

# ASSESSMENT OF GEOMETRIC PARAMETERS OF TIBIOTARSAL BONES IN WHITE KOŁUDA GEESE USING PERIPHERAL **QUANTITATIVE COMPUTED TOMOGRAPHY\***

Anna Charuta<sup>1</sup>, Barbara Biesiada-Drzazga<sup>2</sup>, Małgorzata Dzierzęcka<sup>3</sup>, Ewa Poławska<sup>4</sup>, Matylda Trusewicz<sup>5</sup>, Ross Gordon Cooper<sup>6</sup>

<sup>1</sup>Vertebrates Morphology Department, Siedlee University of Natural Sciences and Humanities, Konarskiego 2, 08-110 Siedlce, Poland

<sup>2</sup>Department of Breeding Methods and Poultry and Small Ruminant Breeding, University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland

<sup>3</sup>Department of Morphological Science, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 166, 02-776 Warszawa, Poland

<sup>4</sup>Institute of Genetics and Animal Breeding, Polish Academy of Sciences, 05-552 Jastrzębiec, Poland Department of Conservative Dentistry, Pomeranian Medical University, 71-466 Szczecin, Poland <sup>6</sup>Eurohouse, Dog Kennel Lane, Walsall WS1 2 BU, England, United Kingdom

\*Corresponding author: anna.charuta@neostrada.pl; anna.charuta@uph.edu.pl

### Abstract

The aim of the study was to evaluate geometrical parameters of tibiotarsal bones in geese depending on age, sex and place in the bone and conditions of breeding. The inner structure of tibiotarsal bones of White Koluda® geese (W31) subjected to intensive breeding was analyzed using peripheral quantitative computed tomography (pQCT). The analyzed bones were derived from birds representing five age groups: one-day-old as well as birds of 2, 4, 6 and 8 wk of age. Bones of 10 males and 10 females were isolated from each group. The analysis was conducted at two levels of the bone: the area of the proximal metaphysis and at half the length of the diaphysis. The proximal metaphysis was selected for the research as it had higher content of the cancellous bone than the distal metaphysis. It was known that the cancellous bone was characterized by a higher rate of metabolic changes. The following geometric parameters were determined: total bone area (TOT A), trabecular area (TRAB A), cortical area (CRT A), cortical thickness (CRT THK C), periosteal circumference (PERI C), endocortical circumference (ENDO\_C) and Strength Strain Index (SSI). It was found that 6 wk of life may be considered as critical in the postnatal development of geese. In the group of 6 wk males in the shafts of the tibial bones, there was a decrease in the values of the following parameters: TOT A, TRAB A, PERI C, ENDO C. Whereas in 8 wk, an attenuation of the values of all geometric parameters of tibiotarsal bones was observed in both sexes (with the exception of CRT THK C), which may have a negative influence on bone resistance to fractures. In this period, a significant decrease in the values of geometric parameters of tibiotarsal bones could be observed, negatively affecting the resistance of the bone to fractures in 8 wk. The values of SSI parameter at the proximal metaphysis of the tibiotarsal bone decreased in both males and females in 8 wk, In turn, at mid-diaphysis, the SSI values decreased only in males in 8 wk. The achieved results and observations justify the use of optimal environmental and nutritional conditions in geese in order to improve the quality of their skeletal system and to minimize the risk of the occurrence of deformities and fractures of the pelvic limb during the breeding.

Key words: bone quality, mineralization, leg deformities, poultry, tibia

<sup>\*</sup>Source of funding: the statutory activity: 244/08/5.

The issues concerning the skeletal development of birds in their postnatal development have not been fully analyzed yet. The number of birds in a flock with serious bone diseases may amount to 30%. Over the last years body weight of geese has increased (Biesiada-Drzazga et al., 2008). Metabolic diseases of limb bones in meat birds lead to an increased mortality in a flock and lower quality of the achieved carcasses. The attempts at the assessment of the quality of the osseous tissue in selected bird species were motivated by the alarming phenomenon of pathological fractures and deformations of pelvic limb bones in farm birds (Crespo et al., 1999; Sanotra et al., 2001; Hester et al., 2004; Horbańczuk et al., 2004; Tatara et al., 2005; Cooper et al., 2008; Barreiro et al., 2009; Lewis et al., 2009; Talaty et al., 2009; Tykałowski et al., 2010; Barreiro et al., 2011). The scale of the phenomenon, entailing considerable economic losses, resulted in a gradual increase in the interest in the skeleton of these birds (Tykałowski et al., 2010; Charuta et al., 2011, 2012 a, b, 2014; Charuta and Cooper, 2012). It is believed that it is caused by, among others, endeavours to ensure the shortest breeding period possible and, in consequence, achieve the highest possible body weight as quickly as possible by means of genetic modification. The rapid increase in the muscle weight of birds compared with their bone mass results in an increased risk of deformations of bones, including in particular tibiotarsal bones (Tatara et al., 2007; Charuta et al., 2011, 2012 b; Charuta and Cooper, 2012). A number of analyses proved that either a tibiotarsal or a tarsometatarsal bone may serve as a model bone in studies on the quality of the skeleton (Charuta et al., 2013 b).

The method commonly applied to evaluate the quality of the tissue bone in animals and humans is computed tomography. Thanks to the method, it is possible to analyze values of the parameters responsible for bone properties such as trabecular and cortical volumetric bone mineral density (vBMD) system in live birds (Tatara, 2006).

The previous research on the development of the skeleton of turkeys (from 3 wk to 16 wk), ducks (from 1 day to 8 wk), chicken broilers (from 2 wk to 6 wk) and ostriches (14-mo-old ostriches) showed significant changes in the values of not only densitometric but also geometric parameters of the osseous tissue in the entire postnatal period (Tatara et al., 2007; Charuta et al., 2011; Charuta et al., 2012 a; 2013 b).

Therefore, this paper presents an analysis of selected geometric parameters of the osseous tissue of tibiotarsal bones, which are considered to be most susceptible to injuries in the studied farm birds, in geese in the postnatal development. It should be stressed that no similar studies have so far been carried out and no satisfactory information on the growth process of bones in the postnatal period and the possibility of interfering in its course has so far been obtained. However, there are papers describing this phenomenon in broilers (Yair et al., 2012; Yair et al., 2013).

