	0.01	0.2653
Heart weight (g)	0.332[a]	0.293[b]	0.295[b]	0.01	0.0474
Hatchability (%)	98.67	98	98	1.58	0.7247

[a,b] Means in row that possess different superscripts differ significantly (P<0.05); ***C:*** *Incubated at 37.6°C throughout;* ***TT1:*** *exposed to heat at 39°C 3 h/d from EA 12 to 14 of incubation;* ***TT2:*** *exposed to heat at 39°C 3 h/d from EA 15 to 17 of incubation*

Table 2*. Effect of cyclical higher incubation temperatures on some blood parameters levels on newly hatched male broiler chicks*

Tablo 2. *Yumurtadan yeni çıkmış erkek etlik civcivlerde döngüsel yüksek inkübasyon sıcaklıklarının bazı kan parametreleri üzerine etkisi*

Parameter	Experimental Treatments			SEM	P-Value
	C	TT1	TT2		
T_3 (ng/ml)	1.65	1.15	1.78	0.09	0.31
T_4 (ng/ml)	1.88	1.55	2.51	0.19	0.37
TG (mg/dl)	42.67	47.83	45.17	13.51	0.28

Means with a row superscript letter did not differ (P>0.05); ***C:*** *incubated at 37.6°C throughout;* ***TT1:*** *exposed to heat at 39°C 3 h/d from EA 12 to 14 of incubation;* ***TT2:*** *exposed to heat at 39°C, 3 h/d from EA 15 to 17 of incubation;* ***TG:*** *Triglycerides,* ***T_3:*** *3,5,3′-triiodothyronine,* ***T_4:*** *L-thyroxine*

(P<0.05). The expression of HSP70 mRNA was numerically lower in TT1 than control group.

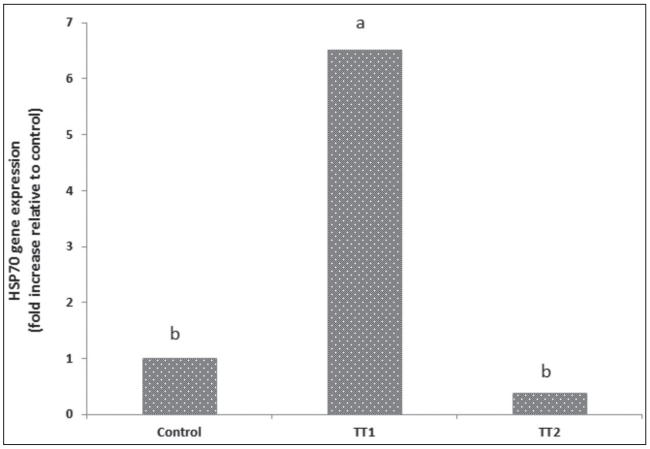

Fig 1. Result of the HSP70 gene expression data (mean ± SD) using real time qPCR according to $2^{-\Delta\Delta ct}$ method. Means with different superscripts differ significantly (P<0.05). Control group (incubated at 37.6°C throughout); TT1: exposed to heat at 39°C, 3 h/d from EA (embryonic age) 12 to 14 of incubation; TT2: exposed to heat at 39°C, 3 h/d from EA 15 to 17 of incubation. [a,b] Means that possess different superscripts differ significantly (P<0.05)

Şekil 1. HSP70 gen ekspresyon verilerinin (ortalama ± SD) 2-ΔΔct yöntemine göre gerçek zamanlı qPCR kullanım sonucu. Farklı üst harf taşıyan ortalamalar arasındaki fark önemlidir (P<0.05). Kontrol grubu (37.6°C'de daimi inkübasyon); TT1: 39°C sıcaklığa maruziyet, EY (embriyonik yaşı) 12-14 arası 3 saat/gün inkübasyon; TT2: 39°C sıcaklığa maruziyet, EY 15-17 arası 3 saat/gün inkübasyon. [a,b] Farklı harf taşıyan ortalamalar arasındaki fark önemlidir (P<0.05)

