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EVALUATION OF DENSITOMETRIC AND GEOMETRIC PARAMETERS OF THE FEMUR IN 14-MONTH-OLD OSTRICHES DEPENDING ON SEX WITH THE USE OF COMPUTED TOMOGRAPHY

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Abstract

The aim of the study was to analyse densitometric and geometric parameters of the compact bone and the trabecular diaphysis of the femur of ostriches with the use of computed tomography. The method has been applied in ostriches for the first time. The study was conducted on the femur of 14-month-old ostriches of both sexes. The obtained results were analysed statistically using one-way ANOVA, with Bonferroni correction for multiple-group comparisons. It was stated that the mineral content (BMC) in the middle of the diaphysis of the femur was significantly higher in males (465.52 mg/mm) than in females (417.67 mg/mm). Similar tendencies were observed for the total bone area (TOT_ A), trabecular area (TRAB_ A), cortical area (CRT_ A), and strength strain index (SSI). The average area of the CRT_A for both sexes was 568.62 mm^2 and was lower than the TRAB_A – 884 mm^2, at $P \leq 0.05$. Moreover, it was found that strength strain index (SSI) of the diaphysis depended on the TOT_A and the TRAB_A. Both features positively correlated with SSI. Pearson's correlation coefficient for both features was $r = 0.97$, at $P \leq 0.01$. Further research concerning the dynamics of changes of densitometric and geometric parameters occurring with age and involving both sexes is recommended, as there are many locomotion problems in ostriches, especially older ones, used intensively on farms for many years.

Key words: ostrich, bone, femur, computed tomography.

In the last years a growing interest in intensive ostrich farming worldwide, including Europe, has been reported (6, 11, 14, 16). One of the reasons of such interest is that these birds provide dietetic meat and high quality skins (13, 20-22). However, in order to develop this relatively new poultry branch, there is a need to broaden the knowledge not only in regard to ostrich breeding and management (5, 12), genetics (15, 11), or reproduction (16), but also locomotory system, as well as anatomy and structure of ostrich bones (7, 8), including the femur, since in ostrich flocks problems with skeleton have been reported. Rotation of the tibial proximal metaphysis and tarsometatarsus (1, 2), and femoral breaks are the main bone deformities in the pelvic limb in ostriches.

It should be stressed that the femur in ostriches, contrary to other species of poultry kept on farms, is a pneumatic bone. It is also the shortest bone of the pelvic limb of ostriches as its length is app. 300 mm, whereas the length of the tibia and tarso-metatarsal bone is 540 mm and 480 mm, respectively (9). In the area of the proximal metaphysis, there is an air opening, where air sacs lead, called femoral diverticula (3). Thus, the description of densitometry and geometric parameters as well as mechanical parameters of the femur of healthy ostriches is necessary for a better understanding of such a high bone resistance to mechanical loading.

The aim of the study was to evaluate densitometric and geometric parameters of the femur of ostriches depending on sex. The obtained results will enrich the information on anatomical structure of the bone as there is little data in the current literature concerning ostrich femur.

Material and Methods

The research was conducted on the femur of the left pelvic limb of healthy 14-month-old ostriches (*Struthio camelus*) (10 males and 10 females). The body weight of 14-month-old males amounted to 116 kg. Females were on average 18 kg lighter. The birds were kept according to the EU standards recommended for this species. The birds were fed standard mixed concentrates STRUŚ PREMIUM (Nutrena, Poland). The slaughtering of birds was permitted by the Local Bioethical Committee for planned procedures on animals. The bones for the analysis were cleaned of the soft tissues, measured, marked, and stored at -25°C.

Afterwards, using computed tomography,

densitometric and geometric parameters concerning the total bone were analysed using a user defined weight function in the middle of the diaphysis of the femur.

The following densitometric parameters were determined: BMC- bone mineral content per 1 mm slice in mg/mm, vBMD - volumetric bone mineral density in mg/cm³ (the mean density of the total bone), CRT_CNT - cortical content per 1 mm of slice (mg/mm) (the mineral content of the pure cortical bone within a 1 mm slice), and CRT_DEN - cortical density in mg/cm^3- the mean density of the pure cortical bone.

The following geometric parameters were determined: TOT_A - bone area in mm^2 (cross sectional area of the bone after the tissue has been peeled off) (Fig. 1), TRAB_A - trabecular area in mm^2 (cross sectional area of the trabecular area after the cortical and subcortical areas have been peeled off), CRT_A - cortical area in mm^2 (the area that is assigned to be pure cortical), CRT_THK_C - cortical thickness in mm, PERI C - periosteal circumference in mm, and ENDO_C - endosteal circumference in mm. SSI - Strength Strain Index, the moment of resistance in mm³, was also defined.