### Material and methods

The study was performed on tibiotarsal bones isolated from White Kołuda® geese (W31). The studied birds represented five age groups: one-day-old birds as

well as birds of 2, 4, 6 and 8 weeks of age; from each group 10 bones were isolated from both males and females. Deformations of the tibial bones were evaluated on live birds.

The geese were bred intensively and fed with a standard KG1 concentrate from the moment of hatching up to 3 weeks of life (20.03% protein, 11.97 MJ/kg, calcium 1.36%, phosphorus 0.49%) and with KG2 mixture from 4 to 8 week (18.10%, 12.13MJ/kg, calcium 0.95%, phosphorus 0.49%) (Biesiada-Drzazga et al., 2008). The geese used the run. Prior to the slaughter the body weight of each bird was determined. The slaughter and collection of material for research were conducted with the approval of the 3rd Ethics Committee concerning experiments on animals of Warsaw University of Life Sciences, Warsaw, Poland (Resolution No. 23/2009).

After the slaughter the analyzed bones were isolated by means of skinning and removing soft tissues with the exception of the periosteum. The tibiotarsal bones were then weighed using the AXIS AD 300 laboratory balance (AXIS Sp. Zoo Gdańsk, Poland) with accuracy of 300 g/0.001 g and kept in the temperature of -25°C in plastic bags to prevent them from drying out.

The analysis of the structure of the bones was performed using a peripheral quantitative computed tomography (pQCT) and XCT Research SA Plus apparatus (Stratec Medizintechnik GmbH, Pforzheim, Germany). The following parameters were determined: total bone area (TOT\_A) (mm²), trabecular area (TRAB\_A) (mm²), cortical area (CRT\_A) (mm²), cortical thickness (CRT\_THK\_C) (mm), periosteal circumference (PERI C) (mm), endocortical circumference (ENDO\_C) (mm), Strength Strain Index (SSI) (mm³). The pQCT analysis was carried out at two positions of the bone: in the area of the proximal metaphysis (18% of the bone length) and at half the length of the diaphysis (50% of the bone length), at the voxel size of 0.07 mm and scanning speed of 4 mm/min. The measurement points were determined based on an initial scanning (20 mm/s). The determined threshold coefficient, differentiating between the compact bone and the trabecular bone, was at 0.900 cm⁻¹.

## Statistical analysis

The analyzed factors included: sex, age and place in the bone. All the data are presented as means  $\pm$  SD. All calculations were performed using the Statistica 9.0 software (StatSoft, Inc. Tulsa, USA). The statistical analysis was performed using the three-way ANOVA and the post-hoc Tukey's test. For all comparisons, P-value  $\leq$ 0.05 was considered as statistically significant. Furthermore, correlations between the analyzed features and body and bone weight were tested using the Pearson's correlation coefficient (P $\leq$ 0.05).

### Results

The results of the performed statistical analyses were presented in Tables 1 to 3. The highest growth in the body weight was observed in males between 4 and 6 wk, whereas in females between 2 and 4 wk.

Table 1. Mean values of body weight (g) and bone weight (g), standard deviation ± SD and coefficient of variation CV (%) in male and female geese at several stages of the experiment

es females males males    CV			Body	Body weight				Bone weight	
x ±SD         CV         x±SD         CV         x±SD         CV           72 a,A±5.83         8.0         65 a,A±5.91         9.0         0.83 a,A±0.05         6.0           731 a,B±58.18         7.9         708 a,B±56.69         8.0         7.78 a,B±0.71         9.1           2220 a,C±199.80         9.0         2130 a,C±191.91         9.0         24.16 b,C±2.17         8.9           3760 b,D±300.55         7.9         3310 a,D±264.23         7.9         34.06 b,D±2.38         6.9           4450 a,E±400.52         9.0         4165 a,E±333.20         8.0         34.85 a,D±2.41         6.9	Age	males		females		males		females	
72 a,A±5.83 8.0 65 a,A±5.91 9.0 0.83 a,A±0.05 6.0 731 a,B±58.18 7.9 708 a,B±56.69 8.0 7.78 a,B±0.71 9.1 2220 a,C±199.80 9.0 2130 a,C±191.91 9.0 24.16 b,C±2.17 8.9 3760 b,D±300.55 7.9 3310 a,D±264.23 7.9 34.06 b,D±2.38 6.9 4450 a,E±400.52 9.0 4165 a,E±333.20 8.0 34.85 a,D±2.41 6.9		x ±SD	CV	S∓X	CV	X±SD	CV	ΩS∓X	CV
731 a,B±58.18 7.9 708 a,B±56.69 8.0 7.78 a,B±0.71 9.1 2220 a,C±199.80 9.0 2130 a,C±191.91 9.0 24.16 b,C±2.17 8.9 3760 b,D±300.55 7.9 3310 a,D±264.23 7.9 34.06 b,D±2.38 6.9 4450 a,E±400.52 9.0 4165 a,E±333.20 8.0 34.85 a,D±2.41 6.9	1 day	72 a,A±5.83	8.0	65 a,A±5.91	9.0	0.83 a,A±0.05	0.9	0.74 a,A±0.07	9.4
2220 a,C±199.80 9.0 2130 a,C±191.91 9.0 24.16 b,C±2.17 8.9 3760 b,D±300.55 7.9 3310 a,D±264.23 7.9 34.06 b,D±2.38 6.9 4450 a,E±400.52 9.0 4165 a,E±333.20 8.0 34.85 a,D±2.41 6.9	2 wk	731 a,B±58.18	7.9	708 a,B±56.69	8.0	7.78 a,B±0.71	9.1	7.38 a,B±0.66	8.9
3760 b,D±300.55 7.9 3310 a,D±264.23 7.9 34.06 b,D±2.38 6.9 4450 a,E±400.52 9.0 4165 a,E±333.20 8.0 34.85 a,D±2.41 6.9	4 wk	2220 a,C±199.80	0.6	2130 a,C±191.91	9.0	24.16 b,C±2.17	8.9	22.00 a,C±1.54	7.0
4450 a,E±400.52 9.0 4165 a,E±333.20 8.0 34.85 a,D±2.41 6.9	6 wk	3760 b,D±300.55	7.9	3310 a,D±264.23	7.9	34.06 b,D±2.38	6.9	32.35 a,D±2.26	6.9
	8 wk	4450 a,E±400.52	0.6	4165 a,E±333.20	8.0	34.85 a,D±2.41	6.9	33.58 a,D±2.35	6.9

a, b – means in rows (sex) designated with different letters are significantly different ( $P \le 0.05$ ). A-E – means in columns (age) designated with different letters are significantly different ( $P \le 0.05$ ).