DISCUSSION

The objective of this study was to evaluate the effect of cyclical higher incubation temperature on hatchability, body and organs weights. A broiler chick spends up 30-40% of its life inside the egg. Thus, each factor or condition that enhance the growth and development during embryonic period can positively affect the post hatch performance of the broiler chicks [16,17]. There are some evidences that showed the embryonic temperature during the last 5 days of incubation could significantly affect post hatch growth and performance [16,18-20]; however, previous studies reported that thermal manipulation had no effect on body weight of both sex [21,22].

In the present study, cyclical higher temperature had no effect on hatchability, body and liver weights of chicks. These observations are in agreement with the findings of Lournes et al.[4] and Leksrisompong et al.[23], who showed that slight changes in incubation temperature cannot affect body weight of chicks and hatchability. The application of higher temperature in this study had no effect on liver weight, whereas application of higher temperature in the study of Leksrisompong et al.[23] resulted in change of liver weight. In our study, cyclical higher temperature was applied, whereas in previous studies [4,18,23] constant higher temperature was applied throughout incubation. This difference may be the reason of conflict between our study and other studies [4,18,23] concerning liver weight. It was reported [20] that deviation from optimum incubation temperature suppresses the development of organs and growth, but in our study, deviation from optimum incubation temperature was cyclical and low, which could not affect body and liver weights.

In this study, heart weight of chicks in control group was significantly higher than heart weight of chicks in TT1 and TT2. Inconsistent with our finding, Yalcin and Siegel [24] reported that heart weight of chicks exposed to higher temperature during embryonic ages from days 10 to 18 of incubation was higher than control numerically. The findings of Leksrisompong et al.[23] and Yalcin et al.[25] concerning heart weight of chicks exposed to higher temperature are in agreement to our result. The lower heart weight in chicks exposed to higher incubation temperatures compared to control group may be explained by low rate of cell division in heart of embryos. These data suggested that certain organs in a critical period during second half of incubation was more sensitive to high temperatures, whereas heart exhibited sensitivity from embryonic ages from 12 to 17 of incubation.

Thyroid hormones are essential for the normal development and differentiation of the embryo. These hormones also have an important role in temperature regulation in chicks by regulating basal metabolic rate. Thyroid hormones are important in several organismal level processes such as hatching [26]. The high perihatch concentrations of thyroid hormones appear to be stimulating a variety of developmental and metabolic processes necessary for successful hatching [13,26]. Plasma thyroid hormone concentrations increase dramatically during late stages of incubation [13,27]. After perihatch peak, plasma thyroid hormones decrease markedly, and then gradually increase during post-hatch life to reach adult concentrations [27]. Thermoregulation is under the control of thyroid hormones. One the mechanisms that induce thermotolerance involves the modulation of heat production through changes in circulating T_3. The ability to reduce plasma T_3 concentration, especially during a thermal challenge, has been suggested to be an improvement in thermotolerance [11,12].

In our study, cyclical higher incubation temperatures had no significant effect on plasma T_3 and T_4 concentrations. These findings are in agreement with the study of Tona et al.[28]. Also, an interesting study [10] showed that plasma T_3 and T_4 concentrations were not affected by high incubation temperatures from embryonic ages from 16 to 18.5 of incubation. Of course, they reported that higher thermal condition resulted in decrease of plasma T_3 concentration at embryonic ages 18 of incubation. Our results were in conflict with previous reports of Wineland et al.[29], who observed a reduction in plasma T_3 at day of hatch in chicks exposed to higher constant incubation temperatures from embryonic age from 17 to hatch. It was demonstrated [22] that continuously or 12 h/day higher temperature during embryonic age from 7 to 16 could significantly reduce thyroid hormones concentrations.

