

Effect of high thermal manipulations in early and late embryogenesis on growth of tibia and femur bones of broilers*

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Abstract

The aim of this research was to determine the effects of thermal manipulations early and late embryogenesis on growth of tibia and femur bones of broilers. For control group eggs 37.5 °C and 55 % humidity were applied on the incubation during 19 days. Incubation temperature was increased to 41 °C and humidity to 65 % for 3 hours on the 8th-10th days of incubation during early embryogenesis. Similarly, incubation temperature was increased to 41 °C and humidity to 65 % for 3 hours on the 16th-18th days of incubation during late embryogenesis. Average tibia bone lengths were determined as 97.57 mm, 99.08 mm and 100.25 mm for control, early and late embryonic groups, respectively, and significant difference were determined between the average values. Also, male broilers (100.27 mm) have higher tibia bone length than females (97.66 mm). The differences between the groups in point of femur bone length was significant, and the highest femur bone length was in the late embryonic group (70.79 mm), the lowest femur bone length in the control (68.93 mm) group.

Key words: Broilers, thermal manipulation, growth, tibia-femur bone

Etlik piliçlerde embriyonun erken ve geç gelişim dönemlerinde yapılan yüksek ısı uygulamanın tibia ve femur kemiğinin büyümesine etkileri

Öz

Bu çalışmada embriyo gelişiminin erken ve geç dönemlerindeki yüksek ısı uygulamanın tibia ve femur kemiklerinin gelişimine olan etkilerinin belirlenmesi hedeflenmiştir. Bunun için, kontrol grubundaki yumurtalara 37.5 °C sıcaklık ve % 55 nem 19. güne kadar uygulanmıştır. Kuluçkanın erken (8-10. günler) ve geç embriyonik (16-18. günler) dönemlerinde ise günlük 3 saat süreyle yumurtalara 41°C sıcaklık ve % 65 nem uygulanması yapılmıştır. Tibia kemik uzunluğu kontrol, erken ve geç embriyonik gruplarında sırasıyla 97.57 mm, 99.08 mm and 100.25 mm olarak ölçülmüş olup gruplar arasındaki farklılık önemli bulunmuştur. Tibia kemiği erkeklerde (100.27 mm) dişilerden (97.66 mm) daha uzun olarak belirlenmiştir. Femur kemiği uzunluğu bakımından gruplar arasındaki farklılık önemli bulunmuş olup femur kemiği uzunluğu en fazla geç embriyonik (70.79 mm) grubunda, en düşük ise kontrol grubunda (68.93 mm) ölçülmüştür.

Anahtar kelimeler: Etlik piliç, ısı uygulama, büyüme, tibia-femur kemiği

Introduction

Chicken meat is an important and cheap source of animal protein for human due to its low fat content, nutritive value and high protein level. Today, a significant increase has been achieved in the production of poultry meat (Warriss, 2010). Increasing the ratio of breast meat, decreasing abdominal fat ratio, improvement of the product quality and product diversity have played an important role in the poultry meat production. In addition to genetic breeding studies, epigenetic adaptation during incubation is used to try to reduce heat stress by high temperature and humidity manipulations in embryonic period in poultry (Yahav, 2004; Yalcin et al. 2005; Narinc et al. 2016). In recent years, applying high temperature and humidity studies has increased in the incubation stage. High temperature and humidity practices in incubation phase increased the resistance of the poultry chickens to heat stress. During the application of heat stress, the temperature threshold of the body changes for heat production and heat dissipation, so the heat stress tolerance of the poultry chickens increases (Yahav, 2000; Nichelmann, 2004). Thermal application studies are basically divided according to incubation stage and postnatal periods. The pre-incubation period is very important period in terms of balancing systems for body temperature. Temperature and humidity of the incubator can cause in the physiological responses of the poultry to high temperature and humidity. Control of early development of the thermoregulatory system during incubation by changes in incubation temperature could be a tool to adapt chicks to higher post-hatch ambient temperatures (Tzschentke, 2007). Higher cyclic incubation temperatures during specific periods of embryogenesis improved the thermotolerance of broilers and reduced the reverse effects of short- or long-term heat stress on broilers (Yalcin et al. 2005; Piestun et al. 2008). The growth rate of the muscle of broilers has displayed an important improvement than body weight improvement (Lilburn, 1994). Developments in the rate of growth of chickens caused many unexpected results, such as an increase in carcass fatness and skeletal diseases (Sullivan, 1994). Besides, genetic selection for fast growth, gender and environmental temperature can affect the skeletal development of poultry (Leterrier and Constantin 1999; Rose et al.

1996; Leeson and Caston, 1993). Yalcin et al. (1995) determined that chicken reared at high temperatures tibia and humerus weight decreased, but the length was not affected. Bruno et al (2000) observed that broiler chickens reared under heat stress conditions indicated a reduction in both the length and width of the tibia, femur and humerus. Also, Eriksen et al. (2003) studied the influence of temperature on bone growth in the chick. Their results indicated that the total skeletal calcium and the true bone length of several long bones vary linearly with the temperature of the chick embryo by as much as 20% per degree centigrade.