Table 2. Mean values of the geometric parameters of the tibiotarsal bones (proximal metaphysis and midshaft) and standard deviation ± SD in male and female geese at several stages of the experiment

	4 I I I	Proximal metaphysis  CV	females  x ±SD  11.12 Aa±0.89  70.55 Ba±5.64  137.03 Ca±10.91  130.72 Ca±10.48  65.00 Ba±5.20  5.02 Aa±0.40  31.66 Ba# ±2.56  61.59 Ca#±4.93  58.85 Ca#±4.71	CV 7:9 8:0 8:0 8:0 8:0 8:0 8:0 8:0 8:0 8:0 8:0	males x±SD 3.79 Aa±0.31 18.96 ABb±1.52 84.92 Bb±6.79 65.00 Bb±5.21 60.61 Ba±4.88 1.70 ABa±0.13 8.53 Bb*±0.68 38.21 ABb*±3.0 3.06 29.27 Ab*±2.32	mid CV CV 8.1 8.0 7.9 8.0 7.6 7.9 8.0 7.6 7.9 8.0 7.6 7.9 8.0 7.6 7.9 8.0 7.6 7.9 8.0 8.0 7.6 7.9 8.0 8.0 7.6 7.9 8.0 8.0 8.0 8.0 8.0 8.0 8.0 8.0 8.0 8.0	midshaff    range   range   range	CV 7.9 8.0 7.7 7.7 7.9 7.9 7.9 7.9 7.9 7.9 7.9 7.9
8 wk 1 day 2 wk 4 wk 6 wk 8 wk	40.12 Aa*±3.10 0.29 Aa±0.02 0.48 Aa*±0.02 11.17 Ba*±0.79 37.14 Ca*±2.77 31.01 Ca±2.38	7.7 6.8 4.1 7.0 7.4	29.26 Aa±2.34 0.49 Aa±0.04 0.77 Aa#±0.06 12.14 Ba#±0.97 33.70 Ca#±2.66 33.96 Ca±2.72	7.9 8.1 7.7 7.9 7.8 8.0	27.31 Aa*±2.18 1.53 Aa±0.12 11.27 Bb*±0.91 18.25 Cb*±1.46 30.56 Db*±2.44 28.29 Da±2.26	7.9 7.8 8.0 8.0 7.9	29.26 Aa±2.34 2.11 Aa±0.16 8.35 Bb#±0.64 20.92 Cb#±1.67 27.14 Db#±2.17 33 96 Da±2 71	7.9 7.5 7.6 7.9 7.9

A-D - means in columns designated with different letters are different (P<0.05).

a, b − means in rows designated with different letters are different (P≤0.05).

\* - identical superscript symbols indicate differences in males between the position in the bone (proximal metaphysis vs. mid-diaphysis); means in rows for males are different  $(P \le 0.05)$ .

# - identical superscript symbols indicate differences in females between the place in the bone (proximal metaphysis vs. mid-diaphysis); means in rows for males are different  $(P \le 0.05)$ .

Table 2 a. Mean values of the geometric parameters of the tibiotarsal bones (proximal metaphysis and midshaft) and standard deviation ± SD in male and female geese at several stages of the experiment