The difference in length of exposure and level of heat leaded to different responses. It can be speculated that thermal challenge caused a decrease in plasma thyroid hormones concentrations but in our study heat challenges were slight and cyclical. In other hand, time interval in the end of heat challenge and day of hatch, in both treatments, was sufficient to compensate this decline. Also the half-lives of T_3 and T_4 are essentially identical and are short (3-9 h) in birds [30]. Response of thyroid hormones to heat takes much time and in this study the length of exposure was not enough for stimulating thyroid hormones. It suggests that slight and cyclical thermal changes during second half of incubation may have no significant effect on plasma T_3 and T_4 concentrations in newly hatched chicks.

Plasma TG levels were slightly higher at the day of hatch in heat treated groups, which were in agreement with Yalcin et al.[31]. Cyclical higher incubation temperature in our study did not affect plasma TG levels of day-old chicks significantly. Our results were in conflict with the findings of Yalcin et al.[32] and Willemsen et al.[10]. Sensitivity of plasma TG levels to changes in thyroid hormones is high [32], hence decrease in plasma T_3 levels leaded to increase of plasma TG levels. As plasma thyroid hormone concentrations had no differences among treatments, hence there were no changes in plasma TG levels.

Of the many HSPs, HSP70 appear to be most closely associated with heat tolerance [2]. Under normal growth conditions, HSP70 is synthesized constitutively; however, its expression increases following thermal challenge. Heart, liver and kidney of broilers are more sensitive to heat stress and HSP70 gene expression increases after 2 h exposure to elevated temperatures [1]. It was showed that the stress-induced responses vary among different tissues [1]. Also, It is clear that after heat preconditioning HSPs are induced during the recovery from the heat stressed period and can work as a repair system in the recovery phase [1]. It was indicated that enhancement in HSP70 expression is evident for periods up to four weeks after termination of the daily heat conditioning episodes [33].

Cyclical higher temperature at embryonic age of 12 to 14 significantly increased liver HSP70gene expression. This indicates embryos in earlier ages have higher capability for synthesis of HSP70 than embryos near hatching. HSP70 gene expression varies in different tissues [1,34], therefore HSP70 gene expression in broiler chicken embryos is tissue and age dependent. In contrast to our result, Givisiez et al.[3] reported that heat-stress had no effect on liver HSP70 gene expression; however, significant effect on HSP70 gene expression of brain and kidney was observed.

The results of this study suggest that cyclical higher incubation temperatures (39°C) for 3 days (embryonic age from 12 to 14) could induce the HSP70 gene expression without negative effects on day-old chicks. It is advisable to industry; however, from the practical viewpoint, the application of cyclical higher temperature need to fully automated, programmable incubators. More detailed studies taking account of different incubation conditions are needed to define the effects of these conditions on thermotolerance and other important health parameters, especially immunity and its related gene expression.

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REFERENCES

1. Yu J, Bao E: Effect of acute heat stress on heat shock protein 70 and its corresponding mRNA expression in the heart, liver, and kidney of broilers. *Asian-Aust J Anim Sci,* 21, 1116-1126, 2008. DOI: 10.5713/ajas.2008.70703

2. Baqchi M, Ireland M, Katar M, Maisel H: Heat shock proteins of chicken lens. *J Cell Biochem,* 82, 409-414, 2001. DOI: 10.1002/jcb.1168

3. Givisiez PEN, da Silva MM, Mazzi CM, Ferro MIT, Ferro JA, Gonzales E, Macari M: Heat or cold chronic stress affects organ weights and HSP70 levels in chicken embryos. *Can J Anim Sci,* 81, 83-87, 2001. DOI: 10.4141/A00-049

4. Lourens A, Van den Brand H, Meijerhof R, Kemp B: Effect of eggshell temperature during incubation on embryo development, hatchability and post hatch development. *Poult Sci,* 84, 914-920, 2005. DOI: 10.1093/ps/84.6.914

5. French NA: Modeling incubation temperature: The effects of incubator design, embryonic development, and egg size. *Poultry Sci,* 76, 124-133, 1997. DOI: 10.1093/ps/76.1.124

