

Bone mineral density and bone quality characteristics of broiler breeders

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This study was carried out at the facilities of the Faculdade de Medicina Veterinária e Zootecnia of UNESP, Botucatu, Brazil, with the aim of evaluating bone quality of broiler breeders. Twenty-three Ross broiler breeder families were used. In the beginning of the study each family consisted of 13 females and 1 male distributed in 23 pens of 5.0m² each, and at the end of the study the average number of females per pen was of 9.34. The management used was that recommended by the genetic company (Agroceres Ross, 2003). On the fourth week of rearing, 84 females removed for bone analyses of the right tibia and femur, using the technique of optical densitometry in radiographic images. These analyses were sequentially carried out in 4, 8, 12, 15, 20, 24, 30, 35, 42, 47, and 52 week-old birds. After each radiographic collection, five birds were slaughtered, and the removed tibiae and femura were submitted to analyses to determine fat-free dry matter, bone strength, and Seedor index (Seedor, 1993). Eggs of the studied birds were collected with aid of nest traps in the same week of radiographic collections. Therefore, it was possible to establish correlations between bone quality and eggshell quality of these birds. There were correlations among bone quality characteristics, but no correlations were between bone quality and external egg quality. Hence, it was concluded there was no effect of egg production on bone quality, probably because there was no need to mobilize the studied bone minerals.

Key Words: bone density, broiler breeder, optical densitometry, Seedor index, shell quality.

Introduction

Breeder broiler calcium requirement is very high, particularly during the period of active eggshell formation. Calcium used for eggshell formation derives directly from the duodenum and jejunum, and indirectly from medullar bone through a bone resorption process (Kienholz *et al.*, 1961; Landauer, 1967; Wilson *et al.*, 1980; Wilson, 1983; Luquetti *et al.*, 2002; Julian, 2005). The proportion of calcium derived from these two sources varies according to the period of the day. During the night, when dietary calcium sources are not available, birds mobilize calcium from the bones, whereas during the day, most calcium comes from the diet. It is known that the higher the contribution of bone calcium for eggshell formation, the worst is eggshell quality.

Medullar bone undergoes through periods of bone deposition and bone resorption. Bone resorption can be minimized when larger particles are fed, such as oyster meal, because these are digested more slowly during the night, extending calcium supply directly from the gastrointestinal tract. According to Julian (2005), the use of calcium in large particles (larger than 0.75mm) is good to maintain eggshell quality, as these particles are stored in the gizzards, ensuring the presence of calcium in the digestive tract during eggshell formation.

However, the supplementation of calcium during the afternoon is controversial. Some authors carried out studies using different particle sizes, forms, and times of calcium particle availability. In some cases, no improvement in eggshell specific weight and resistance, or in hatchability and chick

viability were observed (Robinson *et al.*, 1993; Reis *et al.*, 1995; Zhang & Coon, 1997; Maggioni, 1998).

Phosphorus also has an important role in the normal bone system development of broiler breeders and of embryos, as well as in egg hatchability (Harms *et al.*, 1964; Julian, 2005). Singesen *et al.* (1962) suggested that 0.4% available phosphorus is required for maximum hatchability of birds housed in cages. Later, Wilson *et al.* (1980) determined that broiler breeders required 0.31% phosphorus for normal hatchability. Current recommendations are of 0.35 - 0.45% available phosphorus, depending on the rearing stage of broiler breeders (Agrocere Ross, 2003). Excessive phosphorus consumption, resulting from a combination of dietary phosphorus and other sources, may cause poor eggshell quality, and indirectly worse hatchability (Wilson *et al.*, 1980).

Eggshell quality can be defined by specific weight and its relationship with shell porosity, as there is a positive correlation between eggshell quality and eggshell thickness, and a negative correlation with the concentration of pores (Peebles & Brake, 1987). Therefore, specific weight is an easy method to assess eggshell thickness, and it is widely used to determine its relation with hatchability in broiler breeders. Eggs of old breeders presenting good eggshell quality have the same hatchability as eggs from young breeders (North & Bell, 1990).