				2	Measurement point	ant point			
Analyzed	-		proxin	proximal metaphysis			mid	midshaft	
feature	Age	males		females		males		females	
		x ±SD	CV	x ±SD	CV	x ±SD	CV	x ±SD	CV
	1 day	10.88 Aa±0.78	7.1	11.79 Aa*±0.92	7.8	6.88 Ab±0.55	7.9	7.33 Ab*±0.59	8.0
	2  wk	29.62 Ba*±2.26	9.7	29.59 Ba#±2.38	8.0	15.40 Ab*±1.23	7.9	14.49 Ab#±1.16	8.0
PERI_C	4 wk	33.44 Ca±2.55	7.6	41.40 Ca#±3.27	7.8	29.26 Bb±2.32	7.9	22.38 Bb#±1.79	7.9
(mm)	6 wk	$44.28 \text{ Ca*} \pm 3.44$	7.7	40.53 Ca#±2.24	5.5	28.57 Bb*±2.29	8.0	26.73 Bb#±2.13	7.9
	8 wk	33.06 Ba±3.48	10.5	28.55 Ba±3.34	11	29.15 Bb±2.33	7.9	28.55 Bb±2.28	7.9
	1 day	10.72 Aa±0.57	5.3	11.53 Aa#±0.92	7.9	5.27 Ab±0.42	7.9	5.18 Ab#±0.41	7.9
c f	2  wk	$29.52 \text{ Ba*} \pm 2.31$	7.7	29.40 Aa#±2.35	7.9	9.76 Bb*±0.78	7.9	10.23 Bb#±0.81	7.9
ENDO_C	4 wk	30.01 Ba±2.11	7.0	39.52 Aa#±3.16	7.9	22.08 Bb±1.76	7.9	15.43 Bb#±1.23	7.9
(mm)	6 wk	38.65 ABa*±3.07	7.9	34.89 ABa #± 3.4 2.77	7.6	20.77 BCb*±1.68	8.3	19.33 BCb#±1.55	8.0
	8 wk	26.23 Ba*±2.08	7.9	19.71±1.56	7.9	$20.10*\pm1.61$	8.0	19.71±1.56	7.9
	1 day	0.19 Aa±0.01	5.2	0.351 Aa±0.028	7.9	0.61 Aa±0.05	8.1	0.95 Aa±0.07	7.3
Š	2  wk	$0.56 \text{ Aa} \pm 0.04$	7.1	$0.258 \text{ Aa} \pm 0.021$	8.1	10.36 Ba±0.83	8.0	7.67 Aa±0.61	7.9
SSI	4 wk	40.01 Ba±3.20	7.9	26.02 Bb#±2.08	7.9	45.89 Ca±3.68	8.0	39.71 Ba#±3.17	7.9
(cuiii)	6  wk	$106.76 \text{ BC a}^{*\pm} 8.3$	7.7	$100.07 \text{ BCa\#} \pm 3.4 247.98$	3.3	79.30 Da*±6.32	7.9	68.84 Cb#±5.52	8.0
	8  wk	90.54 Ca*±7.23	7.9	91.75 Ca±7.34	8.0	76.59 Da*±6.16	8.0	89.94 Db±7.19	7.9
	1 day	$0.03 \text{A a}^* \pm 0.003$	10	$0.04 \text{ Aa#} \pm 0.003$	7.5	$0.26 \text{ Aa} \pm 0.02$	9.7	$0.34 \text{ Ab} \pm 0.02$	5.8
CRT	2  wk	$0.02A \ a^* \pm 0.001$	5	$0.03 \text{ Aa#} \pm 0.004$	13	$0.90 \text{ Ba} \pm 0.08$	8.8	$0.68 \text{ Ab} \pm 0.05$	7.3
THK_C	4  wk	$1.10 \text{ Ba} \pm 0.09$	8.1	$0.30 \text{ Aa#} \pm 0.002$	9.0	$1.14 \text{ Ba} \pm 0.09$	7.8	$1.11~Ba\pm0.10$	0.6
(mm)	6  wk	$0.90B \ a^* \pm 0.06$	9.9	$0.90 \text{ Ba} # \pm 0.07$	7.7	$1.24 \text{ Ba} \pm 0.11$	8.8	$1.18 \text{ Ba} \pm 0.08$	6.7
	8 wk	$1.09B a \pm 0.08$	7.3	$1.42 \text{ Cb} \pm 0.13$	9.1	$1.19 \text{ Ba} \pm 0.08$	6.7	$1.41 \text{ Bb} \pm 0.14$	6.6

A-D-means in columns designated with different letters are different ( $P\le0.05$ ).

a, b − means in rows designated with different letters are different (P≤0.05).
\* − identical superscript symbols indicate differences in males between the position in the bone (proximal metaphysis vs. midshaft); means in rows for males are statistically significantly different (P<0.05).

# - identical superscript symbols indicate differences in females between the place in the bone (proximal metaphysis vs. midshaft); means in rows for males are different  $(P \le 0.05)$ .

Table 3. Pearson's correlation coefficient of body weight and morphological and geometric parameters in male and female geese at several stages of the experiment determined in proximal metaphysis and midshaft

				Provinces incompany and and	area mine and			
		Proximal metaphysis	taphysis			Midshaft	haft	
Age	u	males	fem	females	m	males	fen	females
	BW	bone mass	BW	bone mass	BW	bone mass	BW	bone mass
				TOT_A				
1 day	-0.49	-0.16	-0.16	-0.05	60.0	-0.01	0.72	-0.78
2 wk	-0.52	*86.0	0.94	96.0	0.78	0.88	98.0-	66.0-
4 wk	*66.0-	-0.70	0.82	0.79	0.63	0.89	-0.65	0.65
6 wk	-0.43	-0.07	-0.57	-0.92	0.53	0.57	*66.0-	*66.0-
8 wk	*66.0-	*86.0-	*66.0-	*06.0-	0.61	0.77	*66.0-	*06.0-
				$TRAB_A$				
1 day	-0.50	-0.18	-0.16	-0.07	60.0	-0.01	0.71	-0.79
2 wk	-0.88	0.74	0.99	0.98	0.78	0.88	-0.86	66.0-
4 wk	*66.0-	0.71	0.82	0.79	0.63	0.81	-0.65	0.65
6 wk	0.25	0.58	-0.51	-0.95	0.54	0.57	*66.0-	*66.0-
8 wk	*66.0-	*86.0-	*66.0-	*06.0-	09.0	97.0	*66.0-	*06.0-
				$CRT_A$				
1 day	*49.0-	*0.70	-0.33	90.0-	-0.54	0.02	-0.24	-0.44
2 wk	-0.56	09.0-	86.0-	-0.97	0.29	0.47	-0.86	-0.95
4 wk	09.0	-0.25	0.72	0.26	0.57	0.71	0.49	-0.49
6 wk	0.26	-0.11	69:0-	-0.86	0.03	0.04	*6.0-	-0.95*
8 wk	-0.09	-0.29	-0.80	69:0-	0.80	99.0	-0.80	*69.0-

\*Correlations are statistically significant at P<0.05.

Table 3 a. Pearson's correlation coefficient of body weight and morphological and geometric parameters in male and female geese at several stages of the experiment determined in proximal metaphysis and midshaft

