Computer-generated radiological imagery of the structure of the spongious substance in the postnatal development of the tibiotarsal bones of the Peking domestic duck (*Anas platyrhynchos* var. *domestica*)

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ABSTRACT The evaluation of the structure of the spongious substance of the tibiotarsal (TT) bones of the domestic duck aged 4 to 8 wk was performed using radiological analysis. The Trabecula program (Czerwiński, 1994) used in the study identified a map of radiological trabeculae and calculated the number, average volume, density, and width of trabeculae. It was stated that the number of trabeculae differed significantly ($P \leq 0.05$) variant on age, sex, and a unique fragment of the studied bone. Six-week-old hens whose TT bones were most often exposed to deformities and fractures possessed attenuated bone mass. The number of trabeculae per 1 mm^2 during breeding was the lowest (10.34 and 9.54 mm^2 in the proximal and distal epiphyses, respectively). The tibial bones of the 6-wk-old hens also possessed the lowest volume of trabeculae (44.62 and 39.84% for the proximal and distal epiphyses, respectively). Dependant variances between the BW, the number of recognized radiological trabeculae, and the volume, density, and width of trabeculae were calculated using a selected correlation and regression coefficient (r = 0.41; $P \leq 0.05$). Results expounded a unique linear relationship between BW and the volume of trabeculae. Indeed, the larger the BW, the more numerous the trabeculae observed. No significant correlation was determined between the BW and the number of recognized trabeculae nor their density and width. A small number of trabeculae and the lowered density may be the cause of fractures and deformities of the TT bones of the domestic duck.

Key words: bone, domestic duck, tibia, tibiotarsal, Trabecula

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INTRODUCTION

The existing conditions of breeding and farming as well as genetic selection of poultry can lead to slower BW growth with subsequent effects on skeletal growth and development. Within poultry, excessive BW and loss of balance in the growth of the muscle and bone weight lead to deformities and fractures of bones. The deformities concern mainly the tibiotarsal (**TT**) bones and occur in many species of poultry (Orth and Cook, 1994; Cook, 2000; Horbańczuk et al., 2004; Burs et al., 2008; Cooper et al., 2008, 2010). Taking into account the frequency of the occurrence of pathology of the bone tissue of poultry (Gregory and Wilkins, 1989, 1991, 1996; Whitehead, 1992; Whitehead and Wilson, 1992; Gregory et al., 1993; Knowles et al., 1993; Fleming et al., 1994; Keutgen et al., 1999) and observing overwhelming health problems associated with locomotor function and the shape of the duck limbs that are growing intensively, an attempt to identify the pathology was undertaken. This necessitated familiarization with postnatal skeletal development.

Many methods of intravital evaluation of the skeletal system of poultry are available, including radiography (Poulos, 1978), dual X-ray absorptiometry (Hester et al., 2004), digital fluoroscopy (Fleming et al., 2004), quantitative computed tomography (Korver et al., 2004; Tatara et al., 2004, 2005), and computed microtomography (Martinez-Cummer et al., 2006). The latest research also concerns evaluation of bone mineral density and bone mineral content (Barreiro et al., 2009; Talaty et al., 2009a,b, 2010). Trabecula is a program enabling

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the determination of parameters of the bone structure via radiogram analysis (Czerwiński, 1994). The method was widely used in diagnostics of fluorine and osteoporotic changes in humans (Czerwiński, 1994) as well as in veterinary medicine to examine the structure of the pastern bones in horses (Dzierzęcka and Charuta, 2006; Dzierzęcka et al., 2007) and ostriches (Charuta et al., 2008).

The aim of the current investigation was to observe changes in the structure of the spongious substance of the TT bones of the domestic duck, which are most frequently exposed to pathological deformities. Apart from having cognitive value, the achieved results share practical value because they can be used effectively by breeders.

MATERIALS AND METHODS

The research material consisted of radiograms of 100 TT bones of the Peking domestic duck (*Anas platyrhynchos* var. *domestica*), which originates from Roussay, France (STAR 53 HY: female, GL30, × male, GL50). It is a breed of average weight, bred widely in Poland (1998–2010).

