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Morphological Investigations of the Effect of Thermal Manipulation During Embryogenesis on Body Performance and Structure of Pectoral and Thigh Muscle of Ross Broiler Chicken

ABSTRACT

The aim of this study was to determine the optimum timing of thermal manipulation during early, mid, late and long lasting embryogenesis that may result in improvement of body performance and myofiber development (fiber diameter and thickness of perimysium) of pectoral and thigh muscles by a comparative morphometrical investigation on post-hatch day 35. 1440 fertile Ross broiler eggs were divided randomly and equally into 5 treatment groups: control (no TM), TM1, TM2 TM3 and TM4 were thermally subjected to 39°C for 18h with 65% relative humidity daily during embryonic days ED 7–11, ED 11–15, ED 15–18 and ED 7–18 respectively. Out of TM conditions that were investigated, TM1 resulted in a significant improvement of hatchability rate (95.14%) and body performance when compared to the control. On post-hatch day 35, there were significant differences in BW, skinned carcass, breast, thigh and gut weight for all groups when compared to each other with a higher significant in TM1 and TM2 when compared to those of the control. FCR in this study was lower in TM groups when compared to the control group. In TM3 and TM4 the chicks show a significantly lower value of body performance when compared with those of the control group, TM1 and TM2. The histological evaluation of Pectoral muscle revealed myofibers of different diameter (38.39–40.27 μm) and perimysial thickening (17–30.5 μm), while myofibers diameter of thigh muscle ranged between (29–32.9 μm) and the perimysial thickening ranged between (14.36–22.15 μm). Thus, these results indicate that TM during ED 7–11 improved muscle growth and body performance and this finding may be applied by commercial breeders to produce more enhanced broiler chickens.

INTRODUCTION

Incubation temperature during embryogenesis is considered to be the most important factor that may have a significant effect on life performance of embryos and also during their post hatch body performance such as influencing hatchability rate and chick quality (Decuypere & Michels, 1992; Lourens *et al.*, 2005). One of the most suggested strategies that focuses on genetic improvement of muscle growth and growth performance with a low cost, is thermal manipulation strategy during embryogenesis. Thermal manipulation during early or late stages of embryonic development may influence embryonic body weight, hatchability rate and may alter body physiology and performance (Tzschenke *et al.*, 2001; Yahav *et al.*, 2004a; Yahav *et al.*, 2004b; Collin *et al.*, 2005; Piastun *et al.*, 2008a; Piastun *et al.*, 2008b; Al-Zghoul *et al.*, 2013; Loyau *et al.*, 2013). Histogenesis of embryonic pectoral and thigh muscles fibers is directly related to molecular and cellular mechanisms, which proceeds from early embryogenesis and continues up to adulthood until the final mature size and strength

are reached (Olson, 1992; Stockdale, 1992; Ordahl *et al.*, 2000; Koohmaraie *et al.*, 2002; Piestun *et al.*, 2009). Whereas posthatch muscle growth occurs by the increase in myofiber size and the by increase in the volume of cytoplasm without increasing myofiber numbers (Allen & Boxhorn, 1989; Mozdziak *et al.*, 1994; Remignon *et al.*, 1995; Allen *et al.*, 1999; Mozdziak *et al.*, 2000).

To our knowledge, there are no recent studies focused on timing duration of thermal manipulation effects during broiler embryogenesis on body performance and muscle structure properties. Therefore, this study aimed to investigate the effects of thermal manipulation during early, mid, late and long-lasting broiler embryogenesis on muscle structure and body performance parameters (Feed conversion ratio, body and skinned carcass weight). Thus, the results of this research may provide the means of improving growth efficiency and increases overall meat yield production of commercial broiler, without the need to increase feeding or the number of birds slaughtered to cover the market demands.

MATERIALS AND METHODS

Incubation, Hatching, Sampling Plans and Managements.

