Animal Science Papers and Reports vol. 39 (2021) no. 1, 31-46
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of the Polish Academy of Sciences, Jastrzębiec, Poland

Characteristics of pelvic limb long bones in 14-year-old female emu (*Dromaius novaehollandiae*)

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(Accepted February 12, 2021)

Investigation of morphological, geometric, densitometric, and mechanical properties of femur, tibiotarsus, tarsometatarsus, and fibula in 14-year-old female emu was performed. Densitometric measurements were executed using dual energy X-ray absorptiometry and quantitative computed tomography. Geometric properties of femur and tibiotarsus were determined. Mechanical evaluation was performed using three-point bending test. Bone weight, relative bone weight, bone length, and total bone volume were differentiated between all the evaluated bones. The size of femur was

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relatively small in relation to tibiotarsus and tarsometatarsus as expressed by its weight, total bone volume, and percentage contribution to final body weight. Volumetric bone mineral density values measured manually for trabecular and cortical bone compartments were significantly higher in tibiotarsus and tarsometatarsus than in femur, withstanding lower physiological loading (P<0.01). The values of trabecular bone mineral density of distal tibiotarsus and proximal tarsometatarsus were not significantly different (P=0.70), but they were significantly higher than in proximal and distal femur (P<0.05). Manually determined cortical bone area of tibiotarsus and tarsometatarsus were significantly lower than that of femur (P<0.05). Maximum elastic strength and ultimate strength were the highest in tarsometatarsus, then in tibiotarsus and in femur, and the lowest in fibula (all P<0.05). Functional adaptation of body weight bearing bones of pelvic limb in emu to withstand heavier loading is expressed by the highest mechanical endurance in tarsometatarsus then in tibiotarsus and the lowest in femur. Although similar location in pelvic limb bone array, body weight non-bearing fibula showed 50-60 times lower mechanical endurance than tibiotarsus.

**KEYWORDS: emu (*Dromaius novaehollandiae*) / femur / fibula /
tarsometatarsus / tibiotarsus**

Investigations of skeletal system properties in birds have provided valuable information on morphological, densitometric, and mechanical properties of long bones in most frequently reared bird species like chickens, turkeys, geese, ducks, quails and ostriches [Kaczanowska-Taraszkiewicz 2001, Krupski and Tatara 2007, Charuta and Cooper 2012, Charuta *et al*. 2014, Krupski *et al*. 2018, Damaziak *et al*. 2019]. Interest in skeletal system in birds originated from the need to understand the reasons for frequent pathological changes negatively affecting bone tissue properties and animal welfare [Crespo *et al*. 1999, Crespo *et al*. 2000, Tatara *et al*. 2004, Tatara *et al*. 2014]. Considering environmental conditions and nutrition during rearing period in birds, both egg-laying hens and fast-growing meat type poultry species are highly susceptible to occurrence of skeletal disorders [Whitehead and Fleming 2000, Webster 2004, Tatara *et al*. 2014]. Impaired bone tissue formation during systemic growth and development in meat-type poultry results from accelerated body weight gain and massive skeletal muscle development, finally leading to skeletal overloading and bone disorders. Thus, intensive growth and development rate in meat-type poultry leads to an insufficient adaptation of the skeleton to play protective, supportive, locomotory and metabolic functions [Korver *et al*. 2004, Tatara *et al*. 2004, Charuta *et al*. 2011, Charuta *et al*. 2012]. In egg-laying hens, eggshell formation is one of the fastest calcifying processes known in nature and requires about 6 grams of mineral that is deposited in less than 24 hours during the daily egg production cycle [Hincke *et al*. 2010]. Bone stores of calcium in egg-laying hens are utilized extensively for eggshell formation during the reproductive season, when 200-330 eggs are produced in a relatively short period of time. In this period, gastrointestinal absorption of calcium becomes ineffective to provide sufficient amount this mineral to the organism. In egg-laying female birds, physiological adaptation of skeleton to high demand for calcium and phosphorus during egg laying period has resulted in development of the medullary bone compartment, which is considered to be the third type of bone tissue [Whitehead 2004]. Medullary bone is produced on the endosteal surface of cortical bone within the medullary cavity [Dacke *et al*. 1993].

In bipedal emu (*Dromaius novaehollandiae*), femur, tibiotarsus, and tarsometatarsus are the main body weight bearing long bones potentially prone to metabolic and structural disorders; similarly to those observed in other poultry species. Even though emu females produce fewer eggs during the reproductive season than chicken females (10-40 eggs in farm conditions), the weight of their eggs (600-700 g), and eggshells (approximately 13% of egg weight) is significantly higher; their lifespan is also much longer (up to 30 years) than that of egg-laying hens. Thus, it may be considered a possible negative factor affecting mineral metabolism and skeletal system quality in emu [Horbańczuk 2003].

Considering lack of scientific knowledge describing characteristics of pelvic limb long bones in female emu, the aim of this study was to investigate morphological, geometric, densitometric, and mechanical properties of femur, tibiotarsus, tarsometatarsus, and fibula in 14-year-old birds.

Material and methods

Animal handling procedures were performed according to the guidelines for care and use of research animals and were approved by the Local Ethics Committee on Animal Experimentation in Szczecin (The West Pomeranian University of Technology in Szczecin, resolution number 12/2014).

Rearing conditions of emu birds

The study was performed on ten female emus (*Dromaius novaehollandiae*) reared in standard environmental conditions at the experimental facilities of the Department of Poultry and Ornamental Birds Breeding of The West Pomeranian University of Technology in Szczecin, Poland. All birds were reared in a stock from hatching to slaughter at the age of 14 years, after completion of the reproductive period. The emus were housed in a shed with unrestricted access to an open-air free range. Birds were fed granulated complete feed mixtures, based on barley, maize, wheat, and soybean meal, formulated according to the nutritional requirements of the species. The feed contained 18.00% total protein, 6.70% crude ash, 5.20% crude fiber, 2.10% crude fat and 10.63 MJ EMN in 1 kg.