Upon achieving slaughtering maturity, the studied birds were divided into age groups taking into consideration the BW: 4 wk (1,750 g), 6 wk (2,390 g), and 8 wk (2,910 g). The birds were fed ad libitum mixed pasturage for water birds: KB1 (full-value mixture for ducks from the first days until the third week of life) and KB2 (mixture for older ducks from the third week of life until the end of the farming period; Cargil, Siedlce, Poland). The birds were housed in a closed building on deep bedding. Economic slaughter was conducted in poultry slaughterhouse Sedar in Międzyrzec Podlaski in Poland. The 4- and 6-wk-old birds were killed by cervical dislocation and 8-wk-old birds were routinely slaughtered.

To conduct the examination the approval of III Bioethical Commission in Warsaw was granted (no. 55/2008). Prior to slaughtering, birds were weighed and the sex was determined. The bones were isolated and prepared for further analysis. The X-rays of the bones were conducted using EDR 75, with a radiation setting of 50 kv, current intensity of 20 mA, and a distance of 120 cm between the lamp and a Cawo (Cawo SE 4 blue 400) cassette (Primax Berlin GmbH, Berlin, Germany). Fresh bones were placed directly onto a cassette without a dispersion grid. The films were then developed in an automatic darkroom Kodak 35 MX (Kodak, Rochester, NY).

Radiograms were analyzed with the use of the Trabecula program (Czerwiński, 1994). The fragments where the whole bone was visible were scanned. Scans were made at resolutions of 300, 500, and 1,000 dots per inch. Each record was projected as a Bitmap image. The fragments chosen for the analysis of the structure of the spongious substance of the TT bone were rectangular (70 × 100 mm), dissected below the articular surface near the proximal and distal epiphyses (Figure 1).

The Trabecula program, used in the current investigation, analyzed the radiological image at a resolution of 0.096 mm and provided data concerning trabeculae conformation. The program worked on the basis of a compatible algorithm for recognizing radiological tra-

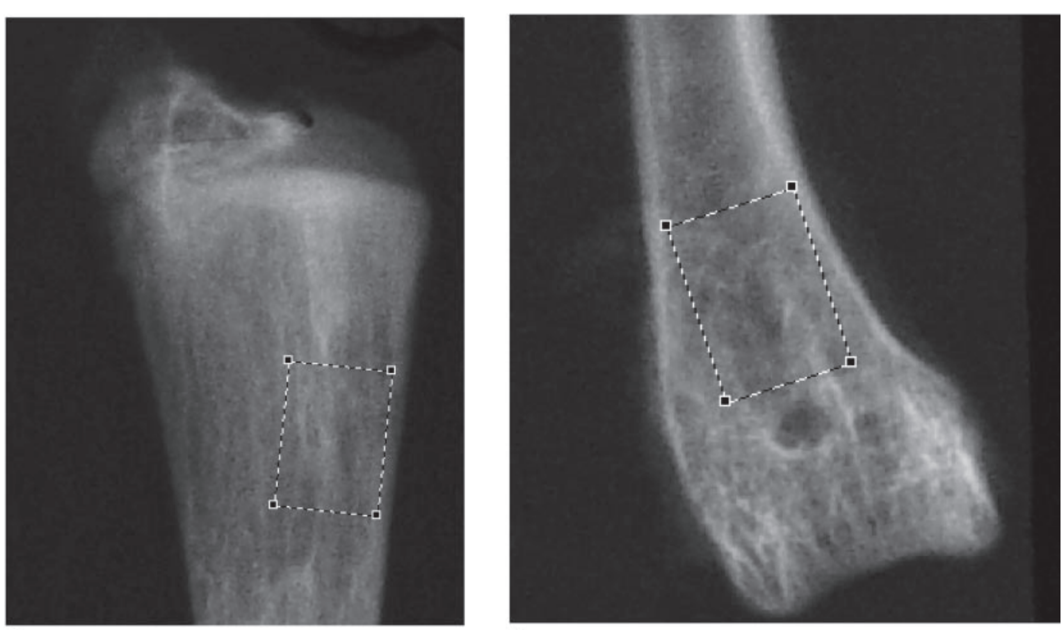

Figure 1. A fragment of a radiogram of the tibiotarsal bone of a domestic duck with a marked area of analysis. A) The proximal epiphysis; B) the distal epiphysis.

