



Bone Densitometry and Calcium Serum Levels in Chickens Treated With Filtered or Unfiltered Water

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ABSTRACT

The aim of this study was to evaluate whether the drinking water of the School of Agricultural and Veterinary Sciences, UNESP, Jaboticabal, Brazil, affected bone mineral density and serum calcium levels of 14-, 21-, and 45-day-old broilers. Bone mineral density (BMD) of the tibiae was assessed using optical densitometry radiographic technique and serum calcium levels. Tibial BMD increased as broilers aged, and achieved its peak at 45 days of age. It was higher in the distal epiphysis of the birds that ingested filtered water ($p < 0.05$) compared with those supplied with unfiltered water. Therefore, it is concluded that filtered water promoted better bone quality in relative to those ingested unfiltered water.

INTRODUCTION

Because bone tissue is being constantly reshaped and replaced, techniques that are able to detect disorders in skeleton mineralization early in poultry life may contribute to their prevention (Charuta & Cooper, 2012).

Bone mineral density (BMD) is a biophysical parameter of experimental and clinical importance that aids bone structure assessment (Louzada *et al.*, 1997). Sequential analyses of variations in bone density are based on the comparison of bone x-ray images with an aluminum scale, because the density of this metal is similar to that of bone hydroxyapatite (Saraiva & Lazaretti Castro, 2002).

The high metabolic activity of broilers makes drinking water more important for their development relative to other species (Bruno & Macari, 2002). Accelerated growth favors metabolic disorders that hinder mineral deposition in the skeleton, resulting in significant bone disease incidence (Angel, 2007).

Moreover, as the skeleton of broilers is still developing during the grower phase, their heavy body weight may cause musculoskeletal disorders (Mendonça Jr., 2009). In this sense, studies on locomotion problems may contribute to improve broiler welfare and to prevent economic losses in the poultry industry (Almeida Paz, 2008). The fast growth rates of modern broilers may cause bones to be less resistant and less rigid (Driver *et al.*, 2005), and therefore their locomotion apparatus is relatively immature, with little compact bone tissue at slaughter age (Mendonça Jr., 2009). Therefore, the early evaluation of BMD provides data on skeleton quality and may aid the diagnosis of common disorders, such as tibial dyschondroplasia in broilers (Almeida Paz *et al.*, 2004), degenerative joint lesions in the femoral region (Almeida Paz *et al.*, 2008, Almeida Paz *et al.*, 2009), and hypocalcemia and osteoporosis in laying hens (Julian, 2005).

Bone mineral density is regulated by remodeling in the adult skeleton and directly influences bone strength. Therefore, bone health depends



on bone mass, geometry and composition, material properties, and microstructure (Clarke, 2008).

Approximately, 98 to 99% of total body calcium and 80 to 85% of phosphorus are located in the bones. When calcium blood concentration decreases, calcium is mobilized from the bones to maintain its physiological blood level (Scott *et al.*, 1982).

As it is a dynamic mineral reserve, the skeletal system provides information on calcium homeostasis (Chang *et al.*, 2008). Because dietary calcium affects in bone development and there are no data in literature on the effects of drinking water calcium level on chicken bone density, the influence of water source on bone density and serum calcium levels of 14- to 45-day-old was investigated.

MATERIAL AND METHODS

The experiment was carried out in an experimental broiler house at the Poultry Sector of of the Animal Science Department of the School of Agricultural and Veterinary Sciences of Unesp, Jaboticabal, SP, Brazil. The experimental procedures were reviewed and

approved by Ethics and Animal Welfare Committee, of that university under protocol n. 000422-08.

In the experiment, 1120 one-day-old male Cobb chicks were distributed according to a completely randomized experimental design in two treatments with 16 replicates each, totaling 32 pens with 35 birds each. Treatments consisted of the supply of filtered water (Hidrofiltros® filter) and unfiltered water from UNESP, Jaboticabal campus, in nipple drinkers.