Left and right bones (femur, tibiotarsus, tarsometatarsus and fibula) were collected from all birds post-mortem, and cleaned from soft tissues. Bone weight and bone length were measured. Relative bone weight (RBW) value was determined for each bone dividing bone weight (in grams) by final body weight (also in grams). Bone samples were placed in plastic bags and frozen for transportation and storage until morphological, densitometric, and mechanical analyses. Four birds were eliminated from the finally evaluated group, one due to an egg retention and presence of exostosis on tarsometatarsus (Fig. 1) in the other three individuals. Left or right bones showing no pathological changes or mechanical injuries were selected from each bird for final statistical comparison of the analyzed bone traits.

Fig. 1. Macroscopic view of exostosis located in the midshaft region of tarsometatarsus in 14-year-old emu after mechanical removal of the periosteum.

Densitometric measurements using dual energy X-ray absorptiometry

All bones were thawed in bags at room temperature for 3 hours and scanned using dual energy X-ray absorptiometry (DEXA) method. Bone mineral density (BMD) and bone mineral content (BMC) were determined for whole bones. Scanning and measuring procedures were performed using Norland XR-46 apparatus (resolution 3.0 x 3.0 mm) with Research Scan software (Norland, Fort Atkinson, USA). All bones were scanned laying on the table in the same position. Femur and tibiotarsus were placed for scanning on the anterior surface of bone on the table, while tarsometatarsus was scanned placed on the table on the posterior surface of bone. Fibula was placed on its lateral surface for scanning procedure.

Densitometric and geometric measurements using quantitative computed tomography

Quantitative computed tomography (QCT) technique and General Electric LightSpeed VCT scanner (64 rows) with OsiriX software for MacPro 29-ZRL computer (GE Medical Systems, USA) were used to determine volumetric bone mineral density (vBMD) of each bone. The measurement of vBMD of femur, tibiotarsus and tarsometatarsus was performed for the trabecular and cortical bone compartments separately, using 1.25-mm thick cross-sectional epiphyseal and diaphyseal (midshaft) QCT scans. Volumetric bone mineral density of the trabecular bone of femur (Td – trabecular bone mineral density) was determined in the proximal epiphysis (9.5% of total bone length measuring from the proximal extremity, Fig. 2A) and the distal epiphysis at approximately 15.0% of total bone length (Fig. 2B), measuring from the distal extremity. The value of Td of tibiotarsus was determined in the distal epiphysis at 4.0% of total bone length (Fig. 2C). In case of tarsometatarsus, Td measurement was performed in the proximal epiphysis of bone at 5.7% of its length (Fig. 2D). The measurements of Td were performed using manually determined region of interest (ROI) for each bone. Volumetric bone mineral density of the cortical bone (cortical bone mineral density – Cd) of femur, tibiotarsus and tarsometatarsus was measured both manually and automatically on mid-diaphyseal scan placed at 50% of total bone length.

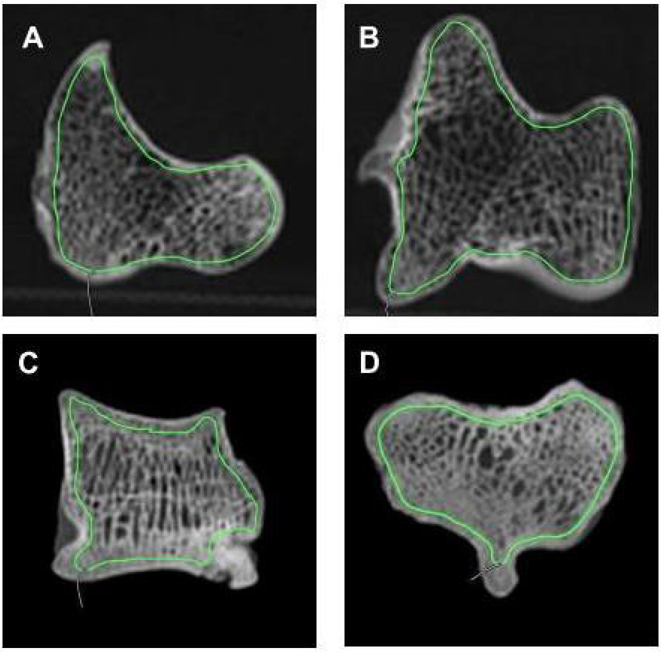

Fig. 2. Measurement of volumetric bone mineral density (vBMD) of the trabecular bone compartment (Td – trabecular bone mineral density) in proximal (A) and distal (B) epiphyses of femur, distal epiphysis of tibiotarsus (C) and proximal epiphysis of tarsometatarsus in 14-year-old emu using quantitative computed tomography (QCT) method. Trabecular bone mineral density measurement was performed within manually determined (by green line) region of interest (ROI). All measurements were performed using General Electric LightSpeed VCT scanner (64 rows) supplied with OsiriX software for MacPro 29-ZRL computer (GE Medical Systems, USA). The measuring scan data were acquired using 1.25-mm thick cross-sectional sequential QCT scans.

Manually determined ROI of the cortical bone was used for the measurement of Cd_{MAN} value (cortical bone mineral density within manually determined ROI; Fig. 3A-3C) and includes the cortical bone compartment. The automatically defined ROI of the cortical bone served for Cd_{AUT} (cortical bone mineral density measured within automatically determined ROI) determination (Fig. 3D-3F) and included both cortical bone and medullary bone compartments, including bony spicules and trabeculae. The volume of interest (VOI) for each ROI for Cd_{AUT} measurements was limited by minimum and maximum density of the measuring scan at 0 and 3071 Hounsfield units (HU), respectively. Cortical bone area (CBA) was measured at the mid-shaft of each bone using both manually (CBA_{MAN}) and automatically (CBA_{AUT}) determined ROI (Fig. 3A-3F). The values of Cd_{MAN}, Cd_{AUT}, CBA_{MAN}, and CBA_{AUT} were determined on the same diagnostic scans of the investigated bones. Total bone volume (Bvol) and mean volumetric bone mineral density (MvBMD) of femur, tibiotarsus, tarsometatarsus and fibula were determined using OsiriX software and MacPro 29-ZRL computer. For Bvol and MvBMD measurements, the VOI for whole bone was limited by minimum and maximum density of the investigated sample at 0 and 3071 HU, respectively. The measurement of Bvol and MvBMD was executed for the whole bones, including all the anatomical structures of the investigated bones. All the densitometric measurements were performed in HU initially and than converted to g/cm^3 to provide data in SI Unit System.

