Abstract

A novel diagnostic method was adapted from studies in horses and humans to postmortem research on ostrich bones. The research made it possible to determine referential values in the method of digital analysis of a radiological image for the tibio-tarsal bone of the ostrich, following exposure to injuries. In the case of a computer-generated analysis of a radiological image, the optimal conditions for taking images were selected, along with their digital record and processing: 0.096 mm/pixel, 256 greyness level (8 bits), and scanning in BMP (a bitmap that is an open format of raster graphics, a block of bytes, which describes an image pixel by pixel). The research was conducted on the rectangular-shaped (160 x 320 points) spongy bone taken about 80 mm below the articular surface near the proximal metaphysis. The research allowed defining parameters of the spongious matrix of the tibio-tarsal bone of healthy 14-month-old ostriches. The average number of trabeculae on lines was 10.41 mm² for both sexes, the average volume was 2.21 %, the density of the trabeculation (the percentage of the surface covered with trabeculae) was 47.44 % for both sexes and the average width of the trabeculae was 0.23 mm. On the basis of the conducted calculations, it is possible to draw a conclusion that the average number of radiological trabeculae, the volume of the trabeculae, and their density do not differ significantly for cocks and hens.

Key words: ostrich, tibio-tarsus, radiography, Trabecula®.

The African ostrich (Struthio camelus australis) is a popular livestock animal principally due to its nutritive and dietic meat and highly valued skin (5, 6, 18, 19, 23). Therefore, ostrich farming has a global perspective (15, 16). One of the problems facing the industry are disturbances in skeletal development up to 6 months of age. During this period, various skeletal problems including leg deformities of the tibio-tarsal and tarsal-metatarsal bones may be caused by factors that include improper feeding and unsuitably balanced diets (6, 7, 8). Younger birds have a bigger proportion of muscle tissue than skeletal mass leading to excessive load and fractures of the bones of the pelvis limb (20). Apart from that, intensive egg production may recruit an increased demand of calcium mobilised from bones to form eggshells and limited locomotor activities may also predispose hens to osteoporosis (27, 29). Considering the widely reported existence of skeletal aberrations in ostriches (1, 2, 17), there is a need to carry out a detailed radiographic analysis of the healthy structure of ostrich bone tissue.

Currently, there are many methods of intravital evaluation of the poultry skeletal system, such as radiography (22), absorptiometry of X-radiation with double energy (24), digital fluoroscopy (13), quantitative computer tomography (26, 28), and quantitative ultrasonography (13). These methods can be utilised to select animals with optimally developed and mineralised skeletal systems for breeding programmes.

One of the new methods using digital analysis of images is Trabecula®. It is a programme describing many parameters of the bone tissue, such as total number of trabeculae per mm², volume, density, and width and height of trabeculae (9). Trabeculae allow intravital evaluation of the bone tissue. Using this programme, probability of fracture can be predicted and it is possible to take some preventive measures (10, 11). The method is widely used in diagnostics of fluorine and osteoporotic changes in humans (3, 14, 21) and in veterinary medicine to study the structure of horses’ pastern bones (12).

The aim of the current research was to adapt a new non-invasive method of bone structural analysis in
humans to a structural study of the tibio-tarsal bone of the ostrich; to determine optimal parameters of analysis using radiological images of ostriches, to show sexual differences between them, and, finally, to define the values of some parameters of the spongious matrix of the tibio-tarsal bone in selected birds.

Material and Methods

The research material consisted of 20 radiographs collected from tibio-tarsal bones of ostriches (10 males and 10 females) approximately 14-month-old. To establish the differences of the studied quantitative traits associated with sex, the obtained results were analysed separately for cocks and hens.

The size of the cassettes and radiograms was 15x40 cm. The image on the radiograms was in natural size. They came from a farm in Stanisławów, located near Warsaw (52° 17’ N, 21° 32’ E). The ambient temperature was –8ºC and relative humidity 90%. Before slaughtering, the birds were weighed precisely using a TP-1500/4 RPT-97262 scale (Lubelskie Fabryki Wag "FAWAG", Lublin, Poland) and their sex was determined after laparotomy. Radiographs were made with an X-ray apparatus EDR 750B, using 50 kVp rays and radiation rate of 20 mAs, considering a distance of 120 cm between the spot of X-ray tube and a Cawo cassette (Cawo SE4 blue 400). The X-ray machine consisted of generator (50 kV, 120kV) and table for radiography equipped with Bucky grid. Only the small spot of the X-ray tube was used for the radiography. The dissected bones were put directly on a cassette without a Bucky grid usage during radiography. The black and white Kodak (M35-MX-OMAT) films were then processed automatically in the Radiodiagnostic unit of a local hospital (Siedlce).

Only high quality pictures were analysed and used in the study. The fragment where the whole bone was seen was scanned. The radiograms were digitalised using an apparatus with a charge-coupled device CCD: 2560 x1920 pixels. The bitmaps received were recorded with a resolution of 0.096 mm/pixel and at a 256 greyness level. The record was made in BMP (a bitmap).

The Trabecula® programme works on the basis of a compatible algorithm of radiological recognising of trabeculae according to the formulated definition focusing on such parameters as the angle and the level of a microdensitometer curve. A segment of a densitometer curve in a quadrilateral shape with a rising stage, a plateau, and a falling arm is seen as a radiological image of trabecula. A map of trabeculae is generated and these are described according to quantity, volume, and density.

