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Study on the Bone Mineral Density of Broiler Suffering Femoral Joint Degenerative Lesions

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ABSTRACT

An experiment was carried out with male and females broilers of two different commercial breeds to evaluate bone mineral density of the right femur head. A number of 600 one-day-old broilers were raised in an experimental poultry house up to 42 days of age at the School of Veterinary Medicine and Animal Science of UNESP, Botucatu, Brazil. After slaughter, three males and three females in each breed in each of the established gross scores were selected. Their femora heads were submitted to gross examination, and subsequently the thighs were submitted to the Veterinary Hospital for radiographic analysis. Femora were also submitted to bone resistance, Seedor index, and dry matter content analyses. All these bone quality characteristics were different between males and females, independent of breed. Breeds presented similar behavior. It was possible to establish correlations between bone quality parameters, and confidence intervals for bone mineral density values, correlating them to femoral degeneration score, which allows characterizing femoral head lesions by radiographic optical densitometry.

INTRODUCTION

The impressive growth of the domestic poultry industry places Brazil in an outstanding position in the world market, resulting in significant profit, but also in some problems for poultry producers. The increasing genetic selection pressure, proper management, and high-density diets have promoted excellent performance results. However, some metabolic disorders currently observed derive from these improvements in genetics and in management. Today, problems like femoral head necrosis in broilers cause significant losses for producers. According to Cook (2000), it is estimated that about 3.2% of broiler production is lost due to skeletal malformations in the US. Also, the lack of a standardized terminology to properly describe the different locomotory anomalies negatively contributes the correct estimation of losses, in addition to the fact that many symptoms caused by these conditions are not clinically visible (Tardin, 1995).

Bone mineral density is one of the most important ways to measure bone quality. Bones have an essential role in normal growth and development of vertebrates. The process of bone development entails a complex sequence of events that interrelated in time and space, leading to bone formation. The continuous addition of cartilage and its subsequent replacement by bone is the essence of the elongation process (Howlet, 1980; Banks, 1991; Thorp, 1992; Almeida Paz *et al.*, 2005).

The study of bone mineral density in commercial poultry by radiographic optical densitometry using the Cromox® Athena-SAI software is very important for poultry production as this modern technique allows the follow-up of bone mass variation at low cost, providing better understanding and assessment of the bone mineralization process.

Bone tissue strength results from calcium and phosphorus deposition in the form of hydroxyapatite during bone mineralization. These two minerals make up 70% of the bone, whereas the remaining 30% consist of organic matter, mainly collagen (Kälebo & Strid, 1988a; Field, 1999; Bruno, 2002).

Hydroxyapatite and aluminum present very similar densities, and therefore studies were conducted relating bone mineralization degree and aluminum density. The authors of those studies concluded that, using radiology, it is possible to compare the amount of calcium and phosphorus in the bone with the aluminum concentration present in a pre-defined scale (Loubel & Dubois, 1973; Kälebo & Strid, 1988a; Kälebo & Strid, 1988b). Studies on bone densitometry using X-ray energy were carried out by researchers in several countries (Schreiweis *et al.,* 2003; Onyango *et al.,* 2003; Korver *et al,* 2004; Hester *et al.,* 2004; Fleming *et al*., 2004; Schreiweis *et al*., 2004), but all of them assessed the tibia and the humerus, or only the tibia.

Bone mineral density may also be measured using bone mineral composition, breaking strength, Seedor index (Seedor, 1995), etc. (Orban *et al,* 1983). Bone volume is considered in the Seedor index, i.e., this and the above measures are important when evaluating bone quality, and are closely related.

Together with tibial dyschondroplasia, femoral degeneration is the main abnormality associated the condition commonly referred as "leg weakness". The incidence of both these diseases is very high, affecting 50-80% of broiler flocks (Bains *et al*., 1998). Studying the incidence of femoral necrosis, Almeida Paz *et al.* (2005) (unpublished data), this condition was found in 64 to 97% of the evaluated birds, and it increased as a function of bird age.

The aim of this experiment was to evaluate the degree of femoral degeneration in broilers of different breeds using radiographic optical images.