		Description	i oral a cho			:JV	Midohoft	
		FIUXIIIIALI	rioxiiiiai iiietapiiysis			IMI		
Age	TI.	males	fem	emales	m	males	fen	females
	BW	bone mass	BW	bone mass	BW	bone mass	BW	bone mass
				PERI_C				
1 day	-0.49	-0.16	-0.15	-0.04	0.10	-0.00	0.73	-0.79
2 wk	*76.0	-0.53	96.0	0.97	0.77	0.87	98.0-	-0.99
4 wk	-0.58	0.27	0.83	0.79	0.65	0.81	89.0-	89.0
6 wk	*86.0-	-0.84*	-0.57	-0.92	0.53	0.57	-1.00*	-0.99*
8 wk	*66.0-	*26.0-	*66.0-	*06.0-	0.61	0.77	*66.0-	*06.0-
				ENDO_C				
1 day	60.0-	0.31	-0.08	-0.04	0.28	-0.02	0.87	-0.41
2 wk	0.97	-0.07	0.97	0.97	0.85	68.0	-0.81	96.0-
4 wk	09.0-	0.26	0.63	0.73	99.0	0.83	69.0-	69.0
6 wk	0.25	0.58	0.70	0.85	0.76	0.80	*66.0-	*66.0-
8 wk	*86.0-	-0.92*	*66.0-	-0.92*	-0.11	0.11	*66.0-	-0.92*
				ISS				
1 day	*89.0-	-0.72*	-0.26	0.02	-0.64	-0.24	-0.08	-0.57
2 wk	86.0	-0.51	-0.89	-0.92	0.32	0.58	-0.87	-0.99
4 wk	66.0	0.71	0.74	0.33	0.57	0.72	99.0	-0.66
6 wk	09.0	0.27	-0.76	-0.80	0.34	0.36	*66.0-	*66.0-
8 wk	-0.42	-0.57	*96.0-	-0.82*		0.87	*96.0-	-0.82*
				CRT_THK_C				
1 day	*89.0-	-0.71*	-0.31	-0.03	·	0.03	-0.52	-0.16
2 wk	86.0	-0.50	-0.91	*/6.0-		-0.37	0.23	0.35
4 wk	0.97	0.82	0.62	0.22	-0.01	-0.01	*69.0	-0.67*
6 wk	86.0	86.0	*89.0-	98.0-	-0.29	-0.30	0.99	0.98
8 wk	0.73*	0.56	0.15	0.21	0.62	0.43	0.15	0.21

\*Correlations are statistically significant at  $P \le 0.05$ .

The highest increase in weight of tibia bones in both sexes occurred between 2 and 4 wk of life (Table 1).

The TOT\_A at the proximal metaphysis was higher than at mid-diaphysis ( $P \le 0.05$ ). The TOT\_A values in males in the postnatal development increased from the first day to the sixth week of life. In eight-week-old males, in turn, TOT\_A values decreased at the two studied places of the bone ( $P \le 0.05$ ). TOT\_A values of males decreased at mid-diaphysis in 6 wk compared to 4 wk ( $P \le 0.05$ ) (Table 2).

The values of the TRAB\_A and CRT\_A in males at the two analyzed places in the tibiotarsal bone increased until the sixth week of life and decreased in eight-week-old birds ( $P \le 0.05$ ). TRAB\_A values of males decreased at mid-diaphysis in 6 wk compared to 4 wk ( $P \le 0.05$ ).

The PERI C at proximal metaphyses of tibiotarsal bones in males increased in the postnatal development until the sixth week of life and decreased in the eighth wk (P $\le$ 0.05). PERI C values decreased by 11.22 mm (P $\le$ 0.05) in 8 wk compared to 6 wk (Table 2 a). In turn, the parameter at the mid-diaphysis of the analyzed bones increased throughout the entire period of observations (P≤0.05). The values of the ENDO C in males increased during the entire postnatal development both at proximal metaphyses and at half the length of diaphyses until the sixth week of life (P≤0.05) and decreased in eight-week-old males, with the value of the parameter decreasing by an average of 0.67 mm at half the length of diaphyses and by an average of 12.4 mm in the area of proximal metaphyses (P \le 0.05). The maximum values of the SSI at the two places of the bone in males were observed in six-week-old birds, with the values of this parameter decreasing in the eighth week of life by 16.22 mm<sup>3</sup> at proximal metaphyses and by 2.71 mm<sup>3</sup> at mid-diaphyses (P≤0.05). CRTH C values decreased in the group of 8 wk males in mid-diaphysis compared to 6 wk males (P≤0.05). SSI values in males in both bone sections attenuated in 8 wk compared to 6 wk ( $P \le 0.05$ ) (Table 2 a).

In females the values of TOT\_A, TRAB\_A, PERI\_C and ENDO\_C at the proximal metaphyses of tibiotarsal bones decreased in the sixth and eighth week of the postnatal development compared to 4 wk ( $P \le 0.05$ ). Moreover, in 8 wk females in proximal metaphyses of the tibiotarsal bone, SSI attenuated compared to 6 wk females by  $8.32 \text{ mm}^3$  ( $P \le 0.05$ ) (Tables 2 and 2 a).

Significant differences between males and females were found in the values of the ENDO\_C in eight-week-old geese and in the values of the SSI in four-week-old birds, with the values of the two parameters being significantly higher in males compared to females ( $P \le 0.05$ ).

Values of Pearson's correlation coefficient of body weight and morphological and geometric parameters in male and female geese at several stages of the experiment determined in proximal metaphysis and midshaft were presented in Tables 3 and 3 a.

In four- and eight-week-old males at proximal metaphyses TOT\_A correlated negatively with body weight, with the values of the correlations being r = -0.99 and r = -0.99 respectively (P $\le$ 0.05), and with bone weight in two- and eight-week-old geese. TRAB\_A correlated negatively with body weight in the fourth (r = -0.99) and eighth (r = -0.99) week of life, and with bone weight in eight-week-old geese in males. Negative correlations were also observed between CRT\_A and body and

bone weight in one-day-old males ( $P \le 0.05$ ). PERI\_C correlated negatively with body weight in the 2nd, 6th and 8th week of life and with bone weight in six- and eight-week-old males in proximal metaphysis ( $P \le 0.05$ ). ENDO\_C in eight-week-old males correlated negatively with body weight and bone weight ( $P \le 0.05$ ).

A negative correlation was observed in one-day-old males in proximal metaphysis between the SSI and body weight and bone weight, with the correlation coefficient being r = -0.68 and r = -0.72, respectively (P $\le$ 0.05). In the group of males, a negative correlation was also observed in proximal metaphyses between CRT\_THK\_C and body weight on the first day of life: r = -0.68, whereas in the 8th week, the correlation was positive and amounted to r = 0.73 (P $\le$ 0.05) (Tables 3 and 3 a). The above-mentioned parameters at mid-diaphyses did not correlate negatively with body weight and bone weight at any stage of the postnatal development in males (P $\le$ 0.05) (Tables 3 and 3 a).