The objective of the research was to evaluate the effects of high thermal manipulations early (EE₈₋₁₀ days) and late (LE₁₆₋₁₈ days) embryogenesis on growth of tibia and femur bones of broilers.

Material and Method

This study was conducted in summer season between June and July months during 6 weeks of age. Eggs used in this study were obtained from commercial firms. Eggs were stored for one day at 16-18 °C and 70-75 % humidity conditions. Eggs were randomly divided into 3 groups. For control group eggs 37,5 °C and 55 % humidity were applied on the incubation during 19 days. Then, incubation temperature was increased to 41 °C and humidity to 65 % for 3 hours on the 8th-10th days of incubation during early embryogenesis. Similarly, incubation temperature was increased to 41 °C and humidity to 65 % for 3 hours on the 16th-18th days of incubation during late embryogenesis. (Bruzual et al. 2000). The eggs were turned through 270° automatically every hour. At the 18th day of incubation, the eggs were transferred to 37.5 °C and 55 % humidity conditions. Hatched chicks were wing-banded and individually weighed at hatching. The chicks were fed with a diet consisting of 12.50 MJ kg⁻¹ metabolic energy and 210 g crude protein kg⁻¹ *ad libitum* and unlimited water was supplied during the experiment. At the end of the experiment at random from each group of 10 males and 10 females broilers selected, and the femoral and tibial lengths and widths were measured in these animals. Much of the adhering tissue was removed from each bone with scissors and blades. The length and width of each bone was measured with dijital caliper (mm ± 0,01) (Applegate and Lilburn, 2002; Birgul et al. 2011).

Statistical methods

Following model was used for statistical analyses:

$$Y_{ijk} = \mu + g_i + c_j + e_{ijk} \quad (1)$$

where Y_{ijk} represents the observation values, μ was the overall mean, g_i is the effect of the i -th manipulation, c_j is the effect of the j -th gender and e_{ijk} is the error term, $\sim N(0, \sigma^2e)$.

Findings and Discussion

Temperature and relative humidity values in terms of weeks were presented in Table 1. The effects of thermal manipulations and gender on length and width of tibia and femur bones were given in Table 2 and 3. Temperature and relative humidity values were ranged between 29.79- 32.46 °C and % 42.73-74.82, respectively.

Table 1. Weekly temperature (°C) and relative humidity (%) values

Week	Temperature±SE ¹	Relative humidity±SE ¹
1	30.54±0.17	65.90±3.53
2	30.93±0.18	73.11±4.31
3	30.22±0.18	74.82±3.52
4	31.74±0.22	70.85±4.52
5	32.46±0.28	42.73±3.48
6	29.79±0.27	56.8±4.56

¹Standard error

As seen in Table 2, the length of tibia was 97.57 mm in the control, 99.08 mm in the early embryogenesis and 100.25 mm in late embryogenesis groups, respectively. Average length of tibia of early and late embryogenesis was significantly longer than those of control group ($P < 0.05$). For all that, significant difference between the genders was found, and tibia length was significantly longer in males (100.27±0.62mm) than females (97.66±0.62mm). Also, there was significant difference among the groups in terms of tibia widths, and tibia width (9.84mm) in the late embryogenesis group was higher than those of control (9.26mm) and early embryogenesis (9.31mm) groups. As presented in Table 3, difference among the groups in terms of lengths of femur was found significant ($P < 0.05$), and highest length of femur value was determined in late embryogenesis group (70.79mm). In contrast, the lowest length of femur value was found in control group (68.93mm). Also, there was determined significant difference between the genders, and length of femur of males was higher than that of females. However, difference among the groups in respect to widths of femur was not significant, in contrast, males have higher value (10.34mm) than those of females (9.16mm).

Kocabagli (2001) reported that lengths of tibia bone vary between 89.4-92.2mm. Also, Barreiro et al.(2011) found that lengths of tibia bone were 32.5mm, 54.36mm and 80.68mm in 8th, 22th and 42th days of age, respectively. Yalcin et al.(1995) stated

that high temperatures reduced tibia bone weight, however, did not affect the length of tibia bones. Again in another study, effects of genotype, age of parent and gender on morphological characteristics and composition of tibia bone were investigated. Tibia lengths of male and females broilers at 32 and 48 days of age were changed between 88.56-98.50 mm and between 115-118 mm changed, respectively. In contrast, tibia widths of male and females broilers at 32 and 48 days of age were changed between 6.20-6.50 mm and between 8.44-9.07 mm (Yalcin et al. 2001). In a study by Quarantelli et al. (2007), lengths of femur bone at 21, 41 and 61 days of age as 51.40-54.57mm, 75.50-79.87mm and 90.50-97.37mm, but lengths of tibia bone as 72.47-96.0mm, 107.5-114.5mm and 132.43-140.83 mm were determines, respectively. High ambient temperature reduced growth of femur and tibia bones of broilers, but did not affect bone fracture resistance, and lengths of femur and tibia bone of broilers grown in low ambient temperature (15°C) was significantly lower than those of grown in optimum ambient temperature (24°C), however there was no significant difference between the ambient temperature groups in point of width of femur and tibia bones (Bruno et al.(2000, 2007). Shim and Pesti (2011) stated that application of different temperatures during incubation had no significant affect on length of the tibia bone for broilers. Curek (2010) reported that there was no significant difference between the low and rapidly growing