All sampling and experimental incubating and hatching management conditions were approved by the King Faisal University Animal Care and Use Committee (KFU-ACUC). 1700 Ross 708 broiler eggs were supplied from a 36 weeks broiler flock age (from Al-Hassa, Saudi Arabia). Broken, unfit small and large abnormal eggs were excluded ($58\text{g} < \text{eggs} < 65\text{g}$) before the first incubation day (1550 uniform size eggs remained) then egg candling was performed at ED7 to excludes the dead embryos and unfertile eggs to end up with 1440 fertile eggs. The remaining eggs were distributed randomly into 5 treatment groups, the first one was the untreated control group that was incubated at 37.8°C with 56% relative humidity (RH) whereas, TM1, TM2 TM3 and TM4 were thermally subjected to 39°C for 18h with 65%(RH) daily during embryonic days ED 7–11, ED 11–15, ED 15–18 and ED 7–18 respectively.

All one-day hatched chicks were recorded and the field study was conducted at the research station farm. Water and feed were supplied ad libitum to the chicks and kept brooding at initial house temperature of $(31 \pm 1^\circ\text{C})$ and the temperature was reduced by an average of $(0.2\text{--}0.3^\circ\text{C})$ per day to achieve a final

house temperature of $(22 \pm 1^\circ\text{C})$ by post hatched day 24. Total live body weight was measured and recorded from 20 chicks per treatment during the first post hatch day and then it was recorded on post-hatch days 14, 21, 28 and 35 of age. Skinned carcass weight was measured from five chicks from each treatment group after humanely euthanatized at post hatch day 35.

Histo-Metrical Examination

For morphometrical analysis of muscles fiber diameter and perimysium thickness, approximately one centimeter of pectoral and thigh muscles tissue samples from all treatment groups on posthatched day 35 were taken and placed overnight in 4% paraformaldehyde for further processing. All trimmed samples were processed for histological examination and stained with H&E and Gomori Trichrome Stains and observed by the light microscopy under 10x and 40x magnification powers. Morphological measurements were taken by Image processing and analysis image J 1.52a analyzer (Wayne Rasband, national institutes of health, USA, <http://imagej.nih.gov/ij>).

Statistical Analysis

Chi-square was applied to analyze hatchability rate. Whereas Data for body performance (Feed conversion ratio, body, skinned carcass, breast and thigh weight), morphometrical analysis of pectoral and thigh muscles fiber (Fiber diameter and Thickness of perimysium) were expressed in means $\pm$ SE. One-way ANOVA followed by all-pairs Bonferroni test were applied to compare different parameters in all treatment groups using IBM SPSS statistics 20 software (IBM software, Chicago, USA). Differences was considered significant at $p < 0.05$.

RESULTS

Effect of thermal manipulation on hatchability

The effect of TM during embryogenesis on hatchability is summarized in Table 1. The total number of hatched eggs after 21 days of incubation was 977 with a total hatchability of 83.15%. Early, mid, late and long-lasting thermal manipulation significantly influenced the hatchability rate. TM1 resulted in improvement of hatchability rate with a higher significant value (TM1 = 95.14%) when compared with the control (92.89%). Thermal manipulation at TM3 and TM4 resulted in adverse effect in the hatchability rate and chick quality (spraddle leg deformity, opened navel (omphalitis), unabsorbed yolk materials and subcutaneous edema)

Table 1 – The effects of TM on hatchability rate in broiler chicks thermally manipulated at 39 °C for 18h during early, mid, late and long lasting embryogenesis.

Groups	Control	TM1 (ED7-11)	TM2 (ED11-15)	TM3 (ED15-18)	TM4 (ED7-18)
No. of eggs at Day18	225	247	241	231	231
Total (hatch eggs)	209	235	217	180	136
Unhatched eggs	16	12	24	51	106
Unhatched eggs%	(7.11%)	(4.86%)	(9.96%)	(22.08%)	(45.89%)
Hatchability	92.89% ^b	95.14% ^a	90.04% ^c	77.92% ^d	58.87% ^e

Control= 37.8°C; TM1= Thermal manipulation from ED 7-11 at 39°C for 18 h; TM2= Thermal manipulation from ED 11-15 at 39°C for 18h; TM3= Thermal manipulation from ED 15-18 at 39°C for 18h. TM4= Thermal manipulation from ED 7-18 at 39°C for 18h.

a-e Within the same row, means $\pm$ SD with different superscripts differ significantly ($p < 0.05$).

when compared with the control group. Furthermore, thermal manipulation in TM2 was significantly higher than those of TM3 and TM4 (90.04%).