A feeding regime of three phases was adopted (1-7, 8-21, and 22-45 days). The diets were based on corn and soybean meal and formulated according to nutritional recommendations and ingredient composition proposed by Rostagno *et al.* (2005), as shown in Table 1.

Birds were submitted to the management practices recommended by the genetic company's manual, and an intermittent lighting program was applied. Until 20 days of age, chicks were brooded using one heating source per pen that was controlled according to birds' behavior. After that, environmental temperature was controlled by side curtain and fan management, when needed.

Table 1 – Ingredients and calculated composition of the experimental diets

Ingredients (%)	Rearing phases		
	1 to 7 days	8 to 21 days	22 to 45 days
Corn	56.69	62.43	66.13
Soybean meal	36.80	29.99	25.99
Soybean oil	2.93	4.43	4.90
Dicalcium phosphate	1.83	1.57	1.42
Limestone	0.86	0.79	0.74
Salt	0.42	0.41	0.38
DL- methionine (99%)	0.22	0.16	0.16
L-Lysine	0.15	0.12	0.18
Vitamin and mineral supplement*	0.10	0.10	0.10
Total	100	100	100
Calculated composition			
Metabolizable energy (kcal/kg)	3.00	3.17	3.25
Protein (%)	21.6	19.0	17.5
Calcium (%)	0.92	0.81	0.74
Available phosphorus (%)	0.46	0.40	0.37
Total phosphorus (%)	0.71	0.63	0.59
Digestible lysine (%)	1.28	1.07	1.01
Total lysine (%)	1.31	1.11	1.05
Digestible methionine + cystine (%)	0.91	0.77	0.73
Total methionine + cystine (%)	0.93	0.79	0.75
Digestible methionine (%)	0.55	0.45	0.42
Total methionine (%)	0.56	0.46	0.44

* Supplied per kg of feed: vit. A - 8000 IU; vit. D3 - 2000 IU; vit. E - 15 mg; vit. K - 1.8 mg; vit. B1 - 1.8 mg; vit. B2 - 6 mg; vit. B6 - 2.8 mg; vit. B12 - 12 mcg; Niacin - 40 mg; Folic acid - 1000 mcg, Pantothenic acid - 15 mg; biotin 600 mg; Se - 0,3 mg; Manganese - 90 mg; Zn - 100 mg; Fe - 100 mg; Cu - 16 mg; Iodine - 1.5 mg.



Birds were vaccinated against Newcastle disease and infectious bursal disease by eye drop on day 8, and received a booster vaccination on day 14.

Filtered and unfiltered water were sampled before the experiment and stored in 1-L plastic flasks. Samples were collected directly from the tap and the filter installed in the pens to evaluate the influence of water treatment system on chemical water quality. Analytical determinations were performed by the Department of Technology from School of Agriculture and Veterinary Sciences, Unesp.

On days 14, 21 and 45, twenty birds per treatment were anesthetized with ketamine and Zoletil®, sacrificed, and their right tibiae were collected, and subsequently submitted to bone densitometry.

An aluminum phantom was used as densitometric reference for the radiographic samples. This phantom or penetrometer (metal alloy 6063, in accordance with the Brazilian Association of Technical Norms) has of 12 steps, ranging between 0.5 and 5.0 mm until the tenth step; the eleventh was 6.0 mm thick, and the twelfth was 8.0 mm thick; each step area was 5x25 mm², and was radiographed simultaneously with the bones.

The penetrometer was placed parallel to the tibia, so that the highest steps were in highest region of the chassis. Radiographs were taken by an X-ray apparatus (Siemens Tridoro 812 E) in the Radiography Sector of the Veterinary Hospital Laudo Natel of the School of Agriculture and Veterinary Sciences. P-MATG/RA Kodak films were used, and chassis measuring 18x24cm, 24x30cm and 30x40cm were used for tibiae collected on days 14, 21, and 45, respectively. Metal chassis were fitted with intensifying screens (Regular Lanex). The films, previously identified by printing devices, were revealed and fixed in an automatic processor (Kodak X-OMAT 200). Radiographs were scanned for reading in BMD software Image Pro Plus® (version 4.1, Media Cybernetics, Silver Spring, MD) at the Morphology and Physiology Department. Densitometry values were expressed in millimeters of aluminum (Al mm), according to Louzada (1997).

