

# Bone mineral density of the tibia and femur of broiler breeders

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The aim of this study was to follow-up the physiological variations in the development of the bone tissue, associating them with the egg production curve. This study was carried out in the facilities of the Faculdade de Medicina Veterinária e Zootecnia of the UNESP, Botucatu, Brazil. Twenty-three families of Ross broiler breeders were used, each family consisting of 13 females and 1 male, distributed in 23 pens of 5.0m<sup>2</sup> each. The management was that recommended by the genetic company manual (Agroceres Ross, 2003), with daily feeding until 6<sup>th</sup> week of age; and birds were fed according to a 5:2 schedule (5 days fed, 2 days of fasting) between 7 and 17 weeks of age, returning to daily feeding starting at 18 weeks of age. The birds did not receive afternoon calcium supplementation. On the fourth week of rearing, 84 females were removed for bone analyses of the right tibia and femur, using optical densitometry in radiographic images technique. These analyses were sequentially carried out in 4, 8, 12, 15, 20, 24, 30, 35, 42, 47, and 52 week-old birds. The egg production curve of the birds was followed-up and associated to bone mineral density results. For bone mineral density evaluation (BMD) birds were divided by weight categories as light, intermediate, or heavy within each data age. BMD values of the tibias were not influenced by weight range, but by the age at collection. On the other hand, interactions were found among femur BMD values and weight and age categories. There was no correlation between eggshell quality and femur BMD. A negative correlation (-0.15) was observed between tibia BMD and eggshell percentage. It was possible to conclude that the egg production has little influence on bone mineral density of the birds probably because there was no need of bone mineral mobilization during the production period, since the observed egg production was similar to that observed under commercial conditions.

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**Key words:** bone; bone density; broiler breeders; egg production; shell quality.

## Introduction

Ossification is a process in which a complex sequence of time and space-related event lead to bone formation. There are two processes of bone development: intramembranous and endochondral ossification. Cell mechanisms of bone formation are identical in both processes: intramembranous ossification is responsible for the ultimate shape of a limited number of bones, which are not pre-formed by cartilage. Endochondral ossification includes the activities responsible for the formation of bones that support weight, and also for the elongation of most of the skeletal mass during growth. The continuous addition of cartilage and its later substitution by bone are the essence of the elongation process (Almeida Paz *et al.*, 2005).

Bone tissue growth and development are linked to global body growth. Skeletal diseases can be triggered when other tissues growth rate increases, particularly the muscle tissues, with no parallel increase in bone tissue growth rate. Some authors, such as Kestin *et al.* (1999) e Rath *et al.* (1999),

observed that management and selection practices emphasizing growth impair poultry bone maturation, strength, and quality, which may cause caged layer disease tiredness, osteoporosis, degenerative bone diseases, and lameness in broiler breeders and broilers. These problems may result in lower egg production, worse egg shell quality, low performance, and high mortality (Leeson & Summers, 2000; Vargas Jr. *et al.*, 2002).

Broiler breeder rearing can be divided into several stages, starting with the embryo development, hatching, rearing, development, and production. Between 22 and 70 days of age, skeletal growth rate is very fast, and 85% of mature size is reached at 56 days of age and at approximately 12 weeks of age, 95% of bone growth potential is achieved. In later stages, besides body growth, there is a concomitant growth of the ovaries. The bone system will still be highly influenced by sexual hormones by the endocrine system and from fast weight gain. At 30 weeks of age, birds reach physiological maturity, and growth is halted. However, the peak of egg production starts, increasing mineral requirements. Egg mass production peaks at 34 weeks of age, and egg production decreases thereafter. As egg production decreases, egg shell quality also decreases (Qin & Klandorf, 1991; Leeson & Summers, 2000; Luquetti *et al.*, 2002). The bone system is then used to supply body mineral requirements, in order to ensure a balance between calcium and phosphorus absorption and requirement.

The egg reaches the uterus in approximately six hours after ovulation, and remains in this segment for 18 to 20 hours. Calcification occurs very slowly. Calcium deposition rate for shell formation reaches a plateau 14 hours after egg formation, and decreases during the last two hours. Egg shell consists of calcium carbonate (Austin & Nesheim, 1990), which passes from the blood to the shell gland, and precipitates as calcium organic salts. Approximately 2 to 35 of the calcified layers are composed by an organic matrix, consisting mainly of protein. Genetic improvement associated to management allows an efficient laying cycle of broiler breeders.