As the laying period progresses, egg weight increases, eggshell becomes thinner, and internal quality worsens. There is also a high incidence of eggs with no shell. At a determined moment, the hen stops laying eggs. Eggshell quality decreases as egg production is reduced. These changes are associated with an increase in egg size and with a lower intestinal absorption of minerals by the birds (Qin & Klandorf, 1991; Leeson & Summers, 2000; Luquetti *et al.*, 2002). Therefore, the bone system is used to supply the mineral needs of the body, ensuring the balance between calcium and phosphorus absorption and requirements.

Bone tissue resistance results from calcium and phosphorus deposition as hydroxyapatite during the process of bone mineralization (Kålebo & Strid, 1988a; Field, 1999; Bruno, 2002).

This study aimed at producing data on bone quality and eggshell quality during rearing and production periods of broiler breeders in order to assess the possibility of associating bone quality with eggshell quality. Modern low-cost techniques, such as optical densitometry in radiographic images, ash content, dry matter, bone strength, and Sedor index, were used.

Materials and Methods

A total number of 280 Ross 308 broiler breeder females, and 40 males were used. Birds were housed in the experimental facilities of FMVZ – UNESP/Botucatu, in a masonry house divided in 24 pens of 5m² each, with an average density of 2.6 birds/m². The house was darkened with a cover with a light retention capacity of 80%, and special dark curtains (black-out curtains). Starting on the 18th week of rearing, birds were submitted to natural light and additional artificial light to provide 17 hours of light per day, and the cover was removed.

Up to 3 weeks of age, birds were brooded using infrared brooding lights, and chick feeders were used. After this age, brooders were removed, and birds were fed using bell feeders and automatic trough feeders, with 15 cm feeder space per bird. At 10 days of age, all birds were debeaked using a standard debeaker.

Birds were fed and had their weight controlled according to the genetic manual guidelines (Agrocere Ross, 2003). Feed was offered daily from week 1 to 6, and from weeks 18 to 52. Feeding followed a 5:2 (5 days with feed and 2 days fasting) during the period between 7 and 17 weeks of age. Afternoon calcium supplementation was not provided.

During grower and developer periods, males were kept in pens separate from females. On week 18, males were divided in 24 pens, with a density of 2.8 birds/m², with a total number of 14 birds per pen. Twenty two pens housed males and females, whereas 2 pens housed replacement males.

During the production period, nest traps were used, with a nest opening for 4.3 females. The collection of eggs used for eggshell quality analysis (egg weight, eggshell percentage, and specific weight) was carried out through nest traps, which allowed the identification of the hens and eggs. Nest traps were placed two days before and two days after collection of eggs for radiographic image.

Feed nutritional levels and rearing stages were determined according the genetic manual recommendations (Agroceres Ross, 2003). Limestone particle size was the same as that used for broiler (smaller than 0.75 mm).

Bone mineral analysis was carried out using optical densitometry in radiographic images, and values were expressed in aluminum millimeters. Analyses started when birds completed 4 weeks of age, and were performed up to 52 weeks of age (4, 8, 12, 15, 20, 24, 30, 35, 42, 47, and 52 weeks of age). A number of 84 birds was chosen at random to compose the studied group. Birds were 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 63 cm. 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 20th degree. Each degree presented an area of 15 x 5 mm. 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 4m for the remaining samples.

After radiographic examination, five birds were randomly selected to be sacrificed, after which their legs were removed for bone strength, bone mineral composition, and Seedor index analysis. The removed legs were dissected, and the bone duly identified according to bird number, right or left leg, and collection number, after which they were frozen in a commercial freezer. Therefore, the first radiographic collection, at 4 weeks of age, used 84 birds; the second collection used 79 birds; and the third used 74, until the 11th collection, at 52 weeks of age, when there were 34 birds.