Blood samples, corresponding to one milliliter of blood, were collected from the axillar vein of 32 randomly selected birds at each rearing phase (14, 21 and 45 days, respectively), for serum calcium determination. Blood was placed in 1.5-mL Eppendorf tubes and centrifuged at 3000 rpm for 15 minutes to obtain the serum. Calcium serum levels were determined using a commercial kit (Labtest®).

The results were submitted to analysis of variance, evaluating tibial regions (proximal epiphysis, diaphysis,

and distal epiphysis) and age factor (14, 21 and 45 days). Means were compared by the test of Tukey at 5% significance level (SAS, 2004).

RESULTS AND DISCUSSION

Bone mineral density (BMD; $p < 0.05$) increased as birds aged (Table 2). Barreiro *et al.* (2011) also found increasing BMD values during the early stages of broilers development, in agreement with Yalçın *et al.* (2001), who stated that the first 16 days of life are crucial for the development of the tibia in broilers, stressing the significant effect of age on bone anatomy.

Independently of treatment used, in this experiment, mean tibial BMD was different among the different sections analyzed: it was higher in proximal and distal epiphysis compared with the diaphysis. Oliveira *et al.* (2009) also observed variations in BMD among sections (proximal epiphysis, diaphysis and distal epiphysis) of the tibia of 21-d-old broilers.

Bone growth and mineralization are intensive physiological processes during the first two weeks of the broiler's life (Julian, 2005). In the present experiment, BMD was higher in diaphysis and proximal epiphysis ($p < 0.05$) of birds which had access to filtered relative unfiltered water when broilers were 14 days old, but there was no effect ($p > 0.05$) of treatments on the BMD of the distal epiphysis (Table 2). On day 21, the BMD of the proximal epiphysis of tibia of broilers that consumed filtered water was higher than those supplied with unfiltered water (Figure 1), but the BMD of the diaphysis and distal epiphysis of birds receiving unfiltered water was higher relative to filtered water ($p < 0.05$; Figure 2).

On day 45, the BMD values of the proximal epiphysis and diaphysis were not different between treatments ($p > 0.05$), probably because there is a pattern of muscle, ligament, and tendon development that depends on bone status and that stabilizes as birds grow. Although Barreiro *et al.* (2009) observed that the proximal epiphysis of the tibiae of 43-d-old broilers presented higher bone mineral density than those obtained in the present study. Araújo *et al.* (2006) reported that different dietary calcium levels did not influence tibial densitometry of 49-d-old layers. According to Almeida Paz and Bruno (2006), the BMD profile of developing long bones is not different among chicken strains.

On day 45, BMD of the distal epiphysis of the broilers that ingested filtered water was higher compared with those supplied unfiltered water ($p < 0.05$), but the treatments did not influence ($p > 0.05$) proximal