broilers in respect to lengths of tibia, and tibia lengths were determined 107.19mm for slow growing and 106.44mm for rapid growing broilers. In the same study, there was significant difference between the winter (107.90mm) and spring (109.97mm) seasons in terms of lengths of tibia bones. Thermally treated eggs of broilers during late embryogenesis (EL₁₄₋₁₇ days), incubation temperature was increased to 41 °C for 4 hours by Badran et al. (2012). The authors concluded that this process provides epigenetic adaptations necessary to deal with the high temperature, and thermally treated group has higher tibia length

than that of control group. In another study, length and width of tibia femur bone of broilers from hatching to at 43 days of age were measured weekly. Significant difference was found between the male and females in point of length of tibia bone at 15 and 35 days of age, and similarly, significant difference was determined between male and females in respect to length of femur bone at 43 days of age. In addition, significant difference was found between male and females in point of width of tibia and femur bones at 28 and 43 days of age in the same study (Applegate and Lilburn, 2002).

Table 2. Effect of thermal manipulation and gender on length and width of tibia bone (mm)

Groups	Length of tibia (mm)			Width of tibia (mm)		
	Left*	Right*	Average±SE	Left*	Right*	Average±SE
Control	98.23±0.65	96.90±1.00	97.57±0.76 ^b	9.32±0.17 ^b	9.21±0.19	9.26±0.16 ^b
EE ¹	99.25±0.65	98.90±1.00	99.08±0.76 ^a	9.13±0.17 ^b	9.49±0.19	9.31±0.16 ^b
LE ²	100.15±0.65	100.34±1.00	100.25±0.76 ^a	9.83±0.17 ^a	9.86±0.19	9.84±0.16 ^a
Gender						
Male	100.69±0.53 ^a	99.83±0.82	100.27±0.62 ^a	9.97±0.14 ^a	10.05±0.15 ^a	10.01±0.13 ^a
Female	97.73±0.53 ^b	97.59±0.82	97.66±0.62 ^b	8.88±0.14 ^b	8.99±0.15 ^b	8.93±0.13 ^b

¹Early embryogenesis, ²Late embryogenesis; * mean±SE, a,b Means in column, with different letters differ significantly at (P<0.05)

Table 3. Effect of thermal manipulation and gender on length and width of femur bone (mm)

Groups	Length of tibia (mm)			Width of tibia (mm)		
	Left*	Right*	Average±SE	Left*	Right*	Average±SE
Control	68.96±0.56	68.90±0.57	68.93±0.55 ^b	9.80±0.16	9.71±0.15	9.76±0.15
EE ¹	69.17±0.56	69.40±0.57	69.28±0.55 ^{ab}	9.64±0.16	9.49±0.15	9.57±0.15
LE ²	70.74±0.56	70.83±0.57	70.79±0.55 ^a	9.86±0.16	9.99±0.15	9.93±0.15
Gender						
Male	70.65±0.45 ^a	70.89±0.46 ^a	70.78±0.45 ^a	10.34±0.13 ^a	10.33±0.12 ^a	10.34±0.12 ^a
Female	68.59±0.45 ^b	68.53±0.46 ^b	68.56±0.45 ^b	9.19±0.13 ^b	9.13±0.12 ^b	9.16±0.12 ^b

¹Early embryogenesis, ²Late embryogenesis; * mean±SE, a,b Means in column, with different letters differ significantly at (P<0.05)

Conclusion

It was concluded that raising the incubation temperature for a short period (3 hours/day) at the early or late part of embryonic development (day 8 to 10 and day 16 to 18 day of incubation) induced beneficial thermal epigenetic physiological changes. This epigenetic thermal adaptation could be employed for coping with post-hatch heat stress. The highest value for length of tibia bone was in late embryogenesis period, whereas lowest width of tibia bone was obtained in this group. In terms of length of femur bone, there was significant difference between the control and late

embryogenesis groups, but no significant differences were detected between the groups in terms of width of femur bones. Obtained results in this study and the findings of the literature show some differences in terms of length and width of tibia and femur bones. Applied different temperature and humidity values and also applying different times have influenced differently obtained results. Consensus could not be reached exactly studies on this subject. Therefore, more research is needed to determine the effects of application of temperature and humidity on the development of skeletal and bone during incubation stage.

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