In eight-week-old females the TOT\_A, TRAB\_A, PERI\_C, ENDO\_C, SSI parameters at proximal metaphyses correlated negatively with body weight and bone weight ( $P \le 0.05$ ). However, considering CRT\_THK\_C, the correlations were negative in 6 wk in relation to body weight r = -0.68, whereas in relation to bone weight, the correlations were negative in 2 wk: r = -0.97 ( $P \le 0.05$ ) (Tables 3 and 3 a). In turn, at mid-diaphyses in females TOT\_A and TRAB\_A, PERI\_C, ENDO\_C and SSI correlated negatively with body and bone weight in the 2, 6 and 8 week of life ( $P \le 0.05$ ). Negative correlations were also found in females between CRT\_A and CRT\_THK\_C and body weight and bone weight ( $P \le 0.05$ ). The highest number of negative correlations between geometric parameters and body weight and bone weight were found in six- and eight-week-old females (Tables 3 and 3 a).

## **Discussion**

The analyzed broiler geese (White Kołuda® geese-W31) were subjected to intensive breeding, as a result of which they attained slaughter maturity in the 8 wk of life, half as quickly as in the case of the traditional, semi-intensive breeding (from 17 to 24 weeks). Thus, as a result of such fast growth and significant increase in body weight, the skeleton of geese was particularly susceptible to all deformations and fractures. The performed analyses made it possible to determine the period in which the geometric parameters, responsible for the optimal mechanical endurance of the skeleton of geese, decrease.

It was found that in eight-week-old male geese the values of all the analyzed parameters, including the strength strain index SSI, decreased at proximal metaphyses. At half the length of diaphysis in 8-week-old males, too, a decrease was observed in the values of all the parameters, with the exception of PERI\_C. Whereas in females, the values of TOT\_A, TRAB\_A and PERI\_C attenuated in 6 wk, two weeks earlier than in males (it was the period of the most intensive increase of the body weight in females). The performed research also proved that 6-week-old female geese were susceptible to deformations and fractures of the tibiotarsal bone (30% of the ana-

lyzed females), with the deformations being observed in the 6 and 8 wk of life, which is at a slightly later stage compared with males (10% of the analyzed males).

The research on the development of tibia bones in geese under semi-intensive traditional breeding (until the 16th week of life) was also conducted using Trabecula® software (Czerwiński, 1994). The Trabecula® program used in the research analyzed the radiological image with a resolution of 0.096 mm and distinguished radiological trabeculae in accordance with the adopted definition. The programme was based on an appropriate algorithm used to distinguish radiological trabeculae in accordance with the definition and based on the specific parameters, including the angle and level of a microdensitometric curve (Charuta et al., 2012 b). The application of these two research methods showed that a critical moment in the development of tibia bones in broiler geese, bred till 8 wk, was 6 wk and concerned mostly females, whereas in a group of geese bred to 16 wk, most deformities and fractures of tibia bones as well as problems with walking were observed in 6-week-old males, in which the lowest number, volume and density of radiological trabeculae was found (Charuta et al., 2012 b).

The Trabecula® software was also used to analyze the tibiotarsal bones of the Peking domestic ducks (Charuta et al., 2011). The study showed that in six-week-old females the number, density and volume of radiological trabeculae at proximal metaphyses decreased. In male ducks, in turn, no statistically significant decrease in the values characterizing the trabecular bone was found, unlike in geese (Charuta et al., 2011, 2012 b).

Growth processes of tibia bones in the postnatal period were also observed in turkeys (Tatara et al., 2007; Charuta et al., 2012 a). The analysis of densitometric parameters with the use of computed tomography showed that vBMD in diaphyses increased from 3 wk in both sexes from 688 mg/cm³ (3 wk) to 532 mg/cm³ (16 wk). The decrease of vBMD had a significant influence on health condition of the birds. Attenuation of vBMD values in metaphyses and diaphyses of tibia bones led to the decrease of bone resistance to fractures. Impaired walking or a total lack of possibility of moving resulting from deformities of tibia bones led to deformities of thoracic muscles, thus decreasing the profitability of poultry production. Crespo et al. (1999) pointed out limb problems in male turkeys. In that case, problems concerned the femur in male turkey of commercial breeding flock.

The research conducted on broiler chickens using pQCT showed that attenuation of vBMD occurred in metaphyses of tibia bones in 4 wk (males). In females, however, vBMD decreased in 6 wk. Thus, in broiler chickens, males were earlier prone to deformities and fractures of tibia bones than females (Charuta et al., 2013 b).

Life cycle changes in bone mineralization and bone size traits of the tibia and humerus were evaluated in commercial male and female broilers using dual-energy x-ray absorptiometry (DEXA) by Talaty et al. (2009). The research showed that tibia continued to grow, especially after the initiation of the growth spurt at 3 to 4 wk of age, as indicated by bone length, width, and BMC, but it did not become denser in minerals after 4 wk of age as its surface area increased.

The frequency of occurrence of limb problems in broiler chickens bred in traditional production systems in Denmark was analyzed by Sanotra et al. (2001). It was

concluded that the prevalence of leg problems in broilers in conventional production systems is very high and compromises the welfare of the birds. One of the main factors responsible is their high growth rate. The risks of the occurrence of these leg problems were significantly influenced by body weight and sex of the chicks. Shim et al. (2012) pointed out limb problems in broiler chickens bred intensively. Fast-growing broilers are especially susceptible to bone abnormalities, causing major problems for broiler producers. The cortical bones of fast-growing broilers are highly porous, which may lead to leg deformities. Leg problems were investigated in 6-week-old Arkansas random-bred broilers (Shim et al., 2002).

Analysing the value of densitometric parameters during the postnatal development, it was stated that attenuating values of vBMD were the sign of insufficient adaptation of tibia bones to increasing body weight and led to the decrease of resistance of tibia bones to fractures.

In research on other species, Tatara et al. (2012 b) stated lack of sexual dimorphism between females and gonadectomized male Polish Landrace pigs. The conducted tests showed lack of sexual dimorphism in the values of following parameters decisive for the bone condition: trabecular BMD, cortical BMD and cross-sectional area, second moment of inertia, mean relative wall thickness, cortical index, maximum elastic strength, and ultimate strength. However, in contrast to the current study, the study on pigs was performed on hormonally impaired males (Tatara et al., 2012).