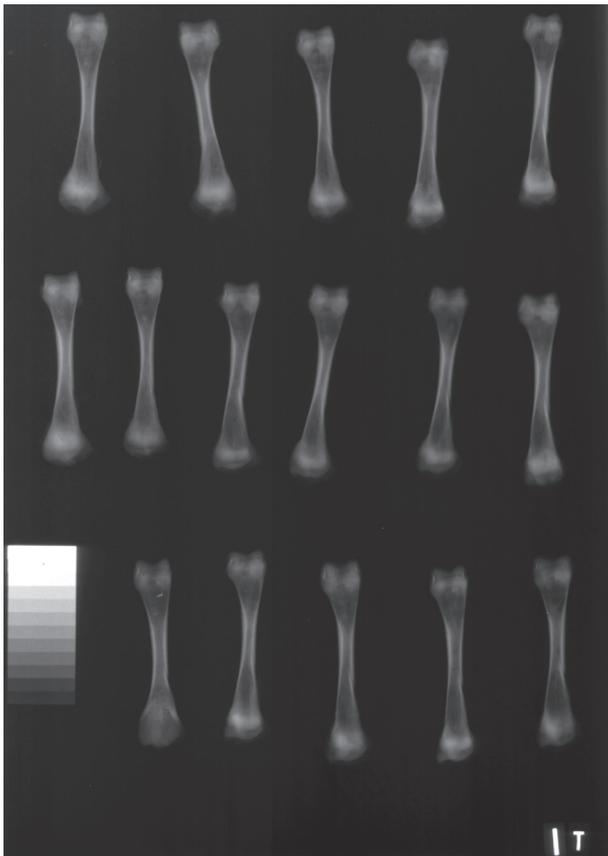


Figure 1 – Radiographic image of the tibia of 21-d-old broilers supplied with filtered water.

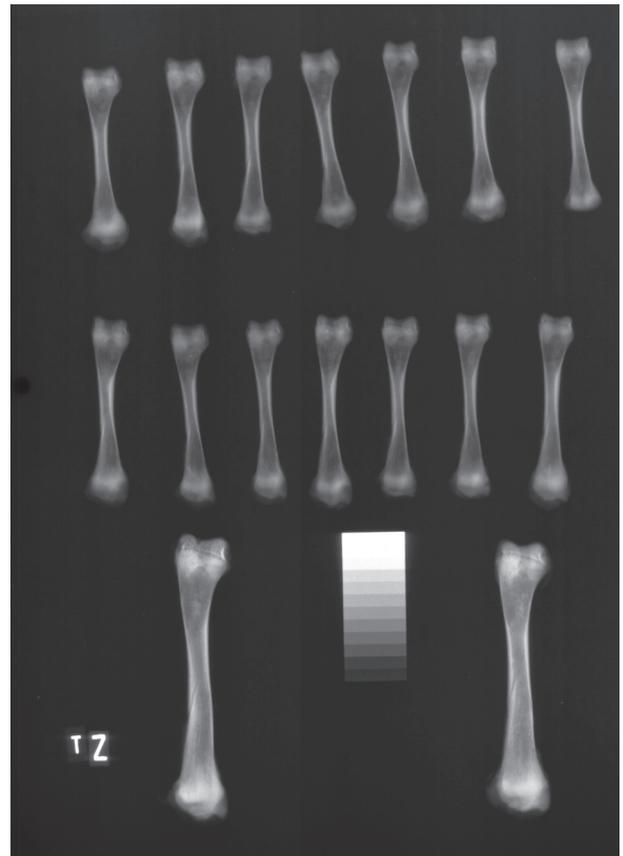


Figure 2 – Radiographic image of the tibia of 21-d-old (A) and 45-d-old broilers supplied with unfiltered water.

epiphysis and diaphysis BMD (Table 2). Because the distal epiphysis of the tibia is subject to higher pressure and impact during movement, it is suggested that the broilers that ingested filtered water may present greater biomechanical stability.

Table 2 – Mean (\pm standard deviation) bone mineral density (mm Al) of the proximal epiphysis, diaphysis and distal epiphysis of the tibiae of broilers supplied with filtered or unfiltered water between 14 and 45 days of age.

Age (days)	Proximal epiphysis	
	Filtered water	Unfiltered water
14	1.72 \pm 0.11 a	1.44 \pm 0.03 b
21	1.73 \pm 0.09 a	1.44 \pm 0.03 b
45	3.09 \pm 0.18 a	3.04 \pm 0.09 a
Diaphysis		
14	1.72 \pm 0.05 a	1.43 \pm 0.04 b
21	1.79 \pm 0.08 b	2.20 \pm 0.11 a
45	2.29 \pm 0.09 a	2.11 \pm 0.09 a
Distal epiphysis		
14	1.35 \pm 0.08 a	1.28 \pm 0.32 a
21	1.40 \pm 0.06 b	1.82 \pm 0.16 a
45	2.73 \pm 0.57 b	2.25 \pm 0.57 a
CV %	22.28	

Means followed by the same lowercase letters in the same row are not different ($p > 0.05$).