Bone tissue strength results from calcium and phosphorus deposition in the form of hydroxyapatite during the process of bone mineralization. These two minerals account for about 70% of bone composition; the remaining 30% consists of organic matter, particularly collagen (Kälebo & Strid, 1988; Field, 1999).

Hydroxyapatite and aluminum have similar densities. Many authors carried out studies aiming at related bone mineralization degree and aluminum density, and concluded that, using radiology, it is possible to compare the amount of calcium and phosphorus deposited in the bone with the amount of aluminum found using a pre-defined scale (Loubel & Dubois, 1973; Kälebo & Strid, 1988).

Some diseases, such as osteoporosis, osteopenia, and osteochondrosis, were studied using bone densitometry measurements in humans and animals (Bourrin *et al.*, 2002; Huang *et al.*, 2002), but few of these studies were carried out in poultry. Louzada (1997) conducted a study using broilers as a model for methodological standardization and clinical application of optical densitometry in radiographic images, comparing aluminum values expressed in mm with calcium percentages in bone specimens. Almeida Paz *et al.* (2004) studied tibial dyschondroplasia in broilers using the same technique, and found correlation between densitometry values and the incidence of this disease.

## Materials and Methods

This study used 280 Ross 308 females and 40 Ross 308 males. Birds were housed in the experimental facilities of Faculdade de Medicina Veterinária e Zootecnia of UNESP-Botucatu, at an average density of 2.6 birds/m<sup>2</sup>. All birds were debeaked at 10 days of age using a standard debeaker. There was no need of corrective debeaking between 10 to 15 weeks of age as no beak deformities were observed.

Feed was formulated based on the nutritional level recommended by the genetic company manual (Agroceres Ross, 2003), and according to the rearing stages, which were divided in 6: grower (0 to 5 weeks), developer I (6 to 13 weeks), developer II (14 to 19 weeks), pre-lay (20 to 24 weeks), lay start (25 to 45 weeks), lay end (46 to 52 weeks),

Whereas cockerels were offered a specially formulated feed. Feed was offered *ad libitum* on the first week, and controlled thereafter. Birds were fed daily from 1 to 6 weeks, and, from week 7 to

week 17, a 5:2 feeding regime was adopted (5 days feeding, and 2 days fasting) at the beginning of the 7<sup>th</sup> week. Feed was again daily offered daily starting on week 18. No afternoon supplementation of calcium was offered.

Data were collected 11 times for optical density in radiographic images in broiler breeders' tibia and femur, on weeks 4, 8, 12, 15, 20, 24, 30, 35, 42, 47, and 52, respecting critical rearing and egg production ages.

On the fourth rearing week, 84 females were taken at random for sample collection. These birds were individually weighed in a 2-g semi-analytical scale. This group was transported to the Veterinary Hospital of FMVZ, where they were submitted to x-ray examination using a portable x-ray apparatus, which was calibrated and applied a focus-film distance of 36 cm. The 47kVp X 2mAs used radiographic technique was applied to the samples collected on weeks 4, 8, and 12 weeks, and this technique was later changed to 47kVp X 4mAs for samples collected on weeks 15, 20, 24, 30, 35, 42, 47, and 52.

All radiographic films were of the same brand, with green background and 18x24 cm frames, equipped with rare earth metal frames. A phantom aluminum scale was placed in the central area of the frame, in parallel and 3.0 cm distant from the studied region. This scale was used as densitometric reference. The phantom consists of 20 degrees, with the first degree being 0.5 mm thick, followed by 0.5 mm variations up to the 20<sup>th</sup> degree. Each degree presented an area of 15 x 15 mm.

Routine radiological procedures were used, and the processes of revelation and fixation were performed in a standard automatic processor. The region standardized for reading was the right tibia proximal epiphysis and the right femur distal epiphysis. Bone mineral density was read using the software CROMOX® ATHENA 3.1. Radiographs were scanned, and the images were analyzed using a 10-mm high and a 35-55-mm wide opening. The reading axis of the bone density of the tibia was 0°, whereas the reading axis of the femur followed the inclination of the bone diaphysis, which can vary between -27° and 32°, based on a 0° inclination horizontal axis. Birds were submitted to fasting until the end of each radiograph collections.

In order to follow up the production curve and to associate it to bone development, five daily egg collections were carried out. Eggs were submitted to specific weight and eggshell percentage analysis using a method adapted from Castelló *et al.* (1989).