MATERIAL AND METHODS

Bone mineral density (BMD), femoral degeneration incidence (FDI), bone strength (BS), Seedor index (SI), bone dry matter content (DM) and performance parameters of 42-day-old male and female broilers from two different genetic lines (lines A and B) were assessed.

A total number of 600 broilers were raised in the experimental broiler house of FMVZ-UNESP/Botucatu, Brazil. Birds were housed in a conventional masonry house, covered with asbestos tiles. The house is 40-m long, 8-m wide, and 3.5-m high, and was divided into 48 pens measuring 3.25 x 1.55m, with a total area of 5m^2 each. The house is equipped with fans, and each pen equipped with a brooder, trough feeders, and bell drinkers.

Birds were submitted to the same management commonly applied in commercial farms, with feed and water offered *ad libitum* and light for 24h a day during the entire rearing period.

Birds were distributed in a completely randomized experimental design in a 2 x 2 factorial arrangement of 2 breeds (A or B) and two sexes (male or female), with three replicates of 50 birds each.

Feeding was divided in four phases: pre-starter (0-7 days), containing 2950Kcal/kg metabolizable energy, 22% crude protein, 0.98% calcium and 0.46% phosphorus; starter (8-21 days), with 3000kcal/kg metabolizable energy, 21% crude protein, 0.93% calcium and 0.44% de phosphorus; grower (22-35 days), with 3100kcal/kg metabolizable energy, 19% crude protein, 0.87% calcium and 0.44% phosphorus, and finisher (36-42 days), with 3300kcal/kg metabolizable energy, 18% crude protein, 0.80% calcium and 0.36% de phosphorus.

At 42 days of age, 180 birds – 45 per treatment – were sacrificed at the Experimental Processing Plant of FMVZ by electrical stunning and bleeding to evaluate the incidence of femoral degeneration. Subsequently, 60 birds were separated for radiographic collection. Birds were individually weighed in a semi-analytical scale with 2.00g accuracy.

After birds were sacrificed, their femur heads were submitted to gross examination for necrosis. The femur was dislocated, and a 1-5 score was attributed. Score 1 corresponded to bone with no lesion; score 2 to lesions where the cartilage was absent in the femur head, but the bone was intact; score 3 to lesions where the femur head had no cartilage and was partially broken; score 4 to lesions where the femur head was very damaged, but its partial outline was still visible; and score 5 to lesions where the head of the femur was completely broken and it was not possible to recognize its outline (Figure 1). This methodology was developed by the company Hybro® and it is not published. Three males and three females from each breed within each gross score were selected for subsequent BMD, BS, SI, and DM analyses.

Bone mineral density – radiographic optical density

Bone mineral density (BMD) analysis of the femora of 42-day-old broilers was carried out. Sixty parts (thighs) were previously selected by gross examination, and belonged to groups of three birds per treatment for each gross score of femoral degeneration.

The thighs with bones, muscle, and skin, were submitted immediately after bird sacrifice to the Veterinary Hospital of FMVZ, where they were radiographed with the aid of a calibrated X-ray apparatus using a 90-cm focus-film clearance, and 36kVp X 1.6mA. Radiological procedures were those commonly used in clinical practice, and developing and fixing were carried out in a standard automatic processor.

The standardized region for reading was the proximal epiphysis (femur head) of the right femur. Readings of the radiographic optical density (bone mineral density) were carried out with the aid of the software CROMOX® ATHENA 3.1 – SIA, and all readings followed the same standard. Radiographs were scanned and the images were analyzed using a 10-mm high and 35-55mm wide opening, depending on bone size, as reading window (Figure 2).

For deboning, parts were duly identified and immersed in boiling water for 10 minutes, and subsequently deboned with the aid of a scalpel, according to the method described by Bruno (2002). With this method, it is possible to extract about 80% of the fat.

Bone strength analyses were carried out at the Department of Rural Engineering of the School of Agronomic Sciences of UNESP, Botucatu, Brazil. A EMIC DL 10000 apparatus was used, regulated to allow a 3.0-cm diaphysis clearance. Bone strength values, in kgf, can only be compared after a clearance is fixed (Almeida Paz, 2006). Specific software records the necessary strength to completely break the bone. Values are expressed in kilograms-force.