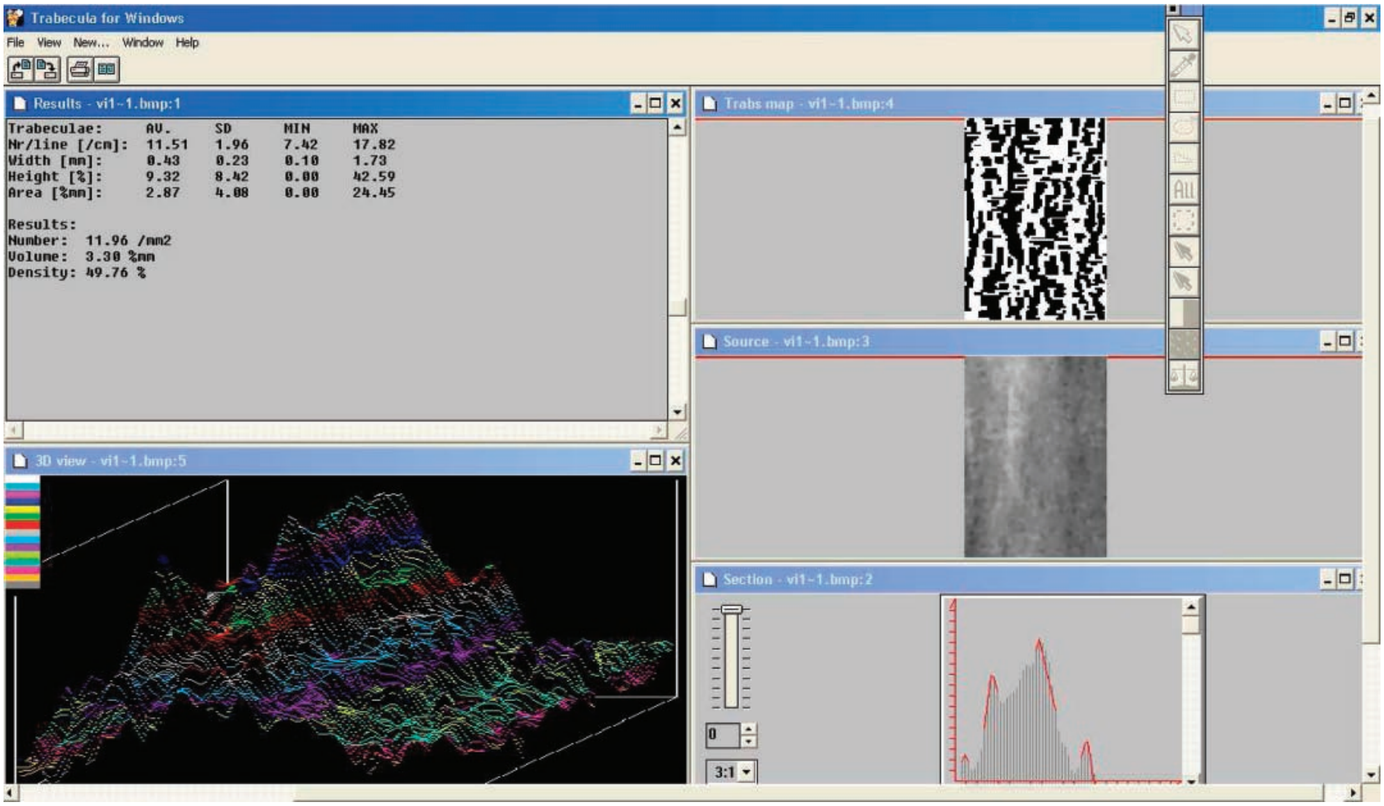

Figure 2. Computer analysis of radiograms with the use of the Trabecula program (Czerwiński, 1994). Color version available in the online PDF.

beculae, according to the formulated definition, focusing on such parameters as the angle and the level of a microdensitometer curve.