Optical density readings in radiographic images (bone mineral density) were carried out using the software CROMOX[®] ATHENA 3.1, and all readings followed the same pattern. Radiographs were scanned, and the images were analyzed using a 10-mm high and 35-45 mm wide opening. A 0° inclination axis was used for tibia readings, whereas femur bone density readings, the reading axis followed the inclination of the bone diaphysis, which varied between -27° and 32°, based on an axis of 0° inclination.

The Seedor index is the value obtained when the bone weight is divided by its length, as proposed by Seedor (1993). It is used as a bone density indicator – the higher the value, the denser the bone. This index does not have a measurement unit. In order to perform this evaluation, the longest length of the bone was measured using a pachymeter, and the weight was obtained using a semi-analytic digital scale, with 0.0001 g precision.

Bone resistance analyses were carried out at the Rural Engineering Department of Faculdade de Ciências Agrônômicas, UNESP, Botucatu campus. Bones were place in ethylic ether for 36 hours for fat removal. An assay apparatus EMIC DL 10000 was regulated to allow a 3cm diaphysis opening. This was the maximum spacing obtained for the smallest bone, and therefore maintained for the other studied bones. In order to compare the bone strength values (kgf), an opening needs to be maintained. The software records the strength needed to completely break the bone, expressing the results in kilograms-force. Specific software allows grouping of up to 10 specimens.

Fat-free dry matter percentage was obtained by weighing the bones in a digital analytical scale, after which they are dried for 72 hours in an oven at 60°C, after which they are removed from the oven and placed on desiccators until reaching room temperature, taking care to avoid environmental moisture. The bones are again weighed, and bone fat-free dry matter is calculated (Bruno, 2002).

Eggshell quality analyses carried out in the present study were specific weight, and eggshell percentage. These two parameters were analyzed according to the methodology previously described by Castelló *et al.* (1989). Eggs used in these analyses derived from broiler breeders belonging to the group in which bone quality analyses were carried out.

Statistical analysis used the software SAEG (1998). Data were submitted to ANOVA, and means were compared by the test of Pearson at 5% significance level.

Results and Discussion

Bone mineral density results were obtained *in vivo*, whereas the remaining results were obtained from bones removed after the birds were sacrificed. Bone quality results, relative to bone mineral

density (BMD), fat-free dry matter (ffDM), ash percentage, calcium and phosphorus percentages, Seedor index, and bone resistance are shown in Table 1.

There were differences ($p < 0.05$) among the evaluated traits, which values increased as bird aged for all bone quality traits, except for ash percentage, which decreased as birds aged.

Tibia BMD values were very similar to those found by Araújo *et al.* (2004), in layers during the second production cycle. One of the treatments used by these authors (low dietary sodium) promoted tibia bone density (8.1 mm Al) equivalent to the femur bone density found in the present study. These results show that the minerals present in the tibia and femur of the studied birds were not removed for eggshell formation, as there was deposition of these minerals in the bones. Bone density decreased only in femura after 47 weeks of age.

On weeks 24 and 30, fat-free dry matter, Seedor index, and bone strength were higher, probably because birds are preparing themselves to start egg production, which happened at 27 weeks of age in the present study. Under the influence of ovarian hormones, the body prepares itself to start production, when the mineral requirements, particularly calcium requirements, increase (Mahmoud *et al.*, 1996; Agrocères Ross, 2000; Lesson & Summers, 2000; Luquetti *et al.*, 2002). The values found for the Seedor index in the present study are lower as compared to broiler values. In studies carried out by Bruno (2002), the values varied between 17.03 and 87.46 for tibiae, and 15.95 and 70.24 for femura. This author also found increasing values of this index as birds aged.

The behavior of bone strength values found here are similar to those of broiler between 42 and 53 days of age. Some authors report bone strength values between 9.24 and 19.61 kgf for tibiae and 9.51 and 20.3 kgf for femura (Lott *et al.*, 1980; Orban *et al.*, 1993; Bruno, 2002).

Table 2 shows the results of the correlations between bone quality traits and eggshell quality traits of the studied birds. Bone quality traits, except for bone strength, presented high correlation with the same characteristics in other bones. In addition, there was high correlation among all bone quality characteristics, except for tibia bone strength, which was correlated only with tibia and femur bone mineral density. Bone ash values had an atypical behavior, with negative correlation with bone mineral density, Seedor index, and fat-free dry matter.