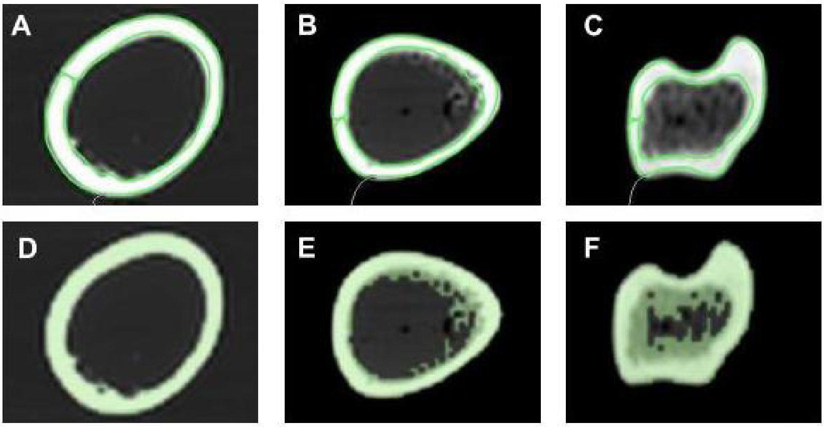

Fig. 3. Measurement of volumetric bone mineral density (vBMD) of the cortical bone compartment (Cd – cortical bone mineral density) in femur (A and D), tibiotarsus (B and E), and tarsometatarsus (C and F) of 14-year-old emu using cross-sectional diaphyseal (midshaft) scans and quantitative computed tomography (QCT) method. Cortical bone mineral density measurement in selected region of interest (ROI) was performed both manually (upper panel) and automatically (lower panel) for the bones on the same QCT measuring scans. Cortical bone mineral density within manually determined ROI (Cd_{MAN}) and cortical bone area (CBA) within manually determined ROI (CBA_{MAN}) were measured within the space between the green continuous lines on the mid-diaphyseal cross-sectional QCT scan placed at 50% of the bone length (A-C). Cortical bone mineral density within automatically determined ROI (Cd_{AUT}) and CBA within automatically determined ROI (CBA_{AUT}) were measured using the determined volume of interest (VOI), limited by minimum and maximum density of the measuring scan at 0 and 3071 Hounsfield units, respectively (D-F). In case of tibiotarsus (E) and tarsometatarsus (F), the measurements of Cd_{AUT} and CBA_{AUT} include significant amount of medullary bone compartment with bony spicules and trabeculae (deeper green shadow), visible as the structures adjacent to the endosteal surface of the cortical bone. All these measurements were performed using General Electric LightSpeed VCT scanner (64 rows) supplied with OsiriX software for MacPro 29-ZRL computer (GE Medical Systems, USA). Measuring scan data were acquired using 1.25-mm thick cross-sectional sequential QCT scans.

Geometrical properties of femur and tibiotarsus were determined on the basis of automatic measurements of horizontal and vertical diameters (both external and internal) of the mid-diaphyseal cross-sectional scan of the bones obtained from computed tomography multiplanar reconstructions. The values of cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were calculated [Brodzki *et al*. 2004, Tatara *et al*. 2005, Tatara *et al*. 2007].

Mechanical evaluation of bones

Mechanical properties of femur, tibiotarsus, tarsometatarsus, and fibula were determined using three-point bending test in Instron 3367 apparatus (Instron, Canton, MA, USA) combined with a computer. The relationship between loading force acting perpendicularly to the longitudinal axis of the evaluated bone and the resulting bone displacement was recorded and presented graphically. The values of maximum elastic strength (Wy) and the ultimate strength (Wf) were determined. The distance between bone supports was set at 40% of the total length of each bone and the measuring head loaded bone samples at the midshaft with a constant speed of 50 mm/min [Tatara *et al*. 2016a]. Femur and tibiotarsus were loaded by the measuring head on the posterior surface of the midshaft, while tarsometatarsus was loaded on its anterior surface. The placement of fibula during the three-point bending test is shown on Figure 4. The measuring head loaded fibula on the posterior surface.

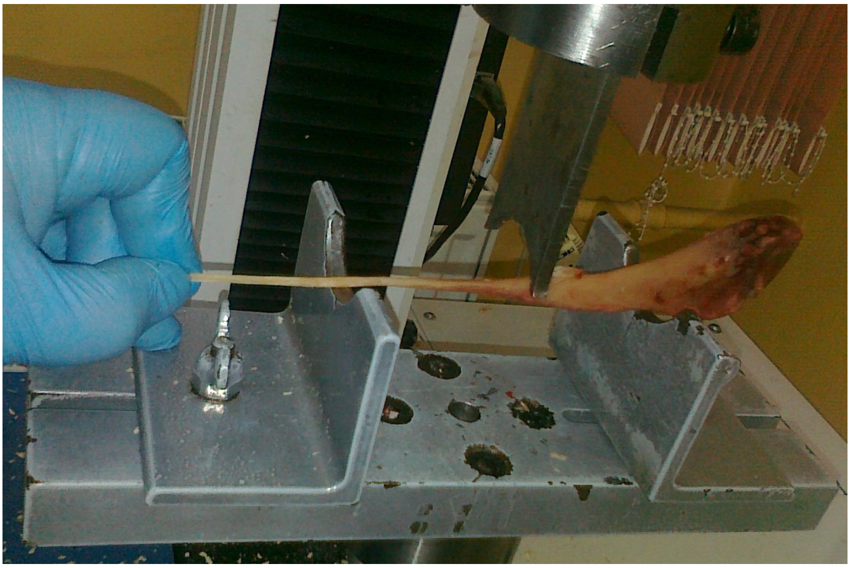

Fig. 4. The placement of fibula on bone supports during the three-point bending test in an Instron 3367 apparatus (Instron, Canton, MA, USA). The measuring head loaded fibula on the posterior surface with constant speed of 50 mm/min.