A segment of a curve with a rising stage, a plateau, and a falling arm (45°) was observed as a radiological trabecula. A map of trabeculae was generated after analyzing 128 successive curves. Then the program calculated and characterized trabeculae for the whole visible area as averages from the analysis of 128 lines (Figure 2).

The program generated the trabecula map and for the entire area of the analysis it calculated the number of recognized radiological trabeculae per square millimeter of the marked analysis area, the average volume of the trabeculae as a percentage of the volume of a cube (maximal and minimal base measured given in % mm), and density given as a percentage of the surface covered with trabeculae as well as the width of the trabecula (μm). To establish differences of the studied quantitative traits associated with sex, the obtained results were analyzed separately for cocks and hens.

The achieved results were analyzed statistically using a 2-factor ANOVA. The number of trabeculae and their volume, density, and width were calculated separately for the proximal and distal epiphyses. The Tukey test at $P \leq 0.05$ and $P \leq 0.01$ was used to compare the averages. The relation between BW and the number of the recognized radiological trabeculae and their volume, density, and width were examined with the use of correlation and regression coefficient (r = 0.41).

RESULTS AND DISCUSSION

Radiological images of 4, 6, and 8-wk-old birds were analyzed with the Trabecula program out of 100 radiograms of the TT bones. The number of radiological trabeculae and their volume, density, and width were analyzed in 4, 6, and 8-wk-old ducks. The achieved results are shown in Table 1.

Average number of radiological trabeculae per 1 mm^2 in the area of the spongious bone of the proximal epiphysis was 11.43, 10.70, and 11.31 mm^2 in 4-, 6-, and 8-wk-old birds, respectively. The number of trabeculae significantly ($P \leq 0.05$) depended on age and sex of birds (Table 1). The highest number of trabeculae in the proximal epiphysis was found in 4-wk-old cocks and the lowest number was found for 6-wk-old hens. At that time fractures and deformities of the TT bones were most frequently observed (Figure 3). It is worth emphasizing that the examined ducks had most TT deformities at the age of 6 wk. Then, a bigger relation of the muscle tissue to the bones led to extensive loading and bone deformities, including fractures of the TT bone, and consequently to growth disorders and skeleton mineralization, which may potentiate attenuation of production effectiveness. It was the result of insufficient adaptation of the skeleton to their big BW caused by growth spurts over a short period of time. Analyzing the group of cocks, a significant ($P \leq 0.05$) relation between the number of trabeculae and age was observed (Table 1). Analyzing the development of the spongious substance of the proximal and distal epiphyses, the proximal epiphysis had a bigger number of trabeculae for both sexes (Table 1). The correlation coefficient between the number of trabeculae and the BW ($r = -0.18$; $P \leq 0.05$) proved that no relationship existed between these 2 features.

Another studied parameter characterizing the TT bone was the volume of trabeculae. On the basis of the evaluation of the bone tissue structure using the Trabecula program, it was determined that the average volume of radiological trabeculae in the proximal epiphysis was 1.39, 2.36, and 2.56% in 4-, 6-, and 8-wk-old birds, respectively. Significant ($P \leq 0.05$) statistical differences were observed between age groups in both sexes. After analyzing sex groups, significantly ($P \leq 0.05$) bigger volume of trabeculae in the proximal and distal epiphyses was observed in cocks (Table 1). The correlation coefficient between the volume of the radiological trabeculae and the BW was $r = 0.41$ at $P \leq 0.05$. The larger the BW, the greater the volume of the trabeculae. The relation may be described with the equation $y = 0.51 + 0.70x$, where y is the relation feature and x is BW. The correlation coefficient suggests that when BW increases by 1,000 g, the volume of trabeculae increases by 70%.