Effect of Thermal Manipulation on Post-Hatch Body Performance Parameters

Effect of TM on feed conversion ratio, body weight, skinned carcass weight, breast and thigh muscle weight, Gut, Liver, Feather and Skin weight of chicks in the post-hatch day 35 are shown in Figure 1 and Table 2.

Figure 1 – The effect of TM during embryogenesis on post-hatch broiler chicks body weight from day 1 to day 35 (n = 20). Control= 37.8°C; TM₁= Thermal manipulation from ED 7-11 at 39°C for 18 h; TM₂= Thermal manipulation from ED 11-15 at 39°C for 18h; TM₃= Thermal manipulation from ED 15-18 at 39°C for 18h. TM₄= Thermal manipulation from ED 7-18 at 39°C for 18h.

a–e Within the same day, means $\pm$ SD with different superscripts differ significantly ($p < 0.05$).

Feed Conversion Ratio (FCR)

The feed conversion ratio varied among all treatment groups and the control as shown in Table 2. The FCR ranged between 1.3 to 1.9. Moreover, the FCR ratio found to be very efficient in all treatment groups when compared to the control.

Body Weight

On post-hatch day 1; the body weight of all TM groups was significantly lower than the control and this continued up to post-hatch day 14. However, on

post-hatch day 14; body weight of TM1 group was significantly higher when compared to the control, while the body weight of TM2, TM3 and TM4 chicks was significantly lower than the control group. Furthermore, on post-hatch days 21, 28 and 35; TM1 remained in higher significant value when compared to the control, while TM3 and TM4 remained in lower significant values when compared to the other treatment groups as shown in Figure 1.

Skinned carcass Weight

On post-hatch day 35, there was a significant increase in skinned carcass weight in TM1 group when compared to all treatment groups. Moreover, there was no significant differences in skinned carcass weight between control, TM2 and TM3, while in TM4 the value was significantly lower when compared to all treatment groups (Table 2).

Breast and Thigh Muscle Weight

On post-hatch day 35, there was significant increases in breast muscle weight in TM1 group when compared to all treatment groups and there were no significant differences between control, TM2, TM3 and even TM4. The significant increase value of thigh muscle weight was recorded in TM1 when compared to those of the control and TM2. In TM3 and TM4 groups, thigh muscle weight was significantly lower when compared with those of TM1, TM2 and control groups as shown in Table 2.

Gut, Liver, Feather and Skin Weight

On post-hatch day 35, there was a significant increase in Gut and Liver weight in TM1 group when compared to all treatment groups. Nevertheless, there was no significant differences in gut and liver weight between control, TM2, TM3 and TM4 treatment groups. In addition, there was no significant differences in feather and skin weight in all treatment groups as shown in Table 2.

Table 2 – Feed Conversion Ratio, Body weight, skinned carcass weight, breast and thigh muscle weight on post-hatch day 35 in broiler chicks subjected to different thermal manipulation treatments during embryogenesis.

