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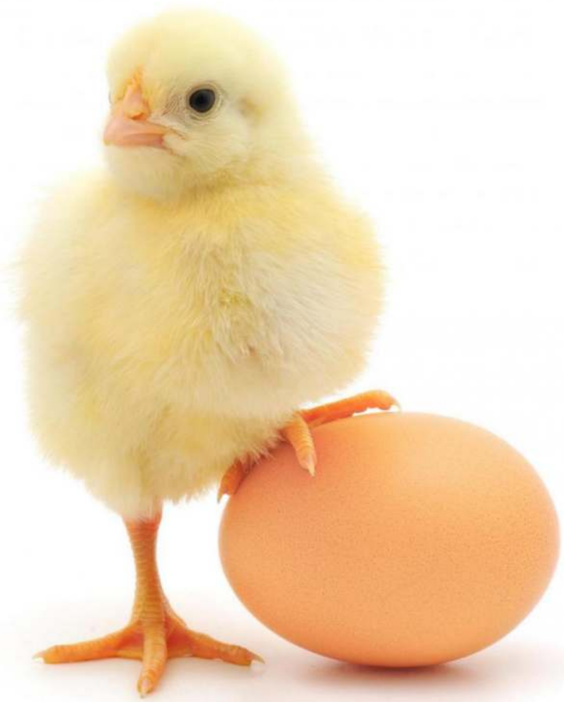

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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Physiologic Values of Broiler Femurs at Different Growth Phases Using Bone Densitometry and Bone Breaking Strength[1]

F.R. Barreiro[2], L.A. Amaral[2], A.C. Shimano[3], J.C.R. Alva[4], J.C. Barbosa[5] and S.M. Baraldi-Artoni[4]
[2]Department of Preventive Veterinary Medicine,
Universidade Estadual Paulista "Julio de Mesquita Filho", Jaboticabal, Brazil, 14870-000
[3]Department of Biomechanic, Medicine and Rehabilitation of Locomotor System,
Universidade de Sao Paulo, Ribeirao Preto, Brazil, 14049-900
[4]Department of Animal Morphology and Physiology,
Universidade Estadual Paulista "Julio de Mesquita Filho", Jaboticabal, Brazil, 14870-000
[5]Department of Statistical Analysis, Universidade Estadual Paulista "Julio de Mesquita Filho",
Jaboticabal, Brazil, 14870-000

Abstract: The objective of this experiment was to determine the normal values of Bone Radiographic Density (BRD) by using the optical densitometry in radiographic images and the Bone Breaking Strength (BBS) of broiler femurs at different ages (8, 22 and 42 d of age). A total of 60 Cobb male broilers were distributed in three age groups of 20 birds. The BRD and the BBS (maxim force and rigidity) values increased ($p<0.01$) over the course of ages, presenting greater values at 42 d of age when comparing to 8 and 22 d of age, evidencing a biomechanical adaptation of femur to growth. This experiment offers results that can be used in other experiments of broilers fed with different nutritional levels and they can also be related to pathological values, allowing the diagnosis of diseases that affect the integrity of the poultry leg.

Key words: Bone breaking strength, bone radiographic density, broiler, femur, growth, physiology

INTRODUCTION

Technologic advancement and utilization of genetic improvement linked to nutrition and handling (Mendonca Junior, 2000) provide the necessary conditions to increase the broiler growth rate in short time. However, these programs of genetic improvement, that bring many benefits to poultry production, have caused damage related to rapid growth (Oliveira, 2006). The lack of standard of the techniques used to diagnose leg disorders in poultries prejudices the right estimative of losses (Tardin, 1995). The femoral degeneration is a consequence of alterations at the growth plate that causes bone mass decrease, affecting until 100 % of the broilers during the grower phase (Cook, 2000). Almeida Paz *et al.* (2007) tested the capacity of the bone densitometry in detecting the bone mass decrease caused by femoral degeneration and concluded that this technique can be used to diagnose this pathology without slaughtering the broilers. Bone densitometry is efficient in the accompaniment of diseases that affect the bone tissue because the injured region presents a higher Bone Radiographic Density (BRD) value during the new bone tissue deposition (Pharr and Bargai, 1997).

The bone resistance is dependent of material, physical and architectural properties of the bones (Rath *et al.*, 2000), as well as other factors such as growth, breed, age and nutrition (Kocabagli, 2001). Bone Breaking Strength (BBS) tests are used to determine the quantity of maxim force that bone is able to support until the rupture (Sa *et al.*, 2004).

The objective of this experiment was to determine the normal values of BRD by using the optical densitometry in radiographic images and the BBS of broiler femurs at different ages (8, 22 and 42 d of age). This experiment offers results that can be used in other experiments of broilers fed with different nutritional levels and they can also be related to pathological values, allowing the diagnosis of diseases that affect the integrity of the poultry leg.