In the studies on tibia model of turkeys conducted by Krupski and Tatara (2006), bone weight, bone length, cortical bone density, bone volume, maximum elastic strength, and ultimate strength were positively correlated. In the study concerning vertebral bones in pigs, a positive correlation was found between the investigated parameters describing bone morphology, namely bone weight and total bone volume (Tatara et al., 2007). Also, tests on correlations between morphometric and densitometric parameters of thigh bones in male Polish Merino sheep conducted by Tatara et al. (2011 b) have shown positive correlations between mean vBMD, trabecular BMD, and cortical BMD.

The solutions aiming at the improvement of the bone quality in geese (Charuta et al., 2012 b), other poultry species (Charuta et al., 2013 a, b; Tatara et al., 2015) and domesticated animals (Andersen et al., 2008; Krupski et al., 2011) may include enriching the animal diet with mineral additives, such as Ca, P, Mg, Cu, Zn (Applegate et al., 2003; Puzio et al., 2004; Angel et al., 2006; Kozłowski et al., 2010; Krupski et al., 2011; Tatara et al., 2011), vitamins (vitamin D) (Tatara et al., 2011 a), precursors and metabolites of amino acids (alpha-ketoglutarate, ornithine alpha-ketoglutarate, 3-hydroxy-3-methylbutyrate) (Tatara et al., 2004, 2006, 2008; Sierant-Rozmiej et al., 2010; Krupski et al., 2011), di- or tri-peptids and to provide animals with optimal density and breeding temperature.

In summary, it should be stressed that the critical moment in the postnatal development of broiler geese is the sixth week of life and concerns mostly males. The values of geometric parameters of the tibiotarsal bones, used as model bones in the study on the skeleton of farm birds, decrease dramatically and significantly. It is corroborated by a decrease of the strength strain index at proximal metaphysic of the tibiotarsal bone both in males and in females. At mid-diaphysis of the analyzed

bones, the value of SSI decreases only in males in the final stage of the fattening, i.e. in the eighth week of life.

The achieved results and observations justify the use of optimal environmental and nutritional conditions in geese in order to improve the quality of their skeletal system and to minimise the risk of the occurrence of deformities and fractures of the pelvic limb during the breeding.

#### References

- Andersen N.K., Tatara M.R., Krupski W., Majcher P., Harrison A.P. (2008). The long-term effect of alpha-ketoglutarate, given early in postnatal life, on both growth and various bone parameters in pigs. J. Anim. Physiol. Anim. Nutr., 92: 519–528.
- Angel R., Saylor W.W., Mitchell A.D., Powers W., Applegate T.J. (2006). Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poultry Sci., 85: 1200–1211.
- Applegate T.J., Webel D.M., Lei X.G. (2003). Efficacy of a phytase derived from *Escherichia coli* and expressed in yeast on phosphorus utilization and bone mineralization in turkey poults. Poultry Sci., 82: 1726–1732.
- Barreiro F.R., Sagula A.L., Junqueira O.M., Pereira G.T., Baraldi-Artoni S.M. (2009). Densitometric and biochemical values of broiler tibias at different ages. Poultry Sci., 88: 2644-2648.
- Barreiro F.R., Baraldi-Artoni S.M., do Amaral L.A., Barbosa J.C., Girardi A.M., Pacheco M.R., Amoroso L. (2011). Determination of broiler femur parameters at different growth phases. Int. J. Poultry Sci., 10: 849–853.
- Biesiada-Drzazga B., Grużewska A., Markowska M. (2008). Impact of feeding based on concentrated mixtures with different shares of soybean and sunflower oil meals on growth and body conformation in geese. Arch. Geflugelkd., 72: 19–24.
- 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. Pol. J. Vet. Sci., 15: 537–545.
- 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 Sci., 90: 830–835.
- Charuta A., Cooper R.G., Pierzchała M., Horbańczuk J.O. (2012 a). Computed tomographic analysis of tibiotarsal bone mineral density and mineral content in turkeys as influenced by age and sex. Czech. J. Anim. Sci., 57: 572–578.
- Charuta A., Dzierzęcka M., Czerwiński E., Cooper R.G., Horbańczuk J.O. (2012b). Sex- and age-related changes of trabecular bone of tibia in growing domestic geese (*Anser domesticus*). Folia. Biol. (Kraków), 60: 205–212.
- Charuta A., Dzierzęcka M., Komosa M., Kalinowski Ł., Pierzchała M. (2013 a). Ageand sex-related differences of morphometric, densitometric and geometric parameters of tibiotarsal bone in Ross broiler chickens. Folia Biol. (Kraków), 61: 211–220.
- Charuta A., Dzierzęcka M., Pierzchała M., Cooper R.G., Poławska E., Horbańczuk J.O. (2013 b). Sex-related differences of morphometric, densitometric and geometric parameters of tibia and tarsometatarsal bone in 14-month-old ostriches (*Struthio camelus*). Poultry Sci., 92: 2965–2976.
- Charuta A., Dzierzęcka M., Pierzchała M., Cooper R.G., Poławska E., Horbańczuk J.O. (2013 c). Sex-related differences of morphometric, densitometric and geometric parameters of tibia and tarsometatarsal bone in 14-month-old ostriches (*Struthio camelus*). Poultry Sci., 92: 2965–2976.
- Charuta A., Tatara M.R., Grużewska A., Pierzchała M., Kalinowski Ł., Trusewicz M., Łuszczewska-Sierakowska M. (2014). Morphological and densitometric re-

