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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Determination of Broiler Femur Parameters at Different Growth Phases[1]

Fabiana Ribeiro Barreiro[2], Silvana Martinez Baraldi-Artoni[3], Luiz Augusto do Amaral[2],
José Carlos Barbosa[4], Annita Morais Girardi[5], Maria Rita Pacheco[3] and Lizandra Amoroso[3]
[2]Department of Preventive Veterinary Medicine, [3]Department of Animal Morphology and Physiology,
[4]Department of Statistical Analysis, [5]Department of Veterinary Clinical and Surgery,
Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil

Abstract: The objective of this experiment was to analyse macroscopically the femur and determine the biochemical values at 8, 22 and 42 days of age, producing basic results that can help to understand the pathogeny of the locomotor problems and the broiler physiologic growth. A total of 60 Cobb male broilers were distributed in three age groups (8, 22 and 42 days of age) of 20 birds. All macroscopic measurements, except the cranial compact layer increased ($p<0.05$) over the course of the ages. The cranial compact layer presented the biggest measure at 22 days of age. The ash percentage increased ($p<0.05$) until 22 days of age, but at 42 days of age this values decreased. Calcium and phosphorus percentage in ash increased ($p<0.05$) until 22 days of age and evidenced constancy from 22 to 42 days of age. The biomechanical adaptation capacity of femur was evidenced by the increase of the macroscopic measures over the course of the ages and the different behavior of cranial compact layer at 22 days of age suggested an adaptation attempt of the immature bone to muscle mass increase. The ash and mineral percentage confirmed that the relative growth occurs with more intensity until 21 days of age. The calcium and phosphorus percentage evidenced the physiologic balance acting on the deposition of these minerals in femur.

Key words: Broiler, mineral, femur, macroscopic analysis, bone ash

INTRODUCTION

The leg disorders of broilers can be caused by many factors, such as genetic inheritance, bird age, broiler density, environmental conditions, nutrition, metabolic diseases, pathogens and mycotoxins (Wu *et al.*, 1993; Naas, 2008). The current broiler breeds have faster growth and higher feed efficiency, being necessary the feed intake control during initial phases to avoid metabolic disorders (Powell and Bittar, 2008). Julian (2005) affirmed that these metabolic disorders are associated with increased metabolism and rapid growth rate which result in the failure of a body system because of the increased work-load. Takita (1998) concluded that broilers presenting rapid growth during the initial phase are more inclined to develop locomotor system anomalies.

Buckner *et al.* (1950) were the pioneers in establish a relation between broiler weight and the femur development, evidencing that more than 98% of the variability in femur length is linked to broiler weight. Guyton (1992) described that the bone tissue suffers continued remodelation, becoming thicker when the weight supported by the bone is higher.

The bone during the growth has regions of particular development with different physiologic characteristics (Applegate and Lilburn, 2002) and it is important to know the physiologic measurements from all femur regions to become possible the growth anomalies diagnosis. Applegate and Lilburn (2002) established a relation between femur growth, broiler age and body weight; observing that the femur presented differences at the regions associated with the linear growth (epiphysis) and at the bone regions more mature near from diaphysis. Bruno (2002) observed that the values of diaphysis length and diameter of broiler femurs were reduced by the feed restriction, whereas Thorp (1992) described that the occurrence of femoral deviation was higher than the tibial and tarsus-metatarsal deviation for broilers fed *ad libitum*.

Some measurements such as bone ash (Garlich *et al.*, 1982) and mineral ash percentage (Akpe *et al.*, 1987) have been used as bone indicators to determine the poultry bone response to different situations. Thorp (1992) compared the diaphysis mineralization of the femur with tibia diaphysis and concluded that the femur mineralization was lower than the tibia one, suggesting that the femur can be considered a vulnerable point of the broiler leg and can be the responsible by the locomotor problems during the final phase of the broiler. Itoh and Hatano (1964) and Dilworth and Day (1965) compared the mineralization of the long bones and concluded that the femur is more sensible to diet changes than tibia, in agreement with Moran Junior and Todd (1994).