The Seedor index, which is the value obtained when bone weight is divided by its length, as proposed by Seedor (1995), is an indication of bone density: the higher the value, the denser the bone. This index does not have a unit. Bones were measured at their longest length, and were weighed using a semi-analytic digital scale.

Performance was evaluated by weekly weighing the birds and the feed, according to the method described by Mendes (1990). The following parameters were evaluated: average initial weight, average body weight, average weight gain, average feed intake, corrected feed conversion ratio, and mortality.

Statistical analyses were carried out using SAEG statistical software (1998) and a 5% significance level. Bone quality and performance data, within each treatment, were submitted to analysis of variance, and the means were compared by the test of Tukey. In order to characterize femoral degeneration, bone mineral density value range was calculated based on variance.

$$IC(\delta_\tau^2) = \frac{\varphi_\tau \hat{\delta}_\tau^2}{\chi^2_{(1-\alpha/2)}} \leq \delta_\tau^2 \leq \frac{\varphi_\tau \hat{\delta}_\tau^2}{\chi^2_{(\alpha/2)}} \qquad \hat{\delta}_\tau^2 = \frac{MSTreat - MS\,Res}{r}$$

Where:
δ^2 = MS Residue
φ_τ = Degrees of Freedom of the Residue

RESULTS AND DISCUSSION

There was no interaction between breed and sex ($p>0.05$) for any of the evaluated parameters.

Table 1 shows the performance data of the studied broilers. Average body weight and weight gain was higher in males, independent of breed, which has been already described in literature (Mendes *et al*., 1993; Cotta, 1994; Moreira *et al*., 2003; Moreira *et al*., 2004). Feed intake was higher in males of breeds until 35 days of age, after which only breed A males presented higher feed intake. However, no differences were found ($p>0.05$) in feed conversion ratio. Males also presented higher mortality, as they are more sensitive to temperature changes, and because of their higher metabolic rate, they tend do have higher mortality due to metabolic disorders (Takita, 1998; Almeida Paz, 2005). Both studied breeds presented similar performances.

Table 2 presents the incidence of femoral degeneration in the 180 sacrificed birds. The incidence percentage ($p<0.05$) was different among scores, but not between sexes. Indeed, this difference among scores was expected, as lower scores are generally more common (Thorp et al, 1997; Almeida Paz et al., 2004; Almeida Paz et al., 2005). Lesion incidence ranged between 81.67 and 85.00%.

Bone quality values (BMD, BS, SI, and DM) were associated to gross examination and bird performance. Table 3 shows the mean values of the bone quality characteristics of the 60 birds used for the collection of radiographic images and other analyses. Mean bone mineral density, bone strength, Seedor index, and dry matter content were different ($p<0.05$) between males and females, independent of breed. This shows that males are more susceptible to metabolic disorders than females, especially because they have higher weight gain. The different behaviors between BMD and BS may be explained by the region where they were measured: bone mineral density was obtained in the femoral head, whereas bone strength was measured in the femoral diaphysis.

The established confidence intervals of bone mineral density were associated to femoral degeneration scores (Table 4), allowing to characterize this abnormality using bone mineral density.

CONCLUSION

The results obtained in the present study allowed us to conclude that the incidence of femoral degeneration is high, higher than 81%; however, it is not influenced by broiler breed or sex, highlighting the importance of this condition in broilers. It was also possible to establish confidence intervals for bone mineral density values and to associate these with the gross scores of the lesions, allowing lesion identification by the use of radiographic optical densitometry.

Table 1. Live performance.