The average density of trabeculae (expressed as a percentage of the volume of a cube, with the maximal and minimal base measured given in % mm) in the proximal epiphysis of 4-, 6-, and 8-wk-old birds was similar and did not show any significant statistical differences. Considering cocks and hens separately, some significant statistical ($P \leq 0.05$) sex differences existed

Table 1. Number, volume, density, and width of radiological trabeculae of the tibiotarsal bone of the domestic duck depending on the area of analysis (proximal or distal epiphysis), age (4, 6, or 8 wk), and sex

Item	Male (±SD)	Female (±SD)	Species average (±SD)
Proximal epiphysis			
Number (mm^2)			
4 wk	$9.18^{a,A} \pm 0.25$	$12.47^{b,B} \pm 0.80$	$11.43^{b} \pm 1.71$
6 wk	$10.94^{b,B} \pm 0.77$	$10.34^{a,A} \pm 0.67$	$10.70^{a} \pm 0.78$
8 wk	$11.57^{b,B} \pm 1.04$	11.22 ± 0.71	$11.31^{b} \pm 0.79$
Average	$10.61^{a,A} \pm 1.16$	$11.48^{b,B} \pm 1.10$	11.14 ± 1.20
Volume (% mm)			
4 wk	$1.51^{a,A} \pm 0.15$	$1.34^{a,A} \pm 0.08$	$1.39^{a,A} \pm 0.13$
6 wk	$2.98^{b,B*} \pm 0.28$	$1.44^{a,A*} \pm 0.13$	$2.36^{b,B} \pm 0.81$
8 wk	$2.78^{b,B} \pm 0.21$	$2.47^{b,B} \pm 0.27$	$2.56^{c,B} \pm 0.28$
Average	$2.55^{b,B} \pm 0.68$	$1.31^{a,A} \pm 0.58$	2.11 ± 0.71
Density (%)			
4 wk	$43.64^{a*} \pm 2.58$	$47.71^{b*} \pm 2.17$	$46.43^{a} \pm 2.96$
6 wk	$47.29^{b} \pm 2.51$	$44.62^{a} \pm 1.02$	$46.22^{a} \pm 2.41$
8 wk	$49.50^{b*} \pm 1.66$	$45.74^{ab*} \pm 2.08$	$46.73^{a} \pm 2.57$
Average	$46.44^{a,A} \pm 3.47$	$45.90^{a,A} \pm 2.44$	46.45 ± 2.61
Width (µm)			
4 wk	$0.457^{ab*} \pm 0.04$	$0.411^{a*} \pm 0.033$	$0.426^{a} \pm 0.041$
6 wk	$0.469^{b} \pm 0.039$	$0.430^{ab} \pm 0.024$	$0.453^{ab} \pm 0.038$
8 wk	$0.440^{a} \pm 0.039$	$0.48^{b} \pm 0.046$	$0.470^{b} \pm 0.047$
Average	$0.469^{b} \pm 0.039$	$0.443^{a} \pm 0.048$	0.449 ± 0.045
Distal epiphysis			
Number (mm^2)			
4 wk	$9.10^{a*} \pm 0.40$	$10.68^{BC*} \pm 0.89$	$10.18^{a} \pm 1.71$
6 wk	$10.61^{b*} \pm 0.44$	$8.14^{a,A*} \pm 0.68$	$9.62^{a} \pm 0.78$
8 wk	$9.61^{ab} \pm 1.07$	$9.54^{A} \pm 1.02$	$9.56^{a} \pm 0.79$
Average	$10.00^{a} \pm 0.89$	$9.64^{a} \pm 1.31$	9.78 ± 1.17
Volume (% mm)			
4 wk	$3.39^{a,A*} \pm 0.19$	$2.48^{a,A**} \pm 0.29$	$2.77^{a,A} \pm 0.51$
6 wk	$4.31^{b,B} \pm 0.50$	$2.56^{a,A**} \pm 0.25$	$3.61^{b,B} \pm 0.91$
8 wk	$3.56^{a,A} \pm 0.19$	$3.57^{b,B} \pm 0.34$	$3.57^{b,B} \pm 0.31$
Average	$3.91^{b,B} \pm 0.68$	$2.94^{a,A} \pm 0.58$	3.32 ± 0.76
Density (%)			
4 wk	$45.76^{a,A} \pm 1.30$	$47.08^{b,B} \pm 2.03$	$46.66^{a,A} \pm 1.90$
6 wk	$44.93^{a,A**} \pm 2.44$	$39.84^{a,A**} \pm 1.33$	$42.89^{b,AB} \pm 3.26$
8 wk	$46.14^{a,A} \pm 2.32$	$45.09^{b,B} \pm 3.76$	$45.37^{b,B} \pm 3.41$
Average	$45.41^{b} \pm 3.47$	$44.60^{a} \pm 2.44$	44.94 ± 3.30
Width (µm)			
4 wk	$0.50^{a*} \pm 0.038$	$0.44^{a,A*} \pm 0.042$	$0.42^{a,A} \pm 0.05$
6 wk	$0.49^{a} \pm 0.05$	$0.51^{b,AB} \pm 0.032$	$0.50^{b,B} \pm 0.042$
8 wk	$0.51^{a} \pm 0.036$	$0.52^{b,B} \pm 0.051$	$0.52^{b,B} \pm 0.047$
Average	$0.501^{a} \pm 0.041$	$0.49^{a} \pm 0.058$	0.49 ± 0.052