MATERIALS AND METHODS

A total of 60 Cobb male broilers were distributed in three groups of 20 birds. The birds were housed from 1 d of age and the first group was bred for 8 d, the second for 22 d and the third for 42 d. The handling was the same as that used in the commercial breed of broilers. The stocking density was 15 birds/m^2 in the pens and rice husk litter was used. The light program during the experiment was 24 h of illumination for all phases. The birds were vaccinated at 8 d of age against Gumboro disease, at 13 d of age against Newcastle disease and at 23 d of age against Gumboro disease (reinforcement). Water and ration were provided *ad*

Corresponding Author: F.R. Barreiro, Ayrton Senna Avenue, 333, Apartment 403 B, Santa Luzia district, Jaboticabal, SP, Brazil, 14870-000

libitum. The rations were of commercial brand Purina do Brasil (Inicina and Nutriengorda, Paulínea, Brazil) and were divided in two types, according to the broiler phase. The starter ration (Inicina) was used until 21 d of age and the grower ration (Nutriengorda) from 22 d to 42 d 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 Ciencias Agrarias e Veterinarias, Universidade Estadual Paulista (FCAV/UNESP) (specific authorization number: 020857-07). The right femurs were obtained after the removal of skin, muscles, ligaments, vessels and epiphysary cartilage.

Densitometric analysis: The densitometric evaluations were realized from radiographic images, using the bones collected at 8, 22 and 42 d of age. The technique used in radiographic taken was developed by Louzada (1994). An aluminum staircase composed of 12 steps was used (metallic alloy 6063, in accordance with the Brazilian Association of Technique Norms). The first step measured 0.5 mm in height, increasing 0.5 mm with each step until the tenth. The 11th step measured 6.0 mm, the 12th step measured 0.8 mm and the step area was 5 x 25 mm^2. The images from femurs and aluminum staircase were obtained together, in the same radiography. The x-ray equipment (Tridoro 812E,116 Siemens, Sao Paulo, Brazil) of the radiology department of veterinary hospital at Faculdade de Ciencias Agrarias e Veterinarias, Universidade Estadual Paulista, (Jaboticabal, Sao Paulo, Brazil) was adjusted for 8, 22 and 42 d of age. A focus-to-film distance of 1 m was used for all radiographic images taken. Kodak films (T-MATG/RA, 120 Kodak, Manaus, Brazil) were used inside the cassettes and measured 24 x 30 cm. The revelation and fixation of the films were made by automatic process, using Kodak equipment (X-OMAT 2000, Kodak). The radiographic images were scanned by a Scanion scanner (Express A3 USB, Ion Industria, Sao Paulo, Brazil) and these images were submitted to the computer program Image-Pro Plus (version 4.1, Media Cybernetics, Silver Spring, MD); the density calibration was made, having the aluminum staircase image as reference. Subsequently, the BRD from right femur was measured at the different regions (proximal epiphysis, diaphysis and distal epiphysis) and at the different ages. The densitometric values were expressed in aluminum millimeters (Al mm).

Bone breaking strength analysis: The BBS determinations were realized with the right femurs collected at 8, 22 and 42 d of age. The bones were submitted to an Essay Universal Machine-EMIC (Equipamentos e Sistemas de Ensaios, Sao Jose dos Pinhais, Brazil) and the maxim force (Newton) and rigidity (Newton/mm) were measured. Supports were positioned at bone epiphysis and a force was applied in central region, always in the same point. The distance between supports was 25 mm for bones of broilers at 8 d of age, 35 mm at 22 d of age and 55 mm for 42 d of age. A cargo cell with capacity of 1000 N was used and the pre-cargo was of 5 N. The movement velocity used was 1 mm/s for all ages. A software registered all the test results.

Statistical analysis: The broilers were distributed in a completely randomized design, with 3 treatments (ages) and 20 repetitions (20 birds) for each treatment. Single factor ANOVA was the statistical method used. Pair-wise comparisons of means were made using Tukey's test procedure. A $p<0.01$ was considered significant. All statistical results were obtained from Agroestat (Department of Statistical Analysis FCAV/UNESP, Jaboticabal, Brazil).

RESULTS AND DISCUSSION

Bone densitometry in radiographic images was used in this experiment because it has easy access, low cost, reliable results and this technique was used with success in other experiments (Onyango *et al*., 2003; Schreiweis *et al*., 2003; Fleming *et al*., 2004; Hester *et al*., 2004; Schreiweis *et al*., 2004; Fleming, 2008; Barreiro *et al*., 2009) to determine the BRD in poultries, but all the cited articles studied tibias and humerus, or only tibias.

Bone densitometry is efficient in the accompaniment of factors that affect the bone tissue because the injured region presents a higher Bone Radiographic Density (BRD) value during the new bone tissue deposition. This increase in BRD values is due to mineralization of dead osteocytes (Cruess and Dumont, 1985; Pharr and Bargai, 1997). The technique of optical densitometry in radiographic images can detect values from 12% of calcium loss in bones (Louzada, 1994).

