PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online) Folia Biologica (Kraków), vol. 61 (2013), No 3-4
© Institute of Systematics and Evolution of Animals, PAS, Kraków, 2013 doi:10.3409/fb61_3-4.211

Age- and Sex-related Differences of Morphometric, Densitometric and Geometric Parameters of Tibiotarsal Bone in Ross Broiler Chickens

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Accepted May 15, 2013

CHARUTA A., DZIERZĘCKA M., KOMOSA M., KALINOWSKI Ł., PIERZCHAŁA M. 2013. Age- and sex-related differences of morphometric, densitometric and geometric parameters of tibiotarsal bone in Ross broiler chickens. Folia Biologica (Kraków) **61**: 211-220.

For the first time computed tomography has been used to analyze densitometric and geometric parameters in proximal metaphyses and the mid-diaphyses of tibiotarsal bones in broiler chickens in posthatching development as influenced by age and sex. The research was conducted on 60 tibial bones of 2-, 4- and 6-week-old broiler chickens (Ross 308) (10 males and 10 females in each age group). Statistical analysis has been conducted with the use of one-way ANOVA and Fisher's exact test. Calculations have been performed separately for each sex, with age as a differentiation variable and separately for each of the developmental stages, with sex as a differentiation factor. Pearson's correlation coefficient have been calculated. Also, relative bone density has been determined. It was observed that volumetric bone mineral density (vBMD) in the diaphyses was two times higher (app. 550 cm^3) than in the proximal metaphyses (app. 230 cm^3) of the tibiae in broiler chickens. In the proximal metaphyses of the tibiotarsal bones, densitometric and geometrical parameters increased with age of the birds. Densitometric parameters (vBMD, BMC) in 6-week-old males displayed (slightly) higher values than in females. It is worth emphasising that in both sexes bone mineral content (BMC) was higher in the tibial proximal metaphyses than in the diaphyses. At the mid-diaphysis, most of the densitometric and geometrical parameters, i.e. bone mineral content (BMC), Strength-Strain Index (SSI), periosteal circumference (PERI_C), endosteal circumference (ENDO_C), cortical area (CRT_A), trabecular area (TRAB_A) and bone area (TOT_A), tended to grow with the birds' age. vBMD (volumetric bone mineral density *in situ*) is the only parameter that did not grow with age. It was also observed that in males in 4 wk, vBMD in the proximal metaphyses displayed the lowest values during posthatching development (217.47 cm^3). Also between 2 and 4 wk of the development, vBMD in the diaphyses decreased from 637.64 cm^3 to 539.12 cm^3, ($P \le 0.05$). What is important is that vBMD and BMC are parameters that play a key role in bone mineralization. However, there was no correlation observed between them. Analysis of Pearson's correlation coefficient showed that BMC in both bone sections displayed positive correlation with all the geometric and densitometric parameters (except for vBMD). It may be concluded that vBMD is independent of other parameters.The strongest correlations were observed between BMC and TRAB_A, and TOT_A in the proximal metaphysis, they were (r = 0.98) and (r = 0.98) respectively, $P \le 0.01$, and between BMC and CRT_A at the mid-diaphysis (r = 0.99), $P \le 0.05$. It was demonstrated that in the group of males, decreasing vBMD in the proximal metaphyses between 2 and 4 wk and in the diaphyses from 2 to 6 wk during posthatching development, as well as decreasing relative bone weight, from 1.03% to 0.79%, led to deformities and breaks of the tibiae, which may have a negative effect on the productivity of the broiler chickens flock.

Key words: Volumetric bone mineral density (vBMD), bone mineral content (BMC), broiler chickens, tibia.

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The internal structure of bones including densitometric and geometric parameters is a fundamental factor counteracting extensive usage of the bone tissue while it performs its basic functions: supportive, locomotor and protective. Modern farming led to a very high pace of birds' growth, particularly to a fast gain of muscle weight. Thus, tibial bones are strongly loaded by muscles and more prone to various mineralization disorders and even fractures (CHARUTA *et al.* 2011). Disorders in the development of pelvic limbs (OLKOWSKI *et al*. 2011; STOFANIKOWA *et al*. 2011) as well as the attenuation of bone density BMD in tibiotarsal bones were observed in many species of poultry: laying hens (JEDRAL *et al*. 2008), broiler chickens (BOND *et al*. 1991; WILLIAMS *et al*. 2004; SHIM *et al.* 2012), ducks (CHARUTA *et al*. 2011, 2012a), geese (CHARUTA *et al.* 2012d), turkeys (CHARUTA *et al*. 2012b, 2012c; CRESPO *et al.* 2000) and quails (CHARUTA *et al.* 2013). The defects were also found in ostriches (COOPER *et al.* 2008; HORBAŃCZUK *et al.* 2004). The problems with lower limb bones concern mainly males (CHARUTA *et al.* 2012c; TALATY *et al.* 2010).