	Control	TM1	TM2	TM3	TM4
FCR	1.93	1.53	1.43	1.30	1.41
BW	2069.6±57.9c	2376.1±47.2a	2147.9± 65.6 b	2025.2± 39.0d	1901.5±61.9 e
Skinned carcass	1287.6±79.8b	1445.0±46.4a	1315.8±69.4b	1280.2±74.6b	1179.4±68.6c
Skinned carcass /BW %	62.2%	67.3%	55.4%	63.2%	62%
Breast muscle	447±14.11 b	486.3±9.29 a	468.3±17.56 b	453.3±10.41 b	451.7±2.89 b
Breast muscle / BW %	21.6%	22.6%	19.7%	22.4%	23.8%
Thighs muscle	183.3±28.87 b	221.7±25.66 a	191.7±2.89 b	150±5.00 c	145±8.66 c
Thigh muscle / BW %	8.9%	10.3%	8.1%	7.4%	7.6%
Gut and liver	196.86±9.69 b	225±12.47 a	218.4±25.99 b	213±12.02 b	204.13±20.15b
Gut and liver / BW %	9.5%	10.5%	9.2%	10.5%	10.7%
Feathers and Skin	323.7±28.74a	381.13±22.18 a	341±21.45 a	328±26.50 a	311.86±28.07 a
Feathers and Skin / BW %	15.6%	17.7%	14.4%	16.2%	16.4%

Control= 37.8°C; TM1= Thermal manipulation from ED 7-11 at 39°C for 18 h; TM2= Thermal manipulation from ED 11-15 at 39°C for 18h; TM3= Thermal manipulation from ED 15-18 at 39°C for 18h. TM4= Thermal manipulation from ED 7-18 at 39°C for 18h.

a-e Within the same row, means ± SD with different superscripts differ significantly ($p < 0.05$).

Morphological Results of Pectoral and Thigh Muscle Fibers Diameter and Perimysium Thickness in The Post-Hatched Day 35

The histological evaluation of the longitudinal sections of pectoral and thigh muscles revealed that pectoral myofibers diameter ranged between (38.39-40.27 µm) and thigh myofibers (29-32.9 µm). However, there was no significant differences in pectoral myofiber diameters in all treatment groups. Thigh myofiber analysis showed that there were significant differences in myofiber diameters between control, TM2 and TM3 when compared with TM1 and TM4 as shown in Table 3. There was also a difference in the thickening of the perimysial network ranged between (17-30.5 µm) and (14.36-22.15 µm) in the pectoral and thigh muscles, respectively (Table 3). The histological evaluation of pectoral perimysium thickening has significant differences between all treatment groups when compared to the control with a higher value in TM1, TM2 as shown in Table 3. Moreover, thigh

perimysium thickening has significant differences between the control and all treatment groups except in TM4, which was similar to the control as shown in Table 3.

DISCUSSION

Walstra *et al.* (2010) reported that thermal manipulation over the constant incubation temperature during embryonic growth and development throughout egg incubation can achieve maximum results on hatchability and can trigger the development of physiological control system and body function of the embryo by increasing their adaptation capacity to heat stress. The finding of the previous studies by other authors have reported that thermal manipulation for 24h during early embryonic day 7-16 or late 16-18 at 39.5°C or 40°C can significantly improve hatchability, body performance and thermotolerance acquisition later in post hatch days (Piestun *et al.*, 2008a; Piestun *et al.*, 2008b).

Table 3 – Morphological effects of thermal manipulation on pectoral and thigh skeletal muscle fibers diameter and perimysium thickness on post-hatched day 35 by light microscopy. a-c Within the treatments, means ± SD with different superscripts differ significantly ($p < 0.05$).

	Control	TM1	TM2	TM3	TM4
P. Muscle Fibers Diameter (µm) Mp 10x	38.39±9.53a	40.27±8.82a	40.12±9.16 a	39.83±9.50 a	39.05±7.52 a
T. Muscle Fibers Diameter (µm) Mp 10x	29.04±5.49a	32.90±7.98b	30.58±6.31 a	29.86±7.88 a	31.11±5.46 b
P. Muscle perimysium Thickness(µm) Mp 40x	17.01±3.77 a	30.50±4.84 b	27.55±4.64 b	24.61±4.74 c	24.32±3.30 c
T. Muscle perimysium Thickness(µm) Mp 40x	17.76±3.32 a	22.15±3.18 b	21.74±1.50 b	21.63±2.74 b	14.36±1.61 a

Control= 37.8°C; TM1= Thermal manipulation from ED 7-11 at 39°C for 18 h; TM2= Thermal manipulation from ED 11-15 at 39°C for 18h; TM3= Thermal manipulation from ED 15-18 at 39°C for 18h. TM4= Thermal manipulation from ED 7-18 at 39°C for 18h.

a-c Within the same row, means ± SD with different superscripts differ significantly ($p < 0.05$).