Statistical analysis

All data are presented as means±SEM. Statistical analysis of data was performed using one-way analysis of variance (ANOVA) and multicomparison post hoc Duncan test in Statistica software (version 13.0). For comparisons of the investigated parameters, statistically significant differences between two means were obtained using the Student *t*-test for dependent variables. Differences showing a *P*-value <0.05 were considered as statistically significant.

Results and discussion

Final body weight of the investigated female emu group was on average 42.17±2.49 kg. Results of the evaluation of morphological, geometrical, densitometric, and mechanical properties of femur, tibiotarsus, tarsometatarsus and fibula are shown in Table 1. Bone weight, RBW and Bvol reached the highest values for tibiotarsus, then for tarsometatarsus and femur, while the lowest values were stated for fibula; the differences were statistically significant between all bones (all P<0.01). Bone length

Table 1. Morphological, geometrical, densitometric and mechanical properties of pelvic limb long bones in 14-year-old female emu

Parameter	Femur	Tibiotarsus	Tarsometatarsus	Fibula
Bone weight (g)	188.1±5.0^{a}	463.8±20.6^{b}	279.6±9.9^{c}	38.5±1.1^{d}
Relative bone weight	0.004545 ±0.000312^{a}	0.011157 ±0.000713^{b}	0.006749 ±0.000473^{c}	0.000932 ±0.000069^{d}
Bone length (mm)	235.3±1.9^{a}	443.6±6.7^{b}	395.6±4.5^{c}	304.5±3.8^{d}
Total bone volume (cm^3)	77.8±2.9^{a}	272.0±17.0^{b}	165.45±6.2^{c}	25.2±1.1^{d}
Bone mineral density (g/cm^2)	0.903±0.024^{a}	1.116 ±0.064^{b}	0.900 ±0.028^{a}	0.713 ±0.029^{c}
Bone mineral content (g)	96.42±2.97^{a}	169.78 ±11.09^{b}	111.9±5.32^{a}	13.48±0.80^{c}
Mean volumetric bone mineral density (g/cm^3)	1.859±0.034^{ab}	1.776 ±0.032^{ac}	1.926±0.048^{b}	1.677±0.047^{c}
Trabecular bone mineral density in proximal epiphysis (g/cm^3)	0.624±0.016^{a}	-	1.385±0.029^{b}	-
Trabecular bone mineral density in distal epiphysis (g/cm^3)	0.573±0.013^{a}	1.399 ± 0.019^{b}	-	-
Cortical bone mineral density within manually determined region of interest (g/cm^3)	2.353±0.083^{a}	2.666±0.043^{b}	2.628±0.040^{b}	-
Cortical bone area within manually determined region of interest (mm^2)	212±17^{a}	171±9^{b}	156±11^{b}	-
Cortical bone mineral density within automatically determined region of interest (g/cm^3)	2.525±0.070^{a}	2.358±0.038^{ab}	2.360±0.073^{b}	-
Cortical bone area within automatically determined region of interest (mm^2)	278±12^{a}	310±16^{ab}	327±16^{b}	-
Cross-sectional area (mm^2)	256±14^{a}	211±8^{b}	-	-
Second moment of inertia (mm^4)	34103±723^{a}	10880±262^{b}	-	-
Mean relative wall thickness	0.213±0.025^{a}	0.288±0.022^{b}	-	-
Cortical index	17.36±1.62^{a}	22.18±1.32^{b}	-	-
Maximum elastic strength (N)	1975±314^{a}	2670±177^{b}	3417±98^{c}	45±2^{d}
Ultimate strength (N)	2750±422^{a}	3973±381^{b}	5533±233^{c}	80±5^{d}

Values are means ±SEM.

$^{ab\ldots}$Statistically significant differences between the compared variables are indicated with the different superscript letters for P<0.05.

reached the highest value for tibiotarsus, then for tarsometatarsus and fibula, while the lowest value was stated for femur; the differences were statistically significant between all bones (all $P<0.001$). Areal BMD and BMC of tibiotarsus were significantly higher when compared to all the other bones (all $P\leq0.001$), while no differences of these values were found between femur and tarsometatarsus (both $P>0.05$). The lowest values of BMD and BMC were stated in fibula when compared to all other bone types (all $P<0.001$). No significant differences of MvBMD were found between femur and tibiotarsus ($P=0.17$), between femur and tarsometatarsus ($P=0.27$), and between tibiotarsus and fibula ($P=0.11$). Mean volumetric bone mineral density reached significantly higher values for femur and tarsometatarsus when compared to fibula (both $P<0.01$). Trabecular bone mineral density in proximal epiphysis of femur was significantly lower when compared to this parameter in distal tibiotarsus ($P<0.0001$) and proximal tarsometatarsus ($P=0.0001$). Trabecular bone mineral density in distal bone epiphysis of femur was significantly lower when compared to this parameter in distal epiphysis of tibiotarsus ($P=0.0001$) and proximal epiphysis of tarsometatarsus ($P<0.0001$). The value of Td in femur was significantly higher in proximal epiphysis than in distal epiphysis ($P=0.03$). Cortical bone mineral density within manually determined ROI of tibiotarsus and tarsometatarsus reached similar values ($P=0.65$), which were significantly higher when compared to this parameter in midshaft of femur (both $P<0.01$). Cortical bone area within manually determined ROI reached similar values in tibiotarsus and tarsometatarsus ($P=0.44$), which were significantly lower than CBA_{MAN} of femur (both $P<0.05$). Cortical bone mineral density within automatically determined ROI of femur was significantly higher when compared to this value measured in tarsometatarsus ($P=0.01$), while no significant difference was found when comparing femur and tibiotarsus ($P=0.07$). The values of Cd_{AUT} of tibiotarsus and tarsometatarsus were not significantly different ($P=0.28$). Cortical bone area within automatically determined ROI of femur reached similar value comparing to tibiotarsus ($P=0.13$) and it was significantly lower when compared to tarsometatarsus ($P=0.03$). The values of CBA_{AUT} in tibiotarsus and tarsometatarsus did not differ ($P=0.42$). The values of A and Ix were significantly higher in femur than in tibiotarsus ($P\leq0.01$), while the values of MRWT and CI in femur were significantly lower comparing to tibiotarsus (both $P=0.04$). The values of Wy and Wf were the highest in tarsometatarsus, then in tibiotarsus and femur, while the lowest were in fibula and the differences were statistically significant comparing all bones (all $P\leq0.01$).