Corresponding Author: Fabiana Ribeiro Barreiro, Department of Preventive Veterinary Medicine, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil

The bone tissue has the highest nutrient mobilization and more accented relative growth until 21 d of age (Macari *et al*., 1994; Takita, 1998). Oliveira (2006) observed that the bird age influences the femur ash percentage of Hybro PG, Isa Label JA57 and Ross 308 broilers, increasing the mineral deposition at the first weeks of age, in agreement with the results found by Bruno (2002) and Skinner and Waldroup (1995).

The calcium is the mineral more prevalent in the bird body, approximately 1.5% of the body composition (Underwood, 1981; Larbier and Leclerq, 1992), representing more than 1/3 of the minerals (Klasing, 1998). The bird skeleton contains 99% of the corporal calcium and 90% of the phosphorus (Mendonca Junior, 2000). The major part of the calcium in birds is used to support the body weight during the growth (Silva *et al*., 2001).

The balance between calcium and phosphorus is very important to bone tissue formation because the mobilization and deposition of these minerals can modify the bone mass (Pizauro Junior, 2002). The phosphorus deposition at bone is synchronized with calcium deposition because these minerals form an insoluble salt (Dell'Isola *et al*., 2003).

Field (2000) described that although the bone ash percentage changes with the age, the calcium amount has the values maintained relatively constant. Barbosa (2005) observed that the femur calcium ash did not change over the course of the ages.

The objective of this experiment was to analyse macroscopically the femur and determine the biochemical values at 8, 22 and 42 days of age, producing basic results that can help to understand the pathogeny of the locomotor problems and the broiler physiologic growth. The parameters more used in researches were determined, allowing the comparison of these physiological values with those obtained from broilers submitted to different handling conditions and fed with other diets.

MATERIALS AND METHODS

A total of 60 Cobb male broilers were distributed in three groups of 20 birds. The birds were housed from 1 day of age and the first group was bred for 8 days, the second for 22 days and the third for 42 days. The handling was the same as that used in the commercial breed of broilers. The light program used during the experiment was 24 h of illumination in all phases. The birds were vaccinated at 8 days of age against Gumboro disease, at 13 days of age against Newcastle disease and at 23 days of age against Gumboro disease (reinforcement). Water and ration were provided *ad libitum*. The rations were of commercial brand (Purina do Brasil-Inicina and Nutriengorda, Cerqueira Cesar St., 178, Jardim Garcia, Campinas, São Paulo, Brazil) and were divided in two types, according to the broiler phase. The initial ration (Inicina) was used until 21 days of age and the growth ration (Nutriengorda) from 22 to 42 days of age. The broilers were killed by cervical dislocation, which is a euthanasia method accepted by the Ethics and Animal Welfare Commission of "Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista" (FCAV/UNESP) (specific authorization number: 020857-07). Femurs were obtained at 8, 22 and 42 days of age after the removal of skin, muscles, ligaments, vessels and epiphysary cartilage.

Macroscopic analysis: The femur measurements at different ages were the weight, length, thickness of compact layer (cranial and caudal portions), thickness of spongy layer, perimeter and diameter of proximal epiphysis, diaphysis and distal epiphysis; using a digital pachimeter, fiber and ruler.

Biochemical analysis: The bones were placed in an oven (55°C) for 72 h to dry them. Subsequently, these bones were grinded in a ball mill and put in mufla oven at 550°C for 3 h and the ash was used to quantify calcium and phosphorus amounts in the bone, by the technique of Silva and Queiroz (2002). The calcium values were determined by atomic absorption in GBC equipment (932AA, Analitica, Assungui St., 432, São Paulo, SP, Brazil), whereas the phosphorus determination was realized by a spectrophotometer (B-395, Micronal, João Rodrigues Machado St., 25, Brooklin, São Paulo, SP, Brazil).

Statistical method: The broilers were distributed in a completely randomized design, with 3 treatments (ages) and 20 repetitions (20 birds) for each treatment. The pair-wise comparisons of means were made using Tukey's test procedure. A p-value <0.05 was considered significant. All of the statistical results were obtained from Agroestat (Department of Statistical Analysis FCAV/UNESP).