In this study, our finding in TM1 and TM2 treatments were compatible with the previous studies in that there were significant positive responses to thermal manipulation during embryonic development; particularly in hatchability percentage in TM1 at ED7-ED11 which was 95.14% when compared with the control that was 92.89%. Moreover, in post-hatch day 14; TM1 group was significantly higher in body live weight, skinned carcass, breast and thigh muscle, gut, liver, feather and skin weights when compared to the control. These results may indicate that thermal manipulation at early-term broiler embryogenesis has the best positive influence on hatchability and body performance. In contrast, other previous studies on the effects of thermal manipulation during early embryonic day 7 up to 16 and late during embryonic day 16 to 18 at 38.8, 39.5°C or 41 for 24h on hatchability and body performance showed significant reduction in hatching rate or only slightly reduced it (Willemsen *et al.*, 2010; Al-Zghoul *et al.*, 2013; Al-Zghoul *et al.*, 2015; Piestun *et al.*, 2015; Al-Zghoul, 2018; Shanmugasundaram *et al.*, 2019; Decuyper & Michels, 1992). On the other hand, our findings in TM3 and TM4 treatments of thermal manipulation at late- or long-term showed the same adverse effect on hatchability rate, chick quality and growth when compared with the control group. The adverse effects on chick quality were represented by spraddle leg deformity, omphalitis, unabsorbed yolk materials and subcutaneous edema. In addition to that our study has shown that thermal manipulation in TM3 and TM4 also resulted in transient growth arrest during their first and second post-hatch weeks of life, however it was followed by immediate compensatory growth after the third week of age. The discrepancies between our results and the previous finding may be attributed to the differences in the incubation profiles and experimental design or on the breed and age of the broilers' flocks' eggs that were used in their experiments.

Skeletal muscle myofibers are differentiated from myoblast and myocyte during embryogenesis and undergo hypertrophy by increasing the myofiber cytoplasm without increasing the myofiber numbers (Halevy *et al.*, 2001; Allen *et al.*, 1979; Chen *et al.*, 2007). This expansion in the cytoplasm will result in the increase in the diameter of the myofiber and subsequently will improve muscle weight. It has been reported by Yahav & Hurwitz (1996) and Yahav (1998) that mild thermal manipulation during early age of embryonic development can enhance hypertrophy of skeletal muscle in later age. Our results showed improvement in

thigh myofiber diameters in TM1 and TM4 treatments that support the previous findings of Yahav & Hurwitz (1996) in that early thermal manipulation enhances the myofiber size late in post-hatch age and subsequently improves muscle weight and hence the improvement of body performance. Piestun *et al.* (2009) reported that late term of thermal manipulation at 39.5°C from embryonic day 16-18 for 3 or 6 h daily could increase the myofiber diameter later by post-hatch day 13 of age. On the other hand, our findings in TM3 treatment showed no significant difference in myofiber diameter when compared to our control group.

This study was undertaken to fill in the gap in the studies that investigate the effect of TM during early, mid and late broiler embryogenesis on body performance and myofiber development of pectoral and thigh muscles (fiber diameter and thickness of perimysium) of Ross broiler chicken in Saudi Arabia. Out of TM conditions that were investigated, TM1 treatment resulted in significant improvement of hatchability rate and body performance (muscle mass, fiber diameter, body and skinned carcass weight) on post-hatch day 35 when compared to those of the control without adversely affecting performance. The outcome of this research may provide means of improving growth efficiency. This may contribute to increased overall meat production, without the need to increase feeding or the number of birds slaughtered to cover the market demands.

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