- search of the tibial bone in the post-natal development in domestic geese. Anim. Sci. Pap. Rep., 32: 251–260
- Cooper R.G., Naranowicz H., Maliszewska E., Tennett A., Horbańczuk J.O. (2008). Sex-based comparison of limb segmentation in healthy and tibiotarsal rotation ostriches aged 14 months. J. S. Afr. Vet. Assoc., 79: 142–144.
- Crespo R., Stover S.M., Droual R., Chin R.P., Shivaprasad H.L. (1999). Femoral fractures in a young male turkey breeder flock. Avian Dis., 43: 150–154.
- Czerwiński E. (1994). Quantitative evaluation of changes occurring in the spongious cortical bone influenced by fluorine and their diagnostics meaning. Postdoctoral dissertation of the Medical College of the Jagiellonian University, Krakow, Poland.
- Hester P.Y., Krueger K.K., Jackson M. (1990). The effect of restrictive and compensatory growth on the incidence of leg abnormalities and performance of commercial male turkeys. Poultry Sci., 69: 1731–1742.
- Horbańczuk J.O., Huchzermeyer R., Parada R., Płaza K. (2004). Four-legged ostrich (Struthio camelus) chick. Vet. Rec., 154: 23, 736.
- Kozłowski K., Jankowski J., Jeroch H. (2010). Efficacy of *Escherichia coli*-derived phytase on performance, bone mineralization and nutrient digestibility in meat-type turkeys. Vet. Med. Zoot., 52: 59–65.
- Krupski W., Tatara M.R. (2007). Interrelationships between densitometric, morphometric, and mechanical properties of the tibia in turkeys. Bull. Vet. Inst. Pulawy, 51: 621–627.
- Krupski W., Tatara M.R., Rosenbeiger P., Niedziela D. (2011). Effects of combined administration with 3-hydroxy-3-methylbutyrate (HMB), calcium vitamin D<sub>3</sub> on skeletal system properties in sheep. Bone, 48: 393.
- Lewis P.D., Danisman R., Gous R.M. (2009). Photoperiodic responses of broilers. III. Tibial breaking strength and ash content. Brit. Poultry Sci., 50: 673–679.
- Puzio I., Bieńko M., Radzki R., Kapica M., Filip R. (2004). Influence of phytase and calcitrol on mechanical parameters of femora in broiler chickens. Med. Weter., 60: 1103–1105.
- Sanotra G.S., Lunda J.D., Ersbolla A.K., Petersena J.S., Vestergaarda K.S. (2001). Monitoring leg problems in broilers: a survey of commercial broiler production in Denmark. World. Poultry Sci. J., 57: 55–69.
- Shim M.Y., Karnuah A.B., Mitchell A.D., Anthony N.B., Pesti G.M., Aggrey S.E. (2012). The effects of growth rate on leg morphology and tibia breaking strength, mineral density, mineral content, and bone ash in broilers. Poultry Sci., 91: 1790–1795.
- Sierant-Rozmiej N., Tatara M.R., Krupski W., Rosenbeiger P., Niedziela D., Studziński T. (2010). Antiosteopenic effect of ornithine alpha-ketoglutarate (OKG) administration in female turkeys. Bone, DOI:10.1016/j.bone.2010.04.219
- Talaty P.N., Katanbaf M.N., Hester P.Y. (2009). Life cycle changes in bone mineralization and bone size traits of commercial broilers. Poultry Sci., 88: 1070–1077.
- Tatara M.R. (2006). Current methods for *in vivo* assessment of the skeletal system in poultry. Med. Weter., 62: 266–269.
- Tatara M.R., Pierzynowski S.G., Majcher P., Krupski W., Brodzki A., Studziński T. (2004). Effect of alpha-ketoglutarate (AKG) on mineralisation, morphology and mechanical endurance of femur and tibia in turkey. Bull. Vet. Inst. Pulawy., 48: 305–309.
- 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. Med. Weter., 61: 225–228.
- Tatara M.R., Śliwa E., Krupski W., Brodzki A., Pasternak K. (2006). Ornithine alphaketoglutarate increases mineralization and mechanical properties of tibia in turkeys. Bone, 39: 100–105.
- 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 Rum. Res., 67: 179–183.
- Tatara M.R., Śliwa E., Krupski W., Worzakowska M. (2008). 3-Hydroxy-3-methylbutyrate administration diminishes fundectomy-induced osteopenia of the lumbar spine in pigs. Nutrition, 24: 753–760.

- Tatara M.R., Krupski W., Jankowski M., Zduńczyk Z., Jankowski J. (2011 a). Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkey. Brit. Poultry Sci., 52: 718–729.
- Tatara M.R., Krupski W., Tymczyna B., Majcher P., Rosenbeiger P., Niedziela D. (2011 b). Interrelationships between densitometric, morphometric, and mechanical properties of the femur in male Polish Merino sheep. Bull. Vet. Inst. Pulawy, 55: 795–799.
- Tatara M.R., Krupski W., Tymczyna B., Łuszczewska-Sierakowska I. (2012). Biochemical bone metabolism markers and morphometric, densitometric and biomechanical properties of femur and tibia in female and gonadectomised male Polish Landrace pigs. J. Pre-Clin. Clin. Res., 6: 14–19.
- Tatara M.R., Krupski W., Kozłowski K., Drażbo A., Jankowski J. (2015). Effects of administration of four different doses of *Escherichia coli* phytase on femur properties of 16-week-old turkeys. BMC Vet. Res., 11: 69. DOI: 10.1186/s12917-015-0385-x.
- Tykałowski B., Stenzel T., Koncicki A. (2010). Selected problems related to ossification processes and their disorders in birds. Med. Weter., 66: 464–469.
- Tymczyna B., Bachanek T., Tatara M.R., Krupski W., Tymczyna-Sobotka M., Łuszczewska-Sierakowska I. (2012). Interrelationships between morphometric, densitometric, and mechanical properties of mandible in 6-month-old male pigs. Bull. Vet. Inst. Pulawy, 56: 177–181.
- Yair R., Uni Z., Shahar R. (2012). Bone characteristics of late-term embryonic and hatchling broilers: Bone development under extreme growth rate. Poultry Sci., 91: 2614–1620.
- Yair R., Shahar R., Uni Z. (2013). Prenatal nutritional manipulation by in ovo enrichment influences bone structure, composition, and mechanical properties. J. Anim. Sci., 91: 2784–2793.

Received: 18 I 2015 Accepted: 3 VIII 2015