In order to evaluate tibia and femur bone mineral density, bird weight class and age were considered as treatments in a completely randomized experimental design, with 33 treatments (3 weight classes and 11 ages). Statistical analysis was performed using ANOVA of the statistical package SAEG (1998). The correlations between these characteristics and bone mineral density measures were calculated using the test of Pearson at 5% significance level.

## Results and Discussion

The body weight data shows that females were about 10% lighter as compared to the weight recommended by the genetic line manual (Agroceres Ross, 2003), and therefore, a growth curve was specially calculated for this flock. On the other hand, males were heavier than the manual recommendations. It is important to stress here that the egg production of the experimental birds cannot be compared to that of a commercial flock, as these breeders were submitted to the stress of radiograph collections. The egg production of these birds was much lower than the estimated egg production of the genetic line manual, in addition to lay starting date, which was on week 27 and not on week 25. egg production peak was also lower (71.00% instead of the estimated 84.5%).

In order to evaluate bone mineral density, breeders were divided into three weight classes: Light (1), intermediate (2), and heavy (3) within each collection age. The results are presented in Table 1. There was an interaction ( $p < 0.05$ ) between live weight and collection age: weight increased according to age within each weight class.

**Table 1.** Live weight (g) and bone mineral density values (millimeters of aluminum) of the tibia and the femur of experimental broiler breeders divided per collection age and weight class.

Weeks	Live weight (g)				Tibia BMD (mm Al)				Femur BMD (mm Al)			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
4	650Cg	755Bh	886Ah	764	1.28	1.37	1.48	1.37e	1.10Ad	1.35Ae	1.59Af	1.34
8	902Cf	1066Bh	1289Ag	1086	2.00	2.31	2.29	2.20cde	2.30Acd	2.52Acde	2.84Aef	2.55
12	1184Ce	1438Bg	1721Aef	1448	2.54	2.46	2.28	2.42cde	3.03Abcd	3.19Ade	3.61Aef	3.28
15	1319dCe	1573Bf	1864Ae	1585	1.89	1.82	1.91	1.88de	2.36Acd	1.90Ae	2.03Af	2.10
20	1512Cd	1847Be	2270Ad	1876	2.13	2.25	1.99	2.12cde	3.07Abcd	3.55Ade	3.35Adef	3.32
24	2182Cc	2640Bd	3173Ac	2665	2.05	4.46	2.09	2.87bcde	3.33Abcd	3.79Ade	4.32Acde	3.81
30	2661Cb	3184Bc	3663bAc	3169	2.19	2.98	3.66	2.94bcde	4.98Cbcd	7.73Babc	10.74Aab	7.81
35	2913Ca	3412Bb	3880Aabc	3402	3.23	3.60	6.70	4.51ab	10.70Bab	9.83Bab	16.94Aab	12.49
42	3083Ca	3412Bb	3880Aabc	3500	3.10	3.67	3.88	3.55abc	6.69Bbcd	6.92Babc	20.02Aa	11.21
47	3110Ca	3562Bab	4024Aabc	3565	3.76	3.78	4.06	3.86abc	5.67Abcd	6.03Abc	6.63Ac	6.11
52	3083Ca	3571Bab	4157Aabc	3603	3.96	3.50	7.63	5.03a	6.95Babc	9.36Bab	12.94Aab	9.75
Mean	2055	2417	2801		2.56	2.93	3.43		4.56	5.10	7.70	

Means followed by different capital letters (row) and different small letters (column) are different according to the Test of Tukey ( $p < 0.05$ ) for each evaluated parameter. 1 = light birds; 2 = intermediate birds; 3 = heavy birds.

Bone mineral density (BMD) of the tibia was not affected by weight class, only by collection age. The lowest values were found at 4 weeks of age, but these were not statistically different from those found up to 30 weeks of age, when the breeders started to produce eggs (19.98%). Tibia BMD between 35 and 52 weeks of age were not significantly different, despite a trend for higher values between 35 and 52 weeks of age. Peak of egg production started at 35 weeks of age (60.74%), which may indicate an increase in calcium deposition in the tibia to supply the requirement for laying. On 52 weeks of age, production was 43.31%, possibly indicating that breeders were depositing calcium in the tibia because production was declining.