Breed	Sex	Parameters				
		Body weightt (g)	Weight gain (g)	Feed intake (g)	FCR	Mortality (%)
				1-7 days		
A	Male	207	163	167	1.16	6.42a
	Female	202	159	167	1.12	3.09b
	Mean	204	161a	167a	1.14b	4.75
B	Male	208	158	163	1.20	3.33b
	Female	202	152	157	1.21	2.63b
	Mean	205	155b	160b	1.21a	3.93
CV		3.59	4.68	4.95	4.00	63.21
				1-14 days		
A	Male	552a	504	630a	1.17	8.09a
	Female	520b	475	584ab	1.19	5.23b
	Mean	536	489	607	1.18	6.66
B	Male	560a	503	596a	1.15	4.80b
	Female	516b	464	556b	1.18	3.82b
	Mean	538	483	576	1.17	4.31
CV		3.61	4.00	4.05	3.14	56.94
				1-21 days		
A	Male	1045a	998	1157a	1.13	9.04a
	Female	940b	895	1007ab	1.10	5.95b
	Mean	992	947	1082	1.12	7.49
B	Male	1048a	1001	1066a	1.05	5.51b
	Female	944b	897	940b	1.02	5.84b
	Mean	996	949	1003	1.03	5.67
CV		2.13	2.26	6.08	5.19	49.47
				1-28 days		
A	Male	1639a	1594a	2009a	1.21	9.23a
	Female	1550ab	1507ab	1749b	1.16	6.42b
	Mean	1594	1550	1879	1.18	7.68
B	Male	1619a	1568a	1881ab	1.15	7.02b
	Female	1425b	1475b	1649b	1.10	6.07b
	Mean	1522	1546	1765	1.13	6.54
CV		2.08	2.08	4.95	3.44	57.34
				1-35 days		
A	Male	2316a	2263a	3398a	1.49	10.45
	Female	2049b	1997b	2968b	1.48	7.14
	Mean	2182	2130	3183	1.49	8.79
B	Male	2254a	2194a	3158a	1.42	9.18
	Female	2020b	1962b	2792b	1.41	7.28
	Mean	2137	2078	2975	1.41	8.23
CV		1.91	1.99	4.02	3.40	46.91
				1-42 days		
A	Male	3034a	2975a	5117a	1.67	11.66a
	Female	2653b	2598b	4539b	1.72	7.14b
	Mean	2843	2786	4828	1.69	9.40
B	Male	2882ab	2822a	4734b	1.58	12.36a
	Female	2604b	2555b	4232b	1.60	8.75b
	Mean	2743	2688	4483	1.59	10.55
CV		2.63	2.68	3.78	2.65	44.72

Means followed by different letters in the same column are different by the test of Tukey at 5% significance. CV = coefficient of variation.

Table 2 - Femoral degeneration incidence in male and female broilers of two different commercial breeds.

Lesion score	Femoral degeneration incidence (%)					
	Breed A			Breed B		
	Male	Female	Mean	Male	Female	Mean
1	13.33	23.33	18.33Ba	10.00	20.00	15.00Aa
2	46.67	46.67	46.67Ca	60.00	50.00	55.00Bb
3	20.00	10.00	15.00Ab	10.00	10.00	10.00Aa
4	10.00	10.00	10.00Aa	10.00	10.00	10.00Aa
5	10.00	10.00	10.00Aa	10.00	10.00	10.00Aa
CV	2.45	3.02		2.67	3.00	

Means followed by different letters capital letter in the same row and different small letters in the same column are different by the test of Tukey at 5% significance. CV = coefficient of variation.

Table 3 - Mean values of bone mineral density (BMD), bone strength (BS), Seedor index (SI), and dry matter content (DM) of the femora of broilers.

Parameter	Breed					
	A			B		
	Male	Female	Mean	Male	Female	Mean
Femoral degeneration (CV 2.99)	2.56a	2.36b	2.46	2.50a	2.40b	2.45
BMD (mm Al)(CV 3.47)	2.48b	3.07a	2.77	2.68b	2.96a	2.82
BS (kgf/cm²)(CV 4.68)	45.19a	34.56b	39.87	45.91a	34.02b	39.96
SI(CV 1.02)	1.87b	2.61a	2.24	2.06b	2.41a	2.23
DM (%)(CV 4.60)	49.21a	45.01b	47.11	47.72a	44.22b	45.97

Means followed by different letters in the same row are different by the test of Tukey at 5% significance.

Table 4 - Confidence intervals of bone mineral density for different lesion scores of femoral degeneration.

Lesion score	Bone mineral density
1	3.57 – 4.33 (± 0.38)
2	2.98 – 3.58 (± 0.30)
3	2.43 – 3.01 (± 0.29)
4	2.16 – 2.47 (±0.16)
5	1.60 – 2.21 (± 0.30)

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