Deformities of tibiotarsal bones in broiler chickens may lead to a decrease in productivity of the flock resulting in bone fractures (RATH *et al.* 2000).

The factors influencing bone disorders may include improperly balanced diet and shortage of nutritional elements. The highest number of metabolic disorders occurs in the cancellous bone tissue (CHARUTA *et al.* 2008, 2011).

Material and Methods

All experiments were approved by the III Local Ethics Committee in Warszawa (application 73/2009 dated 19.11.2009).

The research was conducted on 60 tibiotarsal bones of 2-, 4- and 6-week-old broiler chickens (Ross 308) (10 males and 10 females in each age group). The birds were kept on a traditional bedding. Water and feed were provided *ad libitum*. The birds were fed with full-portion mixtures for broilers according to the age: Starter 1 at 1-3 wk (21% protein, 12 MJ), Grower at 3-5 wk (19% protein, 12.5MJ), Finisher at 5-6 wk (17% protein, 12.5MJ). The average use of the feed on 1 kg of the weight growth was 1.97 kg and the survival rate was app. 94%. Bird density: 10 birds/m^2, max 33 kg/m^2. Bedding type: long-cut rye straw. Bedding thickness: app. 6 cm.

The bones for analysis were cleaned of the muscles. Then, they were weighed on an AXIS AD 300 precise balance, range 300 g/ 0.001g and frozen for storing at -25^{o}C to -30^{o}C. Then, using a high resolution peripheral quantitative computed tomography (pQCT) XCT Research SA Plus (Stratec Medizintechnik GmbH, Pforzheim Germany), the analysis of the densitometric and geometric parameters of the tibiotarsal bones was conducted: vBMD – volumetric bone mineral density (mg/cm^3); density of the bone (*in situ*); BMC – bone mineral content per 1mm slice (mg/mm); mineral content of the total bone within a 1 mm slice; SSI – Strength-Strain Index in mm^3; PERI_C – periosteal circumference in the "circular ring model" in (mm); ENDO_C – endosteal circumference in the "circular ring model" in (mm); CRT_A – cortical area in (mm^2). The area that is assigned to be pure cortical; TRAB_A – trabecular area in (mm^2). Cross sectional area of the trabecular area after the cortical and subcortical areas were peeled off; TOT_A – total bone area in mm^2. Cross sectional area of the bone after the tissue was peeled off.

Computed tomography independently determined the density both of the trabecular bone and the compact bone.

Tomographical analysis of the proximal metaphysis was performed at 18% of the bone length, whereas the analysis of the middle of the diaphysis was performed at 50% of the bone length at the voxel of 0.07 mm and the scanning speed of 4 mm/min. The area for the analysis was determined after initial scanning (20 mm/s) and morphometric bone measurements. The threshold coefficient, differentiating the compact bone from the cancellous bone, was determined at 0.900 cm-1.

Statistical analysis

All data is presented as means ± SEM. The data was found to be normally distributed. Parametric test was used for statistical analysis (one-way ANOVA and Fisher's exact test). Calculations were performed separately for each sex, with age as a differentiation variable and separately for each of the developmental stages, with sex as a differentiation factor. The level of statistical significance was set at P-value ≤0.05. Pearson's correlation coefficient (r) were determined.

Moreover, relative bone weight was calculated as a ratio of body weight and bone weight:

$$\text{Relative bone density} = \frac{\text{bone weight}}{\text{body weight}} \text{ x } 100\%$$

Results

The obtained results have been presented in the following tables: Table 1, Table 2, Table 3, Table 4, Table 5, Table 6.

Two-week-old males weighed app. 60 g more than two-week-old females. The analysis of body mass results in both sex groups showed that the largest increase in body mass during posthatching development occurred between 2 and 4 weeks of life in both sexes. However, it was higher in males, 1185 g, at ($P \leq 0.05$) (Table 1). Compared to females, males displayed statistically larger body mass from 2 weeks until the end of rearing ($P \leq 0.05$). Also tibiotarsal bones in males between 2 and 4 weeks displayed the highest increase of weight during the entire posthatching development period, it was 9.3 g, ($P \leq 0.05$) (Table 1).