In this study, basic morphological, geometric, densitometric, and mechanical properties of femur, tibiotarsus, tarsometatarsus and fibula in 14-years-old female emus were described. All the evaluated morphological parameters were differentiated between all long bone types of the pelvic limb. Considering body weight bearing bones, bone weight, RBW and Bvol reached the highest values in the longest tibiotarsus, then in middle length tarsometatarsus, while the lowest values were noted in the shortest femur. Comparing the observed differences of the morphological traits of bones, the scale of differentiation of the investigated variables was not proportional between them. While bone weight and RBW were higher in similar extent for tibiotarsus by approximately 146% and for tarsometatarsus by 48.5%, when compared to femur, identical comparison of bone length showed 88.5 and 68.1% differences. Comparing tibiotarsus to tarsometatarsus, the values of bone weight and RBW of tibiotarsus were higher by nearly 40%. Total bone volume of tibiotarsus and tarsometatarsus were higher by 245 and 113% comparing to femur, respectively. Moreover, bone length and Bvol of tibiotarsus were higher by 10.8 and 39.2% when compared to tarsometatarsus. Similar morphological differentiation of long bones of pelvic limb was observed in the previous studies on 14-month-old female ostriches, where weight of tibiotarsus was significantly higher than in tarsometatarsus and femur by 36.45 and 43.15%, respectively. Analogical comparison of RBW, bone length, and Bvol have shown differences by 37.68 and 43.16, 10.10 and 43.50, and 42.27 and 47.93%, respectively. The final body weight of the ostrich females was over 2 times higher (91.31 kg) than that of emus in the current study [Tatara *et al*. 2016a]. Based on the previous and current studies, it may be postulated that very similar relationships exist between morphological properties of tibiotarsus and tarsometatarsus in female ostriches and emus. The size of femur in emu in relation to tibiotarsus is much smaller than in ostrich – that is expressed by its 2.5 times smaller bone volume, 1.5 times lower bone weight and RBW, and nearly 1-time lower bone length. Moreover, percentage contribution of tibiotarsus (0.01) and tarsometatarsus (0.006) to final body weight is identical in both these species and evidently lower in case of femur in emu (0.004 *versus* 0.006 in ostrich) [Charuta *et al*. 2015, Tatara *et al*. 2016a, Krupski *et al*. 2018]. The comparison of femur and tarsometatarsus in ostrich females has revealed higher values of bone weight, RBW, bone length and Bvol of tarsometatarsus by 11.8, 9.7, 59.1 and 10.9%, while these differences were 48.6, 48.5, 68.1% and 112.7%, respectively in emu [Tatara *et al*. 2016a]. In the current study on female emu, body weight non-bearing fibula was longer by nearly 30% in comparison to femur; however, its weight and RBW were approximately 5 times lower. Total bone volume of fibula was lower by 67.6% (approximately 3 times) when compared to femur. Comparing fibula to tibiotarsus and tarsometatarsus, the values of bone weight, RBW, bone length, and Bvol were lower by 91.7 and 86.2, 91.7 and 86.2, 31.4 and 23.0, and 90.7 and 84.8%, respectively. In the other studies on meat-type medium size turkey females at the final age of 19 weeks, RBW of femur and tibiotarsus reached 0.0041 and 0.0053 that is less than in female ostriches and emus. Tibiotarsus weight and length were significantly higher by 29.74 and 44.75% in comparison to femur indicating species-related differences of skeletal physiology and anatomy in meat-type bird females [Tatara *et al*. 2005].