[a,b]Means within a column with different superscripts are significantly different ($P \leq 0.05$).

[A,B]Means within a column with different superscripts are significantly different ($P \leq 0.01$).

*Means within a row are significantly different ($P \leq 0.05$).

**Means within a row are significantly different ($P \leq 0.01$).

concerning the density of the trabeculae in the proximal epiphysis (Table 1). The biggest differences were observed in 8-wk-old birds: the density of the trabeculae of cocks was significantly ($P \leq 0.05$) higher at 49.50% mm. Density values significantly ($P \leq 0.05$) depended on the age group, the area of the studied bone, and sex. Interaction of the age group and sex was also significant ($P \leq 0.05$), which means that the values of the feature were different in the examined sexes depending on age. Macroscopic deformations of the TT bone of 6-wk-old hens can be explained by the microscopic structure of the proximal epiphysis of the TT bone. At that age, both the number of trabeculae and their density (44.62% mm) was the lowest in hens. Metabolic bone processes are the most intensive in the spongious substance because it has the biggest area for activity of osteoblasts and osteoclasts, better perfusion, and easier access to the tissue liquids. For this reason, pathological changes and the symptom of decreased bone density become visible in the image of the spongious substance very quickly.

The average width of the radiological trabeculae in the proximal epiphysis in the domestic duck was 0.42, 0.45, and 0.47 μm for 4-, 6-, and 8-wk-old ducks, respectively (Table 1). In the group of hens, the width of trabeculae in the proximal epiphysis increased steadily with age. In the group of cocks, a slight decrease in the width of trabeculae was observed in 8-wk-old individuals (Table 1). The average width of the trabecula in the distal epiphysis was slightly higher and averaged 0.49 μm for the species, and the proximal epiphysis averaged 0.44 μm. The correlation coefficient was $r = 0.25$ ($P \leq 0.05$), which proves that no relation exists between the width of the trabecula and BW.