The Table 1 shows the BRD values of the present experiment. The BRD values increased significantly during the growth, presenting greater values at 42 d of age, when comparing to 8 and 22 d of age. Macari *et al*. (1994) described that the nutrient flow to bone tissue occurs until 21 d of age, however in this experiment the BRD values increased until 42 d of age. Almeida Paz *et al*. (2008) analyzed some densitometric characteristics of broilers with femoral degeneration and observed that broilers with the lower lesion score (score 1) presented BRD values between 3.57 and 4.33 Al mm and broilers with the greater lesion score (score 5) presented values between 1.5 and 2.21 Al mm, at 42 d of age. Oliveira (2006) evaluated the effects of the population density and the genetic group on BRD values of broilers and found values between 0.75 and 0.85 Al mm at 7 d of age; 1.87 and 2.55 Al mm at 21 d of age and 1.85 and 2.75 Al mm at 42 d of age.

Table 1: Values (mean±SD) of bone radiographic density (aluminum millimeters) from proximal epiphysis, diaphysis and distal epiphysis of broiler femurs at 8, 22 and 42 d of age

	Ages				
BRD[1]	8	22	42	F	CV
Proximal epiphysis	1.39±0.23[c]	2.31±0.27[b]	3.29±0.21[a]	302.21	10.46
Diaphysis	1.27±0.18[c]	2.39±0.22[b]	2.82±0.26[a]	245.17	10.58
Distal epiphysis	1.25±0.16[c]	1.96±0.19[b]	2.81±0.25[a]	275.04	10.43

[a-c]Within a row, means without a common superscript differ ($p<0.01$).

[1]Bone radiographic density

Table 2: Values (mean±SD) of bone breaking strength represented by maxim force (Newton) and rigidity (Newton/mm) of broiler femurs at 8, 22 and 42 d of age

	Ages				
BBS[1]	8	22	42	F	CV
Maxim force	23.95±3.30[c]	156.90±20.09[b]	233.90±33.59[a]	281.49	20.31
Rigidity	18.01±3.51[c]	89.79±14.44[b]	109.10±17.54[a]	261.39	18.36

[a-c]Within a row, means without a common superscript differ ($p<0.01$).

[1]Bone breaking strength

Bone resistance is dependent of many factors such as growth, strain, age and nutrition (Kocabagli, 2001). A change in shape, size or orientation of collagen fibers can modify the bone resistance. Bone breaking strength tests are used to express the quantity of force that the bone has capacity to support (Sa *et al*., 2004). The knowledge of the normal values of BBS is important because this values can help the researchers to understand the answer of the bone to different stimuli. Calcium and phosphorus are important inorganic nutrients to maintain the bone resistance (Kocabagli, 2001) and the deficient mineralization can increase the occurrence of fractures during the processing at the slaughterhouse, resulting in condemnation of broiler carcasses. In addition to this situation, broilers with weak legs have worse performance because they have reduced feed intake (Brenes *et al*., 2003; Onyango *et al*., 2003). The capacity of skeleton adaptation to high growth rates stimulates fast alterations in bone dimensions (length and diameter); however these adaptations can result in high fracture incidence and low mechanical resistance (Currey, 1999). Bone resistance can be evaluated using biomechanical properties obtained in engineering tests, such as traction, compression and flexion. Cylindrical bones, as the femurs used in this experiment, are evaluated using flexion tests. This type of test uses 2 supports where the bone is incased in a determined distance and is applied a force in the middle point of the bone. The maxim force is result of the material geometry and composition. The determination of the intrinsic resistance considers the maxim force supported by a material and its geometry (Turner and Burr, 1993).

Currey (2003) demonstrated that bone rigidity is dependent of mineral quantity and that the increase of bone rigidity results in a more fragile bone. This author suggested that the mineral content is the main determinant of the differences in the mechanical properties of the bones.

The BBS values of this present experiment are given in Table 2. The maxim force and rigidity increased with ages, evidencing a biomechanical adaptation of femur to growth. Barbosa (2005) evaluated the femur BBS of different broiler breeds at 21 and 42 d of age and found the following values of maxim force: 210.10 Newton, 189.92 Newton, 207.23 Newton at 21 d of age and 209.21 Newton, 247.25 Newton and 282.05 Newton at 42 d of age. Almeida Paz *et al*. (2008) verified that BBS values increased in the evaluated ages (28, 35, 42 and 49 d). Bruno (2002) analyzed long bones of broilers (femur, tibia and umerus) and concluded that BBS increased with ages, in all evaluated bones.

In conclusion, the physiologic values of broiler femurs at different ages using the optical densitometry in radiographic images and the bone breaking strength were determined and can be used as base for experiments involving femur pathologies and factors that can modify the bone, such as nutrition and handling. The BRD, maxim force and rigidity increased over the course of ages, evidencing a biomechanical adaptation to the growth that was detected by bone densitometry and bone breaking strength.

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