Measurements of areal BMD and BMC in the current study showed the highest values in tibiotarsus and the lowest in fibula. It is surprising that both BMD and BMC values were not differentiated comparing femur and tarsometatarsus, even though the observed evident morphological differences of these bones. The fact that BMD determination using DEXA method may be affected by bone size seems to explain similarity of the densitometric measurements for femur and tarsometatarsus [Tatara 2006, Tatara *et al*. 2016b]. Mean volumetric bone mineral density, determined for all bone structures that possess density equal to water or higher, reached the lowest values in fibula. Similar results of MvBMD were obtained comparing femur with tibiotarsus and tarsometatarsus; however, MvBMD of tarsometatarsus was significantly higher than in tibiotarsus. These results partially correspond to the observations on 14-month-old female ostriches, where MvBMD was higher in tarsometatarsus than in tibiotarsus and the lowest in femur; however, the differences observed in the previous studies were significant between all the evaluated bones [Tatara *et al*. 2016a]. As shown on Figure 2, femur was the only one bone of the pelvic limb possessing two reference positions in the proximal and distal epiphyses for measurements of Td. The value of Td was lower in the distal bone epiphysis by 8.2% when compared to the proximal epiphysis. In contrast to emu, reference measurement of Td of femur in ostrich is feasible only in the proximal bone epiphysis [Tatara *et al.* 2016b]. In case of tibiotarsus in adult emu, the distal epiphysis served as an optimal reference position to measure Td, while the proximal epiphysis of tarsometatarsus was used for such measurements. Similarly to emu, both in ostriches and turkeys, the reference measurement of Td for tibiotarsus is recommended in the distal epiphysis, due to substantial presence of bone marrow, fat tissue and air-containing empty spaces in the proximal epiphysis. As shown in the previous studies on ostriches and turkeys, macroscopic and radiological examinations have revealed that anatomical arrangement of the trabecular bone do not cover entire area of the cross-sections of tibiotarsus alongside all length of its proximal epiphysis [Charuta *et al*. 2013, Rosenbeiger 2015]. It is interesting that the values of Td measured in tibiotarsus and tarsometatarsus reached very similar values which may result from comparable action of compressive, torsional and bending forces on these bones functionally connected at the ankle. Moreover, very comparable loading by body weight of the distal epiphysis of tibiotarsus and proximal epiphysis of tarsometatarsus may be considered as a factor responsible for similar volumetric bone mineral density in both these adjacent bone structures. This hypothesis is in accordance with the results of the previous studies on 14-month-old ostriches, where Td in the same epiphyses of tibiotarsus and tarsometatarsus of females has reached mean values 1.41-1.42 and 1.40-1.41 g/cm^3, respectively [Charuta *et al*. 2015]. In more recent studies on 14-month-old ostrich females, Td of distal tibiotarsus (1.461 g/cm^3) has reached very comparable values to those in proximal tarsometatarsus (1.443 g/cm^3) [Krupski *et al*. 2018]. Considering Td measurements in bone epiphyses acting on two different joints (distal femur and distal tibiotarsus) in 19-week-old female turkeys, significantly higher Td values were obtained for tibiotarsus (1.327 g/cm^3 *versus* 1.171 g/cm^3) [Tatara *et al*. 2005]. The comparison of Td in proximal femur and distal tibiotarsus in female ostrich has also shown significantly higher values for tibiotarsus (1.461 *versus* 0.668 g/cm^3) [Krupski *et al*. 2018]. Thus, the current and the previous studies on emu, ostrich and turkey females confirm increasing vBMD values of the trabecular bone compartment in accordance to peripherally growing pelvic limb bone loading by body weight. Clearing, Td in middle-loaded femur reached the lowest values in comparison to Td measured in heavy-loaded tibiotarsus and tarsometatarsus [Tatara *et al*. 2005, Charuta *et al*. 2015, Krupski *et al*. 2018]. Analogical findings may be drawn when considering the manually measured Cd which was significantly lower in the midshaft of femur in comparison to tibiotarsus and tarsometatarsus. However, CBA_{MAN} was significantly lower in tibiotarsus and tarsometatarsus when compared to femur. Thus, significantly higher Cd of tibiotarsus and tarsometatarsus in emu may results both from heavier bone loading by body weight and lower cortical bone surface than in femur exposed to withstand lower compressive, torsional and bending forces. Regardless the factors responsible for the observed densitometric differences in the bones, it was proven in the current study that both the trabecular and the cortical bone compartments of the long bones of pelvic limb show adaptive metabolic and structural response to physiological loading in accordance to the Wolff's law [Wolff 1986, Frost 2004]. Considering the automatic measurements of Cd_{AUT} and CBA_{AUT}, the obtained results evidently differ from these obtained manually. The mean value of Cd_{AUT} in tarsometatarsus was significantly lower than in femur by 6.5%. While Cd_{AUT} was higher by 7.3% than Cd_{MAN} in femur, the opposite differences were noted for these parameters for tibiotarsus and tarsometatarsus, reaching 11.5 and 10.2%, respectively. Moreover, CBA_{AUT} of tibiotarsus was not different in comparison to this parameter in femur and tarsometatarsus. The observed discrepancy between manual and automatic techniques of CBA and vBMD measurements for cortical bone compartment may be explained by the presence of significant amount of the medullary and trabecular bone structures, located on the endosteal bone surface of the medullary cavity of tibiotarsus and tarsometatarsus, as shown on Figure 3. Thus, the automatic determination of Cd_{AUT} and CBA_{AUT} may be useful to distinguish medullary bone compartment presence and its evaluation in birds. In case of femur, computed tomography cross-section of the midshaft does not show noticeable presence of medullary bone, bony spicules and trabecular bone structures, adjacent to the cortical bone compartment (Fig. 3). The limitation of the ROI for automatic measurements by minimum and maximum density of the measuring scans at 0 and 3071 HU resulted in higher mean values of Cd_{AUT} in comparison to Cd_{MAN} that may be considered as a methodological disadvantage and result overestimation. Thus, the automatic measurements of Cd_{AUT} in this particular experimental model using female emus show important limitations and should be reserved for other bird and mammal species [Tatara *et al*. 2016b].

Analysis of geometrical parameters in the current study was possible only for femur and tibiotarsus showing oval shape of the computed tomography cross-sections of the midshaft. While A and Ix reached higher mean values in femur by 17.6 and 68.1% in comparison to tibiotarsus, MRWT and CI were higher in tibiotarsus by 35.2 and by 27.8%, respectively. On the contrary to the observations in the current study, the results of measurements of A in female ostriches in the previous studies by Krupski *et al*. [2018] have revealed lack of differences of this parameter for femur and tibiotarsus. The value of Ix in femur was 3.2 times higher than in tibiotarsus showing comparable differences to these observed in female emu where 3.1 times higher value of Ix was obtained in femur than in tibiotarsus. The scale of the differences of MRWT and CI between femur and tibiotarsus was higher in ostriches than in emu reaching 144.1 and 80.5% respectively. Comparing absolute values of A of femur and tibiotarsus between emu and ostrich females, the parameters were higher in ostriches by 101.5 and 148.8%. The differences of Ix were analogical and reached 243.5 and 236.2, respectively. It is interesting that in ostriches, the oval shape of tarsometatarsus in the midshaft enables determination of the geometrical parameters of bones. Noteworthy is the fact that MRWT and CI are similar for tibiotarsus and tarsometatarsus, while A and Ix have reached significantly lower values in tibiotarsus by 27.2 and 36.6% [Krupski *et al*. 2018]. In turkey females at 19 weeks of life, the value of A has not shown significant differences for femur and tibiotarsus; however, Ix has shown significantly higher value in femur by 34.1%. Similarly to the current study, the previous study on turkeys has shown significantly higher values of MRWT and CI by 44.7 and 34.6% in tibiotarsus than in femur. Even though in turkey females final body weight and weight of femur was over 5 times lower, and bone weight of tibiotarsus was over 10 times lower, the absolute values of MRWT and CI for femur (0.206 and 16.89) and tibiotarsus (0.298 and 22.73) in those birds were very comparable to these obtained in the current study, proving very similar geometrical arrangement of bone wall in the midshaft, determined by bone tissue mass deposition, and external and internal bone diameters [Tatara *et al*. 2005]. Similarly to emu, the shape of tarsometatarsus in turkeys limits determination of the geometrical properties of this bone [Tatara 2006].