RESULTS

All the macroscopic measurements (Table 1), except the cranial compact layer, presented significant differences ($p<0.05$), increasing over the course of the ages (8, 22 and 42 days of age). The cranial compact layer presented distinct behavior, because the biggest measure was observed at 22 days of age and not at 42 days of age. The results of the biochemical values are presented at Table 2. The ash percentage increased until 22 days of age, but at 42 days of age this values decreased. The calcium and phosphorus percentage in ash presented the same tendency, increasing until 22 days of age ($p<0.05$) and evidencing constancy from 22 to 42 days of age.

Table 1: Values (mean±standard deviation) of weight, thickness of compact layer (cranial and caudal portions), thickness of espongy layer, perimeter and diameter of proximal epiphysis, diaphysis and distal epiphysis of broiler femurs at 8, 22 and 42 days of age

Measurements	8 days	22 days	42 days
Weight (g)	0.98±0.10c	4.64±0.37b	14.96±1.35a
Length (mm)	32.15±1.83c	54.36±1.75b	80.68±1.42a
Cranial compact layer (mm)	1.43±0.33c	2.79±0.59a	2.19±0.76b
Caudal compact layer (mm)	0.67±0.28c	1.64±0.45b	2.00±0.44a
Proximal epiphysis spongy layer (mm)	5.97±0.59c	11.38±1.25b	17.97±0.95a
Distal epiphysis spongy layer (mm)	2.43±0.37c	3.84±1.10b	6.34±1.04a
Proximal epiphysis perimeter (mm)	32.65±2.15c	49.80±2.70b	74.60±3.83a
Diaphysis perimeter (mm)	18.10±3.11c	28.10±3.40b	37.80±3.48a
Distal epiphysis perimeter (mm)	33.05±1.98c	52.20±2.70b	79.65±3.91a
Proximal epiphysis diameter (mm)	8.13±0.65c	12.60±1.23b	18.61±1.56a
Diaphysis diameter (mm)	2.83±0.24c	5.35±0.61b	8.38±0.87a
Distal epiphysis diameter (mm)	8.17±0.49c	14.41±0.81b	21.90±0.87a

a,b,c Means within a row lacking a common superscript differ ($p<0.05$)

Table 2: Values (mean±standard deviation) of ash percentage and calcium and phosphorus in ash of broiler femurs at 8, 22 and 42 days of age

Age	Ash (%)	Calcium (%)	Phosphorus (%)
8	33.38±1.82b	26.35±5.60a	11.78±2.71a
22	36.61±3.19a	22.22±3.09b	7.78±2.02b
42	33.95±2.71b	19.35±3.59b	6.76±1.50b

a,b,c Means within a column lacking a common superscript differ ($p<0.05$)

DISCUSSION

The bone during the growth phase pass by alterations and when the muscle mass increases the bone becomes thicker. Buckner *et al*. (1950) were the first authors that mentioned the relation between the bird weight and femur development, evidencing that above 98% of the femur length variability occurs in function of the broiler weight.

Growing bones present regions different physiologically, with particular characteristics of development (Applegate and Lilburn, 2002) and it is important to know the physiologic measurements of each femur region to identify anomalies on its growth. Thorp (1992) described that the lateral deviation of the femur was greater, when compared with tibiotarsus or tarsometatarsus one in birds fed *ad libitum*. Theoretically, the occurrence of femoral deviation contributes for angular defect at femoral growth plate and other localities due to overload of these regions. This same author compared the mineralization at the femur diaphysis with the tibia one and observed that the mineralization was lower in femur, suggesting that this bone can be considered a vulnerable point of broiler legs and can be the main responsible by long bones abnormalities in the last part of the growth phase. Itoh and Hatano (1964) and Dilworth and Day (1965) compared the long bones mineralization and concluded that femur is more sensible to changes in diet than tibia, in agreement with Moran Junior and Todd (1994). Applegate and Lilburn (2002) described the femur development of broilers, considering the age and weight and observed that the femur showed differences in the regions associated with the linear growth (epiphysis) and near from diaphysis. In this present experiment, it was observed increase in the values at epiphysis and diaphysis from 8 to 42 days of age, evidencing a biochemical adaptation of the bones to support the muscle mass deposition during the growth. The different behavior of cranial compact layer observed at 22 days of age suggests an adaptation attempt of the immature bone to the new bone conformation. The great interval between the values of the different macroscopic parameters from 8 to 42 days of age shows the fast metabolism of the femur what can explain the higher sensitivity to changes in diet of this bone, as cited by some authors (Itoh and Hatano, 1964; Dilworth and Day, 1965; Moran Junior and Todd, 1994). Bruno (2002) concluded that the length of femur and diaphysis diameter were reduced by feed restriction; varying from 43 to 81.3 mm and from 3.8 to 9.6 mm at 14 to 42 days of age, respectively. At this present experiment these values varied from 32.15 to 80.68 mm for femur length and from 2.83 to 8.38 mm for diaphysis diameter between 8 and 42 days of age. This experiment determined the physiologic values of femur measurements more used in researches, allowing to compare with those obtained from broilers submitted to different treatments and presenting pathological alterations.