The fragments chosen for the analysis of the structure of the spongious substance of the tibio-tarsal bone were rectangular-shaped (160 x 320 points), dissected approximately 80 mm below the articular surface near the proximal metaphysis (Fig. 1). To establish which of the parameters were optimal while analysing, the structure of the tibio-tarsal bones and number of trabeculae in given horizontal lines on the marked surface of the radiogram were determined with a naked eye. Thereafter, the number thereof was compared with the results achieved using the Trabecula® programme, which depended on the selection of individual parameters (vertical angle - 20° degrees, level – 40%, and width – 200%). The level was measured in percentages because we analysed radiograms - the greyness scale digitally (from the darkest point to the lightest one - the percentage was the threshold value <gradient> of the difference between the minimum and maximum of the optical density of the analysed image).

As the resolution of a human eye is similar to the resolution of the Trabecula® programme, the results were comparable (10,41 mm²).

According to this algorithm, the programme automatically analysed the trabeculae surface, densitometer curves and generated a map of recognised trabeculae, thereafter calculating their characteristics for the whole surface as an average from the analysis of the given 320 horizontal lines on the horizontal image. Because a rectangle with basis of 160 points and the height of 320 points were analysed, the scan contained 320 lines and each line had length of 160 points. As the result, we achieved a list of trabeculae for each line of the horizontal cross-section of the image, on the basis of which a map of trabeculae was created. The programme generated the trabecula map and for the entire area of the analysis it calculated: the number of radiologically recognised trabeculae per mm² of the marked analysis area; the average volume of the trabeculae as a percentage of the volume of a cube with the maximal and minimal basis measured and given in % mm and density given as a percentage of the surface covered with trabeculae as well as the width of the trabecula (Fig. 2).

In order to achieve the objective values of bone measurements, an arithmetic average was calculated (\( \bar{X} \)) marking the minimum and maximum parameters of the studied bones (number, volume, density, width) as well as the standard deviation (SD). The results were analysed statistically by \( t \)-Student test using Windows XP (95% CI) with \( \alpha = 0.05 \). The \( t_{\text{emp}} \) value was compared with the tabular value. The averages differed significantly when:

\[
\left| t_{\text{emp}} \right| > t_{\alpha, \upsilon} \text{ with } \upsilon = (n_1+n_2)-2 \text{ and } n_1, n_2 \text{ - number of attempts.}
\]

The averages, which differed significantly, were marked with different letters.

Birds used for the study were routinely slaughtered and the bones were gained after slaughtering. Therefore, the approval of Bioethical Commission was not needed. The research was granted by the University of Podlasie, Siedlce, Poland (Research project 1036/07/w). The University received the funds from the State Committee for Scientific Research (KBN), Warsaw, Poland.
Fig. 1. A scanned fragment of a radiogram of the tibio-tarsal bone of the 14-month-old ostrich with the marked area of analysis indicated.

Fig. 2. Computer analysis of a radiological image of the spongious substance structure of the tibio-tarsal bone of one of the ostriches.

The image contains:
- on the left: at the top parameters of the radiographic trabeculae calculated by the programme for the whole marked area, at the bottom a 3-dimensional image of the bone structure in the form of a graph with microdensitometrical curves.
- on the right: at the top a map of trabeculae discovered in the marked area, below - a fragment of the original image generated by the computer, at the bottom: the radiological image of the discovered trabeculae on one of the microdensitometrical curves.
**Results and Discussion**

Determining the parametric values in healthy ostriches allowed us to compare the above parameters with those existing in individuals experiencing skeletal structural disorders or abnormalities. The research was first conducted among humans (9, 10) and horses (11, 12). The current research showed that the adaptation of the Trabecula® is a useful tool for studying the structure of the spongious substance of the tibio-tarsal bone of ostriches.

A resolution of 0.096 mm/pixel in the form of a matrix with a 256 greyness level (8 bits) recorded in Bitmap was recognised as optimal. The most credible image of trabecula maps in the Trabecula® programme in relation to the original radiogram for the tibio-tarsal bone of ostriches was achieved for the following parameters: an angle of 20° degrees, a level of 40%. The results of the radiographic analysis using the Trabecula® programme are shown in Table 1.

The average number of trabeculae analysed radiologically was 10.42 per mm^2 for cocks and 10.40 mm^2 for hens. Their volume was 2.29% for cocks and 2.14% for hens, and their density was 47.72% for cocks and 47.93% for hens. Using these selected parameters, the average volume – 2.21%, the average density (percentage of the area covered with trabeculae) – 47.44 % for both sexes, and the average width of trabeculae - 0.23 mm.

The results achieved are similar for both sexes and did not show any statistical differences. The *t* value for the average number of radiological trabeculae, their volume, density, and width were 0.0726; 0.69; 0.766; and 1.41, respectively.

The results achieved for the skeletal structure on an example radiogram, depending on various parameters, are shown in Table 1. The parameters of bone trabeculae were: angle – 20° degrees, level - 40%, and width - 200%. On the basis of all the calculations, it is possible to draw a conclusion that an average number of radiological trabeculae, their volume and density does not differ significantly amongst cocks and hens. As it is the first study of that kind conducted on ostriches, there are no references in the literature, which would support or reject our findings. Recently, the research has been conducted, which allowed us to get to know the macroscopic structure of the pelvic limb bones of ostriches as far as length and width of the bones are concerned (4).

The present study made it possible to determine referential values in the method of digital analysis of a radiological image of the tibio-tarsal bone of ostriches previously subjected to mechanical injuries. The results achieved can be classified and charted into standard parameters whilst establishing the structure of the spongious substance of the bone when using digital image analysis. The conducted research can be extended to the analysis and observations of other bones in ostriches, for example wings and neck.

<table>
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<tr>
<th>Number of trabeculae per mm²</th>
<th>Volume of trabeculae (%)</th>
<th>Density of trabeculae (%)</th>
<th>Width of trabeculae (mm)</th>
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<tr>
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Note: The averages (comparing cocks and hens) did not differ significantly, which implies that there is no sexual dimorphism.
References