BMD values found for broiler breeders are high as compared to broiler values. Tibia BMD values found by Almeida Paz *et al.* (2004) for broilers were between 1.46 and 1.77 mm Al. When studying tibia BMD of 53-day-old broilers, Louzada (1997) found values between 1.77 and 1.96 mm Al. These values are consistent to those found in 4 to 15-week-old female breeders.

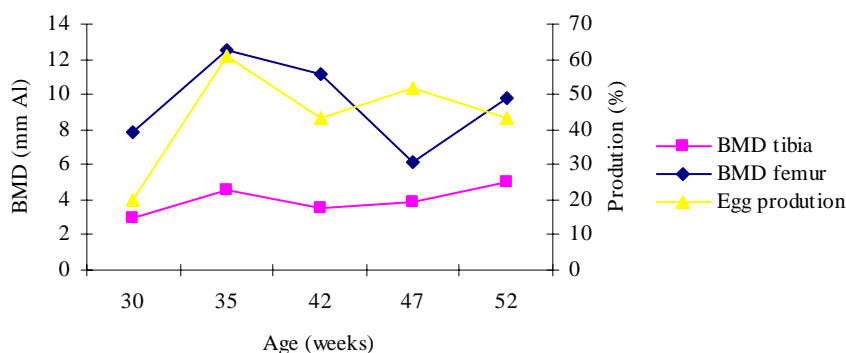
Weight class influenced only femur bone mineral density (BMD), with an interaction ( $p < 0.05$ ) between collection age, live weight, and BMD. Weight increased as age increased, but live weight did no influence BMD until 24 weeks of age (pre-laying period). After 30 weeks of age, body weight affected BMD, with higher BMD values observed with higher weights. There was an exception at 47 weeks of age, when weight class had no effect on BMD values – at this age, there was a sudden increase in egg production (from 42.55% in the previous week to 51.94% during this week), when a decrease in BMD level was also observed. According to Julian (2005), layer femur is the main bone responsible for calcium supply for eggshell formation when dietary calcium is not available. According to that author, the femur of calcium-deficient birds is fragile and porous.

As previously shown, female weight in the present study was lower than the average weight recommended by the genetic company. Taking into consideration that only the collection at 30 weeks of age representing the laying period, with a 19.98% production rate, and that the light-weight breeders were not producing eggs, it is possible that the increase in femur BMD values at this age and weight class are due to a higher calcium mobilization for eggshell formation (Wilson, 1991). This may be an indication of a possible reduction in BMD values on week 47, when production increased 9.34% in a single week.

Eggshell percentage had a similar behavior as that observed for egg weight, with a gradual increase in these values as a function of collection age. Similar results were found by Luquetti *et al.* (2002), when studying the effect of breeder age on eggshell quality. In the study of Pedroso *et al.*

(2005), an increase in egg weight was also found. The specific egg weight presented the highest values in the first and in the last collection (30 and 52 weeks), whereas weeks 35 and 42 presented the lowest values, and collection on week 47 was not different from the other weeks.

Graph 1 shows the egg production curve and the tibia and femur bone mineral density curves of the experimental birds. Tibia BMD curve follows the egg production curve, whereas the femur BMD curve decreases on week 47, when egg production increased.



**Graph 1.** Bone mineral density and egg production of broiler breeders.

Table 2 shows the correlations between egg external quality traits and bone mineral density. Significant correlations were observed as to eggshell quality, with the highest value for the correlation between specific egg weight and eggshell percentage, as expected, because specific egg weight is influenced by eggshell weight (Castelló *et al.*, 1989).

**Table 2.** Pearson correlations ( $P < 0.05$ ) between eggshell quality and bone mineral density of breeders.

	Egg weight	Specific weight	Eggshell (%)	Tibia BMD	Femur BMD
Egg weight	1,00				
Specific gravity	0,27	1,00			
Eggshell (%)	-0,45	0,69	1,00		
Tibia BMD	-*	-	-0,15	1,00	
Femur BMD	-	-	-	0,67	1,00

\* Non-significant correlations are expressed as “-“.

According to Julian (2005), the use of bone calcium for eggshell formation occurs when the available dietary calcium does not supply the requirements. When there is no available dietary calcium, the bird removes calcium from the medullar bones, which can cause bird's death. The feeds used in the present study followed the recommendations of the genetic line manual, with no afternoon calcium supplementation. The results showed that birds do not need to remove bone calcium for eggshell formation, as tibia and femur bone mineral density presented low or no correlation with eggshell quality.

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