Measures such as bone ash (Garlich *et al*., 1982) and quantity of ash minerals (Akpe *et al*., 1987) have been used as indicators of poultry bone quality. The bone mineral density can be measured by determination of the bone mineral composition (Almeida Paz and Bruno, 2006), quantifying the bone ash percentage (Grier *et al*., 1996) and the minerals such as calcium and phosphorus in bone ash (Kienzle *et al*., 1998). In this present experiment, the highest values of ash percentage observed at 22 days of age, comparing with 8 and 42 days of age, can be explained by higher nutrient flow to bone tissue (Macari *et al*., 1994) and higher relative skeleton growth in broilers (Takita, 1998) until 21 days of age. Oliveira (2006) observed that the bird age influenced the femur ash percentage of Hybro PG, Isa Label JA57 and Ross 308 breeds, increasing the mineral deposition during the first weeks, in agreement with the results found by Bruno (2002) and Skinner and Waldroup (1995).

Calcium is the mineral more prevalent in the bird body and composes approximately 1.5 % of the weight (Underwood, 1981; Larbier and Leclerq, 1992).The bird skeleton constitutes 99% of the calcium reserve and 90% of the corporal phosphorus (Mendonca Junior, 2000). The majority of the calcium (99%) is at bone in form of hydroxyapatite (Brown, 1994). The balance between calcium and phosphorus is important for the bone tissue formation, because the deposition of these minerals in bone can increase its mass (Pizauro Junior, 2002). The phosphorus deposition follows the same tendency of the calcium deposition, characterizing the dependence between these minerals that can form insoluble salts (Dell'Isola *et al*., 2003). This tendency was observed in this present experiment, when phosphorus percentage presented the same behavior of the calcium percentage.

Field (2000) described that although the bone ash percentage varies with age, the calcium percentage presents constant values. This author also affirmed that the calcium quantity suffers low variation among the different species and in the different anatomic location. Barbosa (2005) did not observe tendency to variation in calcium percentage from femur ash along the ages. The variation in calcium percentage observed in this present experiment is in disagreement with some authors who affirm that the values have the tendency to remain constant along the ages. However, the variation observed from 8 to 22 days of age can be due to the more intensive bone remodelation and consequent higher mineral requirement of femur in response to the faster broiler growth in this short period. Barreiro *et al.* (2009) determined the biochemical values of broiler tibias at different ages and concluded that the growth phase demanded a higher mineral exigency leading to increased calcium and phosphorus values in ash and ash percentage at 22 days of age. The same tendency between calcium and phosphorus observed in this experiment demonstrates the physiologic balance of the mineral deposition at femur.

In conclusion, biomechanical adaptation capacity of the femur was evidenced by the increase of macroscopic measures over the course of the ages (8, 22 and 42 days of age). The different behavior of cranial compact layer at 22 days of age suggests an adaptation attempt of the immature bone to muscle mass increase. The ash and mineral percentage increased until 22 days of age, because the relative growth occurs with more intensity until 21 days of age, moving more minerals to bone tissue. Calcium and phosphorus presented the same behavior in all ages, demonstrating the physiologic balance acting on the deposition of these minerals in femur.

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