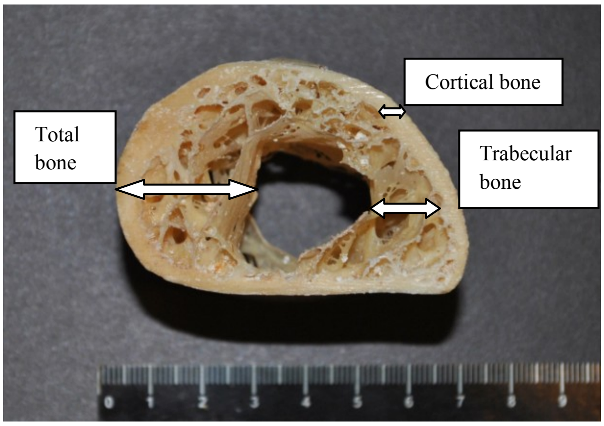

Fig. 1. The cross-section in the middle diaphysis of the femur. Geometric parameters of bones presented graphically

Applying a high resolution peripheral quantitative computed tomography (pQCT) allowed to determine SSI. This non-invasive examination is based on the assumption that the resistance of long bones depends on the quality of the compact bone tissue, particularly on its density and location on the circle of the cross section. First, cross-sectional bending (xCSMI) or twisting (pCSMI) moment of inertia were specified, characterising geometric shape of the cross-section in relation to the three-dimentional coordinate system crossing a geometric middle of the bone. The calculation included specifying the total voxel area (the smallest element of the space, the equivalent of a pixel in a two-dimentional graphics) of a layer of a compact tissue multiplied by a squared distance from a selected axis. The programme to calculate SSI (the moment of resistance) applied a tomographically specified vBMD and the bone radius (Figs 2 and 3).

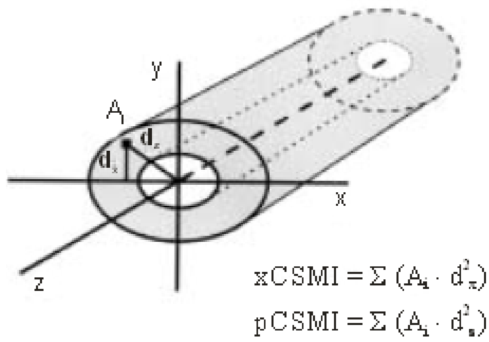

Fig. 2. Schematic presentation of the cross-sectional moments of inertia related to bending (xCSMI) or twisting (pCSMI) calculated as a sum of areas of given voxels (Ai) and squared distance from a given axis: bending (dx) and twisting (dz) (17)

$$SSI = \sqrt[3]{\frac{\sum_{m=1}^{n}\left[A \cdot d^2 \cdot \frac{vBMD}{avBMD}\right]}{EcMx}}$$

Fig. 3. The formula to calculate the indicator of mechanical resistance (SSI).

where: A – area of the sector (voxel), d – distance from the geometric centre, vBMD –volumeric mineral density of the sector (voxel), avBMD – density according to physiological standards (1,200 mg/cm3), EcMx – maximal radius along the X axis

Tomographic analysis was performed at 50% of the bone length. The examination was performed at the voxel size of 0.07 mm³ and scanning speed of 4 mm/min. The analysed areas were determined by initial scanning (20 mm/s), after morphometric measurements of the femur. The threshold coefficient, which differentiates the compact bone from the cancellous bone was set at 0.900 cm^{-1}. The thickness of the analysed slices was 0.07 mm.

Statistical analysis for comparison of traits within section of bone according to animal gender was performed using one-way ANOVA, with Bonferroni correction for multiple-group comparisons (SAS Institute. 2008). Moreover, the Pearson's correlation coefficient was calculated for geometric and densitometric traits of femur middle section.

Results

The weight of the femur ranged from 470 g to 490 g in males and from 430 g to 480 g in females.

The analysis of densitometric, geometric, and mechanical parameters characterising the femur of the ostriches is presented in Table 1.