6. Deeming DC: Yolk sac, body dimensions, and hatchling quality of ducklings, chicks, and poults. *Br Poultry Sci* 46, 560-564, 2005. DOI**:** 10.1080/00071660500255042

7. Smith LDe, Bruggeman V, Debonne M, Tona JK, Kamers B, Everaert N, Witters A, Onagbesan O, Arckens L, De Baerdemaeker J, Decuypere E: The effect of nonventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. *Poultry Sci,* 87, 551-560, 2008. DOI: 10.3382/ps.2007-00322

8. Decuypere E, Bruggeman V: Endocrine aspects of development: new challenges for the control of incubation process. *Worlds Poultry Sci J,* 61, 278-283, 2005. DOI: 10.3382/ps.2010-00853

9. Bensaude O, Dubois PM, Van-Trung N, Morange M: Protein denaturation during heat-shock and related Stress. **In,** Schlesinger MJ (Ed): Stress Proteins Induction and Function. 89-99, Springer Berlin Heidelberg, Germany, 1990. DOI: 10.1007/978-3-642-75815-7_8

10. Willemsen H, Kamers B, Dahlke F, Han H, Song Z, Ansari Pirsaraei Z, Tona K, Decuypere E, Evaraert N: High- and low- temperature manipulation during late incubation: Effects on embryonic development, the hatching process, and metabolism in broilers. *Poultry Sci,* 89, 2678-2690, 2010. DOI: 10.3382/ps.2010-00853

11. Yahav S, Collin A, Shinder D, Picard M: Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. *Poultry Sci,* 83, 1959-1963, 2004. DOI: 10.1093/ps/83.12.1959

12. Yahav S, Rath RS, Shinder D: The effect of thermal manipulations during embryogenesis of broiler chicks *(Gallus domesticus)* on hatchability, body weight and thermoregulation after hatch. *J Therm Biol,* 29, 245-250, 2004. DOI: 10.1016/j.jtherbio.2004.03.002

13. Decuypere E, Bruggeman V: The endocrine interface of environmental and egg factors affecting chick quality. *Poultry Sci,* 86, 1037-1042, 2007. DOI: 10.1093/ps/86.5.1037

14. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real time quantitative PCR and 2-ΔΔct method. *Methods,* 25, 402-408, 2001. DOI: 10.1006/meth.2001.1262

15. SAS Institute. SAS/Stat User Guide: Ver.9.1. SAS Institute Inc., Cary, NC., USA, 2010.

16. Hulet R, Gladys G, Hill D, Meijerhof R, El-Shiekh T: Influence of egg shell embryonic incubation temperature and broiler breeder flock age on post hatch growth performance and carcass characteristics. *Poultry Sci,* 86, 408-412, 2007. DOI: 10.1093/ps/86.2.408

17. Ozaydin T, Celik I: Effects of high incubation temperature on the body weight and yolk consumption of two commercial broiler strain. *Acta Sci Vet,* 42, 1253, 2014.

18. Piestun Y, Druyan S, Brake J, Yahav S: Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. *Poultry Sci,* 92, 1155-1163, 2013. DOI: 10.3382/ps.2012-02609

19. Piestun Y, Harel M, Barak M, Yahav S, Halevy O: Thermal manipulation in late-term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. *J Appl Physiol,* 106, 233-240, 2009. DOI: 10.1152/japplphysiol.91090.2008

20. Ipek A, Sözcü A: The effects of high setter and hatcher temperatures during incubation on slaughter weight and carcass yield in broilers. *Turk J Vet Anim Sci,* 39, 450-454, 2015. DOI: 10.3906/vet-1406-30

21. Piestun Y, Shinder D, Ruzal M, Halevy O, Brake J, Yahav S: Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. *Poultry Sci,* 87, 1516-1525, 2008. DOI: 10.3382/ps.2008-00030