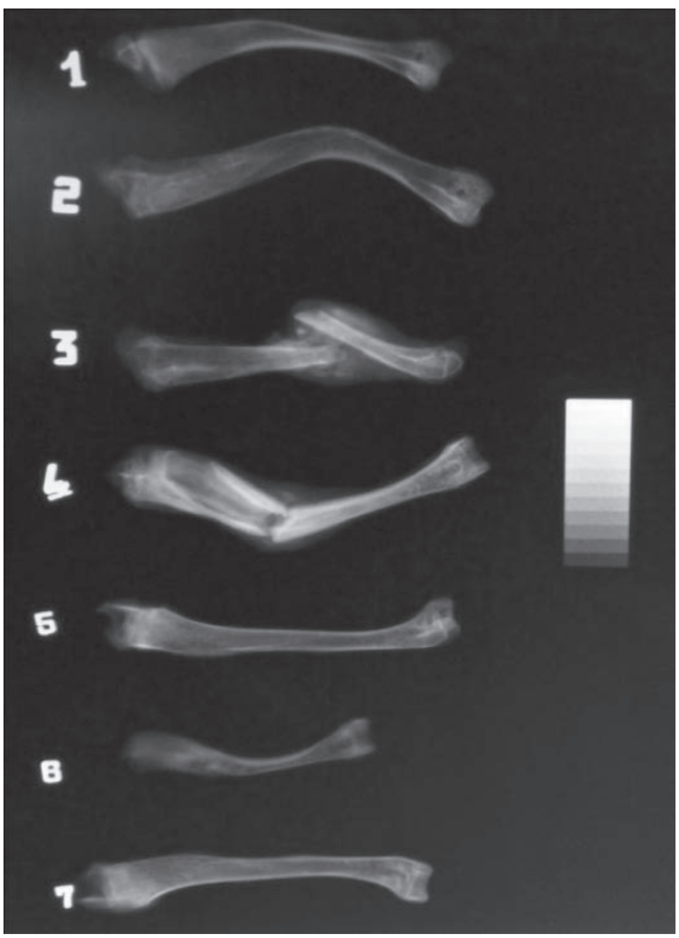

Figure 3. Deformations of the tibiotarsal bones of 6-wk-old females. 1, 2, 5, 7: deformed curved tibiotarsal bones taken from lame birds. 3, 4: fractures to the tibiotarsal bones. 1, 6: shortening of the tibia. All birds intravitally showed the features of lameness of the tibiotarsal bones.

Martinez-Cummer et al. (2006), using computed microtomography, studied the structure of the spongious substance of single comb white Leghorn hens and determined particular parameters characterizing the bone trabecula of the diaphysis of the right femoral harvested at 66 wk of age, including trabecular number (9.01/mm), trabecular separation (0.0166 mm), and trabecular thickness (0.0984 mm). Talaty et al. (2009a,b) touched upon the problem of skeleton mineralization. The authors investigated bone mineral density and bone mineral content and stated that bone mineralization was similar among crosses of meat-type chickens and had little influence on the gait score of male broilers. Barreiro et al. (2009) examined bone radiographic density from tibial ash of Cobb broilers at 8, 22, and 43 d of age. They claimed that the tibia proximal epiphysis presented higher bone radiographic density values compared with other bony regions.

In summary, farm birds develop many problems connected with the skeletal system, such as deficiency, deformity, fractures, and infections, leading to high death rate (Julian, 1998; Knowles and Wilkins, 1998). Among all the illnesses leading to limb deformities in poultry, the most frequent abnormality is dyschondroplasia of the tibial bone, which is one of the most important metabolic illnesses in poultry. It attacks long bones of fast-growing birds bred for their flesh (broilers of chickens, turkeys, and ducks; Poulos, 1978; Whitehead, 1992; Coelho et al., 1997; Peters et al., 2002; Ledwaba and Roberson, 2003). The changes are usually located in the cartilage of the proximal epiphysis of the tibial bone. Histological examination shows involution of the cartilaginous tissue, which is void of vessels and decalcified (Peters et al., 2002; Webster et al., 2003).

It has also been stated that the tendency to develop dyschondroplasia depends on sex of birds. It is strictly connected with hormonal metabolism during the process of bone formation, especially with the relation of androgens and estrogens in the blood (Coelho et al., 1997). In the present study, similar dependences were observed in a group of 6-wk-old birds (females had a smaller number of trabeculae). At that age, the lowest number of trabeculae and the lowest density of trabeculation, a result of mineralization disorders, were observed. The width of trabeculae at this age is the highest. This suggests evidence of spongious bone tissue remodeling, probably caused by the growth of the BW or disorders of the hormone economy. Deformed tibial bones made hens unwilling to move and, upon being forced to move, they showed a very rigid or limping gait.

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