Table 1
Densitometric, geometric, and mechanical parameters of the femur of 14-month ostriches of both sexes

Parameters	Sex						Pooled sexes		
	Males			Females					
	Mean values ± SEM			Mean values ± SEM			Mean values ± SEM		
BMC (mg/mm)	465.52 *	±	23.58	417.67*	±	24.58	441.59	±	17.38
vBMD (mg/cm^3)	259.80	±	17.45	226.58	±	19.45	239.69	±	13.75
TOT_A (mm^2)	2,019.93*	±	44.79	1,894.87*	±	45.78	1,957.40	±	32.37
TRAB_A (mm^2)	912.60*	±	20.13	855.40*	±	21.19	884.00	±	14.99
CRT_CNT (mg/mm)	640.03*	±	21.01	580.31*	±	20.05	610.17	±	14.18
CRT_DEN (mg/cm^3)	1,065.0	±	6.32	1,079.2	±	7.76	1,072.1	±	5.48
CRT_A (mm^2)	600.54*	±	15.82	536.69*	±	16.21	568.62	±	11.46
CRT_THK_C (mm)	4.19	±	0.15	3.82	±	0.18	4.01	±	0.12
PERI_C (mm)	159.08	±	2.02	154.27	±	2.12	156.68	±	1.50
ENDO_C (mm)	133.27	±	2.57	130.33	±	2.88	131.80	±	2.04
SSI (mm^3)	18,542.43*	±	331.5	16,033.62*	±	330.9	17,288.03	±	234.0

BMC - bone mineral content; vBMD - volumetric bone mineral density; CRT_CNT- cortical content; CRT_DEN - cortical density; TOT_A - total bone area; TRAB_A - trabecular area; CRT_A cortical area; CRT_THK_C - cortical thickness; PERI C - periosteal circumference; ENDO_C - endocortical circumference; SSI - strength strain index;
* P≤0.05

On the basis of the conducted analysis performed using computed tomography, it was found that the BMC in the middle of diaphysis of the femur of male ostriches amounted to 465.5 mg/mm and was significantly higher than in females (by 47.8 mg/mm) (P≤0.05). Computed tomography enabled to evaluate a real density of bones. It was stated that vBMD was also higher in males (259.80 mg/cm^3), which probably resulted in a higher index of a predicted residence to fractures (SSI) in this group of birds.

Another studied parameter was the TOT_A. In the middle of the diaphysis of the femur, TOT_A was higher in males by 125.06 mm^2 (P≤0.05). Computed tomography enabled to calculate the area for the cortical and trabecular bone separately. It was stated that the area of the TRAB_A in diaphyses of the femur was higher than the area of the CRT_A and amounted to 884.00 mm^2 and 568.62 mm^2, respectively (Table 1). It should be emphasised that SSI was higher in males by 2,509 mm^3 (P≤0.05). On the basis of the study, it can be stated that such a high resistance of the femur in ostriches (18,542.43 mm^3) depended on the vast area of the trabecular bone (Table 1).

Pearson's correlation coefficient between the BMC and vBMD amounted to r = 0.45 (P≤0.01). It was found that BMC changeability correlated positively with CRT_CNT, r = 0.55, and with CRT_THK_C, r = 0.49 (P≤0.01). It should be noted that BMC correlated positively with the area of the CRT_A, r = 0.55, at P≤0.01. However, it correlated negatively with the TRAB_A, r = - 0.26, at P≤0.01 (Table 2). Thus, the growth of the mineral content resulted in an increase in the area of the compact bone in the middle of the diaphysis of the femur. The vBMD correlated positively with the CRT_THK_C, r = 0.91. The vBMD, similarly to the BMC and TRAB_A, correlated negatively, r = - 0.95, at P≤0.01, as well as with the CRT_A, r = - 0.24, at P≤0.05. Positive correlations were also observed between SSI and TOT_A (r = 0.97), TRAB_A (r = 0.97), CRT_CNT (r = 0.47), CRT_A (r = 0.58), PERI_C (r = 0.97) and ENDO_C (r = 0.95) at P≤0.01.

Discussion

The conducted study has shown that several of the examined parameters in the internal structure of the femur turned out to be significantly higher in males: BMC, TOT_A, TRAB_A, CRT_CNT, CRT_A, and SSI, at P≤0.05.

It should be stressed that the BMC in the whole femur was measured with the use of a densitometer in the previous study by Dzierzęcka and Charuta (10) and was higher in males (265.2 g) than in females (247.97 g). In another research (10), a densitometer was used and the following values of density of the femur were achieved: 1.329 g/cm^2 for males and 1.201 g/cm^2 for females.