Comparing mechanical properties of bones in the current study, the functional adaptation to higher loading by body weight has resulted in the highest values of Wy and Wf in tarsometatarsus followed by those noted in tibiotarsus, and the lowest values were obtained in femur. The values of Wy and Wf in body weight non-bearing fibula were nearly 60 and 50 times lower than in tibiotarsus, showing similar location in the skeleton of the pelvic limb. Comparing the absolute values of Wy in emu and ostrich females, the values of this parameter in femur, tibiotarsus and tarsometatarsus were nearly 4, 3 and 2 times higher in the ostriches. Analogical differentiation between these bones was observed for Wf and the values were 3.4, 2.5 and 1.5 higher in ostrich females, respectively [Krupski *et al*. 2018]. In female turkeys, the value of Wy of femur was significantly higher by nearly 10% in comparison to tibiotarsus, while Wf of femur was significantly lower by 8.5%. The absolute values of Wy and Wf of femur were 4.5 and 4.4 times lower in female turkeys than in emus. For tibiotarsus, the values of the mechanical parameters were approximately 6.7 and 5.9 times higher in emu [Tatara *et al*. 2005].

In conclusion, this study presented morphological, geometric, densitometric and mechanical properties of long bones of the pelvic limb in aged female emus. Similarities and differences of the investigated parameters of bones were shown in relation to ostrich and turkey females. All the morphological parameters were differentiated between femur, tibiotarsus, tarsometatarsus and fibula. The size of femur in emu is relatively small that is expressed by its weight, total bone volume and percentage contribution to final body weight. Femur was the only bone showing possibility for measurements of Td in two reference positions, namely in the proximal and distal epiphyses. Volumetric bone mineral density values measured manually for trabecular and cortical bone compartments were significantly higher in tibiotarsus and tarsometatarsus than in femur withstanding lower physiological loading by body weight. Distal epiphysis of tibiotarsus and proximal epiphysis of tarsometatarsus, functionally connected at the ankle, reached very similar values of Td. The values of Wy and Wf of fibula were nearly 60 and 50 times lower than in tibiotarsus showing similar location in the pelvic limb bone array. Functional adaptation of body weight bearing bones of the pelvic limb in emu to withstand heavier loading is expressed by the highest mechanical endurance in tarsometatarsus followed by that achieved in tibiotarsus and the lowest as noted in femur. Based on the results obtained in the current study, fibula in emu may be recommended as an experimental model bone, body weight non-bearing bone, for further studies on bone metabolism regulation in birds, providing morphological, densitometric, and biomechanical data.

REFERENCES

1. BRODZKI A., SILMANOWICZ P., TATARA M.R., BRODZKI P., ORZELSKI M., 2004 – Cortical index (CI) of dogs' femur assessment in determining mechanical and geometrical properties of the skeletal system. ***Medycyna Weterynaryjna*** 60, 1056-1060.
2. CHARUTA A., COOPER R.G., 2012 – Computed tomographic and densitometric analysis of tibiotarsal bone mineral density and content in postnatal Peking ducks (*Anas platyrhynchos var. domestica*) as influenced by age and sex. ***Polish Journal of Veterinary Sciences*** 15, 537-545. DOI: 10.2478/v10181-012-0083-6
3. CHARUTA A., COOPER R.G., PIERZCHAŁA M., HORBAŃCZUK J.O., 2012 – Computed tomographic analysis of tibiotarsal bone mineral density and content in turkeys as influenced by age and sex. ***Czech Journal of Animal Science*** 57, 573-580.
4. CHARUTA A., DZIERZĘCKA M., MAJCHRZAK T., CZERWIŃSKI E., COOPER R.G., 2011 – 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*). ***Poultry Science*** 90, 830-835. DOI: 10.3382/ps.2010-01031
5. CHARUTA A., DZIERZĘCKA M., PIERZCHAŁA M., COOPER R.G., POŁAWSKA E., HORBAŃCZUK J.O., 2013 – Sex-related differences of morphometric, densitometric, and geometric parameters of tibia and tarsometatarsal bone in 14-month-old ostriches (*Struthio camelus*). ***Poultry Science*** 92, 2965-2976. DOI: 10.3382/ps.2013-03076
6. CHARUTA A., TATARA M.R., GRUŻEWSKA A., PIERZCHAŁA M., KALINOWSKI Ł., TRUSEWICZ M., ŁUSZCZEWSKA-SIERAKOWSKA I., 2014 – Morphological and densitometric research of the tibial bone in the post-natal development in domestic geese. ***Animal Science Papers and Reports*** 32, 251-260.
7. CHARUTA A., TATARA M.R., KRUPSKI W., POŁAWSKA E., OGŁUSZKA M., PIERZCHAŁA M., ŁUSZCZEWSKA-SIERAKOWSKA I., 2015 – Bone morphometric, densitometric and mechanical properties in 14-month-old ostriches fed experimental diet enriched with linseed. ***Animal Science Papers and Reports*** 33, 383-398.