CV = coefficient of variation

The present results also indicates that the broilers fed filtered water may have stronger bones, providing adequate support to muscles, tendons, and ligaments. This was observed in experiment of Barreiro *et al.* (2011), who mentioned that immature bone develops to support muscle mass accretion in the femur of broilers at different growth stage

Although maximum bone mineralization during growth increases dietary calcium requirements (Driver *et al.*, 2005), mean calcium serum values remained constant, independently of treatment ($p > 0.05$; Table 3), in agreement with Barreiro *et al.* (2009). Since diet components influence mineral balance, affecting calcium blood levels (Schoultens *et al.*, 2002), and because negative calcium balance modifies bone architecture (Siris *et al.*, 2004), it is suggested that the experimental diets were nutritionally balanced, supporting calcium homeostasis of the birds.

Table 3 – Mean (\pm standard deviation) calcium serum levels (mg/dL) of broilers supplied with filtered or unfiltered water between 14 and 45 days of age.

Age (days)	Filtered water	Unfiltered water
14	7.94 \pm 1.96	7.55 \pm 1.14
21	7.90 \pm 1.45	8.05 \pm 1.12
45	8.93 \pm 2.03	9.05 \pm 1.66
Mean	8.26 \pm 1.10	8.22 \pm 0.89



The chemical analysis (Table 4) shows that two types of water used during the experiment were according to Conama Regulation 396, Brazil (2008).

The pH values were in the range recommended by the regulation legislation, between six and nine. The slightly alkaline pH is suitable for water consumption by birds. However, pH extremes may interfere with immunization and medication supplied via drinking water (Counotte, 2000).

Fluoride levels were below the maximum allowed levels, favoring bone physiology, because, according to Raffi *et al.* (1997), excessive amounts of fluoride can cause bone weakness in poultry. Similarly, manganese levels (Table 4) were below allowed limit for drinking water (0.5 mg/L) established by Palhares (2005). According to Faria *et al.* (2009), manganese activates several enzymes and is essential for the development of the bone organic matrix, reproduction, metabolism of carbohydrates and lipids, immune system, brain function and cell integrity. Manganese deficiency can cause nervous symptoms in young birds, perosis and chondrodystrophy.

The water supply of Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, presented good chemical quality, with toxic and contaminant level below the maximum recommended levels or absent.

Table 4 – Chemical analysis of the filtered (T1) and unfiltered water (T2) used as drinking water for the broilers during a period of 45 days

Parameters	T1	T2	Unit
pH	7.25	7.83	Dimensionless
Conductivity	169.80	166.70	µS/cm a 25°C
Total hardness	49.00	49.00	mg/L CaCO ₃
Alkalinity of hydroxides	0.00	0.00	mg/L CaCO ₃
Alkalinity of carbonates	0.00	0.00	mg/L CaCO ₃
Alkalinity of bicarbonates	191.00	194.00	mg/L CaCO ₃
Sodium	20.00	20.00	mg/L Na
Potassium	3.00	2.10	mg/L K
Calcium	18.20	18.10	mg/L Ca
Magnesium	0.84	0.80	mg/L Mg
Copper	<0.01	<0.01	mg/L Cu
Total iron	0.01	0.01	mg/L Fe
Manganese	<0.01	<0.01	mg/L Mn
Zinc	0.01	0.01	mg/L Zn
Ammonium	0.024	0.015	mg/L NH ₃
Nitrites	0.006	0.008	mg/L NO ₂ -
Nitrates	<0.001	<0.001	mg/L NO ₃ -
Fluorides	<0.01	<0.01	mg/L F-
Sulfates	0.1	0.1	mg/L SO ₃ -

CONCLUSIONS

Bone mineral density values of broilers increase according to age. Good water quality affects organic calcium balance in broilers, favoring the maintenance of serum calcium levels between 14 and 45 of age and bone mineral density of the distal epiphysis of the tibia of 45-d-old broilers.

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