In current research a bigger area of the TRAB_A in males resulted in a higher metabolic activity, faster remodelling of bones on the surface of the trabecular tissue, which was situated close to the bone marrow. The trabecular bone contrary to the cortical bone had a loose structure. The structure of trabeculae supported the cortical bone from the marrow cavity.

Table 2
Pearson's correlation coefficients for analysed features of the femur in the studied ostriches

Parameters	BMC	vBMD	TOT_A	TRAB_A	CRT_CNT	CRT_DEN	CRT_A	CRT_THK_C	PERI_C	ENDO_C	SSI	Body weight	Bone weight
BMC	x	0.45**	-0.26**	-0.26**	0.55**	0.09	0.55**	0.49**	-0.17	-0.26**	-0.20*	0.27**	0.51**
vBMD	0.45**	x	-0.93**	-0.95**	-0.12	0.38**	-0.24*	0.91**	0.94**	-0.97**	-0.91**	-0.04	0.34**
TOT_A	-0.26**	-0.93**	x	1.00**	0.34**	-0.35**	0.46**	-0.79**	0.99**	0.98**	0.97**	0.17	-0.25
TRAB_A	-0.26**	-0.95**	1.00**	x	0.33**	-0.36**	0.46**	-0.80**	0.99**	0.98**	0.97**	0.16	-0.25**
CRT_CNT	0.55**	-0.12	0.34**	0.33**	x	-0.32**	0.92**	0.20**	0.39**	0.26**	0.47**	0.38**	0.38**
CRT_DEN	0.09	0.38**	0.35**	-0.36**	-0.32**	x	0.05	0.50**	-0.34**	-0.39**	-0.28**	0.06	0.33**
CRT_A	0.55**	-0.24*	0.46**	0.46**	0.92**	0.05	x	0.06	0.52**	0.40**	0.58**	0.38**	0.32**
CRT_THK_C	0.49**	0.91**	-0.79**	-0.80**	0.20*	0.50**	0.06	x	-0.78**	-0.86**	-0.70**	0.06	0.44**
PERI_C	-0.17	0.94**	0.99**	0.99**	0.39**	-0.34**	0.52**	-0.78**	x	0.99**	0.97**	0.19	-0.20**
ENDO_C	-0.26**	-0.97**	0.98**	0.98**	0.26**	-0.39**	0.40**	-0.86**	0.99**	x	0.95**	0.14	-0.27**
SSI	-0.20*	-0.91**	0.97**	0.97**	0.47**	-0.28**	0.58**	-0.70**	0.97**	0.95**	x	0.19	-0.21*
Body weight	0.27**	-0.04	0.17	0.16	0.38**	0.06	0.38**	0.06	0.19	0.14	0.19	x	0.15
Bone weight	0.51**	0.34**	-0.25	-0.25**	0.38**	0.33**	0.32**	0.44**	-0.20**	-0.27**	-0.21*	0.15	x

Symbols as in the Table 1. * Correlation is significant at P≤0.05. ** Correlation is significant at P≤0.01

The trabecular area, which was higher in males and amounted to 912.60 mm^2, allowed bones to resist mechanical loading, to which the skeleton was exposed during everyday activities. The study confirmed the observations by Reich *et al.* (19), indicating that the resistance of the diaphysis of the femur SSI depended on the TOT_A and TRAB_A. These both features positively correlated with SSI. Correlation coefficient for both features amounted to $r = 0.97$, at $P \leq 0.01$. These differences result from different physiological roles of the sexes and their different body mass. Hormones and cytokines have a major influence on the formation of particular types of the bone tissue. Brook (4) has demonstrated that the growth hormone facilitates compact bone formation, whereas steroid hormones influence the formation of the trabecular bone. Rath *et al.* (18), on the other hand, have showed that testosterone increases bone strength in young chickens. From the veterinary and biological perspective, future research on the influence of hormones on the formation of the cortical and trabecular bone tissues in ostriches, depending on the sex and compared with the bird species already investigated, would present a significant value within the field of comparative anatomy.

Summing up, it should be emphasised that applying modern methods of research, such as computed tomography (for the first time in ostriches) indicated that males differ significantly from females. The changes concern BMC, TOT_A, TRAB_A, CRT_A, and SSI. Further research within this scope concerning the dynamics of changes of densitometric and geometric parameters with age for both sexes is recommended. This is a very significant aspect as there are many problems concerning locomotory system in ostriches, especially older ones, used intensively on farms for many years.

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