22. Piestun Y, Halevy O, Yahav S: Thermal manipulations of broiler embryos-The effect on thermoregulation and development during embryogenesis. *Poultry Sci,* 88, 2677-2688, 2009. DOI: 10.3382/ps.2009-00231

23. Leksrisompong N, Romero-Sanchez H, Plumstead PW, Brannan KE, Brake J: Broiler incubation. 1. Effect of elevated temperature during late incubation on body weight and organs of chicks. *Poultry Sci,* 86, 2685-2691, 2007. DOI: 10.3382/ps.2007-00170

24. Yalçın S, Siegel PB: Exposure to cold and heat during incubation on developmental stability of broiler embryos. *Poultry Sci,* 82, 1388-1392, 2003. DOI: 10.1093/ps/82.9.1388

25. Yalçın S, Özkan S, Siegel PB, Yenisey Ç, Akşit M: Manipulation of incubation temperatures to increase cold resistance of broilers: Influence on embryo development, organ weights, hormones and body composition. *J Poultry Sci,* 49, 133-139, 2012. DOI: 10.2141/ jpsa.011117

26. Darras VM, Van der Geyten S, Kühn ER: Thyroid hormone metabolism in poultry. *Biotechnol Agron Soc Environ,* 4 (1): 13-20, 2000.

27. DuRant SE, Carter AW, Denver RJ, Hepp GR, Hopkins WA: Are thyroid hormones mediators of incubation temperature-induced phenotypes in birds? *Biol Lett,* 10, 20130950, 2014. DOI: 10.1098/rsbl.2013.0950

28. Tona K, Onagbesan OM, Bruggeman V, Collin A, Berri C, Duclos MJ, Tesseraud S, Buyse J, Decuypere E, Yahav S: Effects of heat conditioning at d 16 to 18 of incubation or during early broiler rearing on embryo physiology, post-hatch growth performance and heat tolerance. *Arch Geflügelk,* 72 (2): 75-83, 2008.

29. Wineland MJ, Christensen VL, Yildrum I, Fairchild BD, Ort DT, Mann KM: Incubator environment interacts with genetic line of broiler at the plateau stage to affect embryo plasma thyroxine and triiodothyronine concentrations. *Int J Poultry Sci,* 5, 714-722, 2006. DOI: 10.3923/ijps.2006.714.722

30. Rudas P, Pethes G: The importance of the peripheral thyroid hormone deiodination in adaptation to ambient temperature in the chicken *(Gallus domesticus). Comp Biochem Physiol,* 77, 567-571, 1984. DOI: 10.1016/0300-9629(84)90230-5

31. Yalçın S, Cabuk M, Bruggeman V, Babacanoglu E, Buyse J, Decuypere E, Siegel PB: Acclimation to heat during incubation. 1. Embryonic morphological traits, blood biochemistry, and hatching performance. *Poultry Sci,* 87, 1219-1228, 2008. DOI: 10.3382/ps.2007-00435

32. Yalcın S, Bruggeman V, Buyse J, Decuypere E, Cabuk M, Siegel PB: Acclimation to heat during incubation: 4. Blood hormones and metabolites in broilers exposed to daily high temperatures. *Poultry Sci,* 88, 2006-2013, 2009. DOI: 10.3382/ps.2008-00505

33. Wang S, Edens FW: Heat conditioning induces heat shock proteins in broiler chickens and turkey poults. *Poultry Sci,* 77, 1636-1645, 1998. DOI: 10.1093/ps/77.11.1636

34. Zhen FS, Du HL, Xu HP, Luo QB, Zhang XQ: 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. *Br Poultry Sci,* 47, 449-455, 2006. DOI: 10.1080/00071660600827690

35. Moraes VMB, Malheiros RD, Bruggeman V, Collin A, Tona K, Van-As P, Onagbesan OM, Buyse J, Decuypere E, Macari M: Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. *J Therm Biol,* 28, 133-140, 2003. DOI: 10.1016/S0306-4565(02)00049-9