8. CRESPO R.S., STOVER M., DROUAL R., CHIN R.P., SHIVAPRASAD H.L., 1999 – Femoral fractures in a young male turkey breeder flock. ***Avian Diseases*** 43, 150-154.
9. CRESPO R.S., STOVER M., TAYLOR K.T., CHIN R.P., SHIVAPRASAD H.L., 2000 – Morphometric and mechanical properties of femora in young adult male turkeys with and without femoral fractures. ***Poultry Science*** 79, 602-608. DOI: 10.1093/ps/79.4.602
10. DACKE C.G., ARKLE S., COOK D.J., WORMSTONE I.M., JONES S., ZAIDI M., BASCAL Z.A., 1993 – Medullary bone and avian calcium regulation. ***Journal of Experimental Biology*** 184, 63-88.
11. DAMAZIAK K., CHARUTA A., NIEMIEC J., TATARA M.R., KRUPSKI W., GOZDOWSKI D., KRUZIŃSKA B., 2019 – Femur and tibia development in meat-type chickens with different growth potential for 56 days of rearing period. ***Poultry Science*** 98, 7063-7075. DOI: 10.3382/ps/pez445
12. FROST H.M., 2004 – A 2003 update of bone physiology and Wolff's Law for clinicians. ***The Angle Orthodontist*** 74, 3-15.
13. HINCKE M.T., NYS Y., GAUTRON J., 2010 – The role of matrix proteins in eggshell formation. ***The Journal of Poultry Science*** 47, 208-219. DOI: 10.2141/jpsa.009122
14. HORBAŃCZUK J.O., 2003 – *The ostrich*. Warszawa, Poland: Auto-Graf.
15. KACZANOWSKA-TARASZKIEWICZ E., 2001 – Wpływ wieku i płci na wzrost kości kończyn i rozwój ich cech mechanicznych u przepiórki (*Coturnix coturnix Pharaoh*). ***Medycyna Weterynaryjna*** 57, 510-514.
16. KORVER D.R., SAUNDERS-BLADES J.L., NADEAU K.L., 2004 – Assessing bone mineral density in vivo: quantitative computed tomography. ***Poultry Science*** 83, 222-229. DOI: 10.1093/ps/83.2.222
17. KRUPSKI W., TATARA M.R., 2007 – Interrelationships between densitometric, morphometric, and mechanical properties of the tibia in turkeys. ***Bulletin of the Veterinary Institute in Pulawy*** 51, 621-626.
18. KRUPSKI W., TATARA M.R., CHARUTA A., BRODZKI A., SZPETNAR M., JÓŹWIK A., STRZAŁKOWSKA N., POŁAWSKA E., ŁUSZCZEWSKA-SIERAKOWSKA I., 2018 – Sex-related differences of bone properties of pelvic limb and bone metabolism indices in 14-month-old ostriches (*Struthio camelus*). ***British Poultry Science*** 59, 301-307. DOI: 10.1080/00071668.2018.1454587
19. ROSENBEIGER P., 2015 – The influence of alpha-ketoglutarate (AKG) on bone properties of tibia in turkeys under conditions of experimental osteopenia that was induced by dexamethasone. PhD Thesis. Lublin, Poland: University of Life Sciences in Lublin.
20. TATARA M.R., 2006 – Current methods for in vivo assessment of the skeletal system in poultry. ***Medycyna Weterynaryjna*** 62, 266-269.
21. TATARA M.R., KRUPSKI W., CHARUTA A., 2016b – Quantitative computed tomography (QCT) applications in studies focused on quality determination of mineralized tissues in poultry. Proceedings of the 10th Turkey Science and Production Conference, Chester, United Kingdom, 36-47.
22. TATARA M.R., KRUPSKI W., CHARUTA A., BRODZKI A., JÓŹWIK A., STRZAŁKOWSKA N., POŁAWSKA E., CHMIELOWIEC K., HORBAŃCZUK J.O., 2016a – Morphological, densitometric and mechanical properties of pelvic limb bones in 14-month-old female ostriches (*Struthio camelus*). ***Poultry Science*** 95, 2421-2426. DOI: 10.3382/ps/pew188
23. TATARA M.R., MAJCHER P., KRUPSKI W., STUDZIŃSKI T., 2004 – Volumetric bone density, morphological and mechanical properties of femur and tibia in farm turkeys with leg deformities. ***Bulletin of the Veterinary Institute in Pulawy*** 48, 169-172.

24. TATARA M.R., ROSENBEIGER P., CHMIELOWIEC K., CHARUTA A., KRUPSKI W., 2014 – Skeletal system disorders in breeding poultry. ***Magazyn Weterynaryjny*** Suppl. 1, 378-384.
25. TATARA M.R., SIERANT-ROŻMIEJ N., KRUPSKI W., MAJCHER P., ŚLIWA E., KOWALIK S., STUDZIŃSKI T., 2005 – Quantitative computed tomography for the assessment of mineralization of the femur and tibia in turkeys. ***Medycyna Weterynaryjna*** 61, 225-228.
26. TATARA M.R., TYGESEN M.P., SAWA-WOJTANOWICZ B., KRUPSKI W., MAJCHER P., HARRISON A.P., 2007 – Bone development: the effect of short-term alpha-ketoglutarate administration on long term mechanical properties of ribs in ram lambs. ***Small Ruminant Research*** 67, 179-183. DOI: 10.1016/j.smallrumres.2005.09.031
27. WEBSTER A.B., 2004 – Welfare implications of avian osteoporosis. ***Poultry Science*** 83, 184-192. DOI: 10.1093/ps/83.2.184
28. WHITEHEAD C.C., 2004 – Overview of bone biology in the egg-laying hen. ***Poultry Science*** 83, 193-199. DOI: 10.1093/ps/83.2.193
29. WHITEHEAD C.C., FLEMING R.H., 2000 – Osteoporosis in cage layers. ***Poultry Science*** 79, 1033-1041DOI: 10.1093/ps/79.7.1033
30. WOLFF J., 1986 – The Law of bone remodeling. Berlin, Germany: Springer-Verlag.