Egg quality characteristics were correlated with few bone quality traits, with egg weight showing the most frequent, but weak correlation.

These data suggest that birds do not have to resort to the calcium present in the tibia and femur for eggshell formation. This is explained by the fact that bone calcium is used for eggshell formation when dietary calcium is not available (Maggioni, 1998; Agrocères Ross, 2003; Julian, 2005), which apparently did not happen in the present study, despite the use of small-particle limestone and the lack of afternoon supplementation of calcium.

Under the conditions of the present study, it is possible to conclude that the studied birds presented denser and stronger bones as they aged. Bone mineral density increased as these minerals were not mobilized for eggshell formation, and therefore, egg production had little influence on bone quality.

Table 1. Mean values of bone mineral density (BMD), fat-free dry matter (ffDM), Seedor index, and bone strength (BS)

Age (weeks)	Characteristic							
	BMD (mm Al)		ffDM (%)		Seedor index		BS (kgf)	
	tibia	femur	tibia	femur	tibia	femur	tibia	femur
4	1.37e	1.34g	52.09cd	50.60bc	3.72c	4.06d	6.89d	8.89d
8	2.20cde	2.55efg	56.67bcd	59.95abc	5.30c	5.87cd	8.62cd	11.24cd
12	2.42cde	3.28ef	47.54d	45.36c	5.96c	5.55d	9.79cd	14.09cd
15	1.88de	2.10fg	55.77bcd	55.90abc	6.34bc	6.34cd	10.40bcd	15.01bcd
20	2.12cde	3.32ef	52.82cd	47.25bc	6.21c	6.60bcd	11.92bc	17.06abcd
24	2.87bcde	3.81e	71.44a	70.07a	10.09a	10.44a	20.44ab	20.30ab
30	2.94bcde	7.81cd	66.68ab	60.66abc	10.94a	10.75a	21.21ab	27.49ab
35	4.51ab	12.49a	59.71abc	57.94abc	9.37ab	9.77ab	17.08b	28.48a
42	3.55abc	11.21ab	67.95ab	62.91ab	10.51a	11.88a	17.61b	24.03ab
47	3.86abc	6.11d	63.69abc	61.05abc	11.86a	11.89a	28.54a	28.83a
52	5.03a	9.75bc	68.52a	64.35a	11.68a	12.43a	20.04ab	23.66abc

Means followed by different letters in the same row indicate significant difference ($p < 0.05$) by the test of Tukey.

Table 2. Correlations among tibia and femur bone density and eggshell quality of broiler breeders.

	ffDM (tibia)	Seedor (tibia)	Str (tibia)	BMD (tibia)	ffDM (femur)	Seedor (femur)	Str (femur)	BMD (femur)	Egg Weig.	Shell %	Sp. Weight.
ffDM (tibia)	1.00										
Seedor (tibia)	0.83	1.00									
Str (tibia)	-	-	1.00								
BMD (tibia)	0.32	0.53	0.44	1.00							
ffDM (femur)	0.88	0.73	-	0.27	1.00						
Seedor (femur)	0.83	0.95	-	0.49	0.79	1.00					
Str (femur)	0.52	0.65	-	0.47	0.46	0.62	1.00				
BMD (femur)	0.35	0.58	0.43	0.67	0.23	0.50	0.44	1.00			
Egg Weight	0.38	0.33	-	-	0.24	0.20	-0.31	-	1.00		
Shell %	-	-	-	-0.15	0.23	-	-	-	-0.45	1.00	
Sp. Weight.	0.21	-	-	-	0.18	-	-	-	0.27	0.69	1.00

Pearson correlations at 5% significance. ffDM = fat-free dry matter percentage; Seedor = Seedor index; Str = bone strength; BMD = bone mineral density; Shell % = eggshell percentage; Sp. Weight = specific weight. Non-significant correlations are not shown, and are expressed as “-”.

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