Based on body mass and bone weight, relative bone weight was calculated. It is a very important factor, which indicates bone strength and deformity risk or even bone break incidence (Table 6). Indexes presented in the table show that relative bone weight in males decreased between 2 and 6 weeks of posthatching development. In females, on the

Table 1

Mean values ($\bar{x}$) and ± SEM of densitometric and geometric parameters in the proximal metaphysic of tibiotarsal bones of males and females depending on age. Mean values of body weight and bone weight

Analysed feature/Age	Male ($\bar{x}$ ± SEM)	Females ($\bar{x}$ ± SEM)
Densitometric parameters		
vBMD (mg/cm^3)		
2 wk	227.34 ^{a} ± 4.87	223.55^a ± 6.37
4 wk	217.47 ^{a} ± 5.44	239.45 ^{a} ± 5.2
6 wk	229.20 ^{a} ± 5.44	222.61 ^{a} ± 6.37
BMC (mg/mm)		
2 wk	.38 ^{a} ±1.71	4.98 ^{a} ± 1.48
4 wk	28.11 ^{b} ± 1.91	24.36 ^{b} ± 1.21
6 wk	43.28 ^{c} ± 1.91	35.99 ^{c} ± 1.48
Geometric parameters		
SSI (mm^3)		
2 wk	0.23 ^{a} ± 1.77	1.08 ^{a} ± 2.91
4 wk	2.76 ^{b} ± 1.98	6.55 ^{a} ± 2.38
6 wk	14.27 ^{c} ± 1.98	20.63 ^{b} ± 2.91
PERI_C (mm)		
2 wk	23.87 ^{a} ± 1.01	16.63 ^{a} ± 1.27
4 wk	40.23 ^{b} ± 1.15	35.61 ^{b} ± 1.03
6 wk	48.52 ^{c} ± 1.15	45.30 ^{c} ± 1.27
ENDO_C (mm)		
2 wk	23.80 ^{a} ± 1.11	16.13 ^{a} ± 1.38
4 wk	39.97 ^{b} ± 1.25	34.89 ^{b} ± 1.13
6 wk	47.55 ^{c} ±1.25	43.99 ^{c} ± 1.38
CRT_A(mm^2)		
2 wk	0.28 ^{a} ± 0.48	1.30 ^{a} ± 1.1
4 wk	1.69 ^{a} ± 0.54	3.83 ^{a} ± 0.90
6 wk	7.02 ^{b} ± 0.54	8.97 ^{b} ± 1.10
TRAB_A (mm^2)		
2 wk	20.49 ^{a} ± 3.19	9.2 ^{a} ± 3.8
4 wk	58.06 ^{b} ± 3.57	45.3 ^{b} ± 3.1
6 wk	85.00 ^{c} ± 3.7	73.6 ^{c} ± 3.8
TOT_A (mm^2)		
2 wk	45.58 ^{a} ± 7.19	22.13 ^{a} ± 8.45
4 wk	128.99 ^{b} ± 8.04	101.89 ^{b} ± 6.90
6 wk	189.38 ^{c} ± 8.04	164.99 ^{c} ± 8.45
Body weight (g)		
2 wk	395 ^{a} ± 20.450	339 ^{a} ± 9.23
4 wk	1580 ^{b} ± 22.864	1341 ^{b} ± 13.10
6 wk	2550 ^{c} ± 22.864	2263 ^{c} ± 32.59
Bone weight (g)		
2 wk	4.0 ^{a} ± 0.371	3.4 ^{a} ± 0.20
4 wk	13.3 ^{b} ± 4.15	10. ^{b} ± 0.07
6 wk	20.9 ^{c} ± 0.41	20.31 ^{c} ± 0.52

$^{a, b, c}$ Means within a column with different superscripts are significantly different ($P \leq 0.05$).

other hand, relative bone weight reached its lowest value in 4 weeks.

Tables 1 and 2 present the values of densitometric and geometrical parameters of the two analyzed sections in the tibiotarsal bone.

It was observed that in the first two weeks, the investigated traits displayed higher values in both bone sections in males as compared to females ($P \leq 0.05$). The exception were CRT_A and SSI in metaphyses, whose values were higher in females. In the case of these parameters, statistically significant differences were observed between both sexes in 2 weeks ($P \leq 0.05$).

Based on the analysis of densitometric parameters it was also concluded that in both sexes vBMD was two times lower at the mid-diaphysis than in the metaphyses (Tables 3, 4).

Tibial proximal metaphyses in 4 wk of posthatching development of males displayed a decrease in vBMD. However, no statistically significant differences in vBMD were observed between different age groups of males (Table 1). In females, on the other hand, vBMD decreased in 6 wk but there were no statistically significant differences between this group and 2-week- and 4-week-old females. The obtained results show that in males vBMD at the mid-diaphysis decreased during the entire posthatching development period, i.e. from 2 weeks to 6 weeks. The difference in vBMD in males between 2 and 6 wk was as much as 98 mg/cm^3, at ($P \leq 0.05$) (Table 2).

Table 2

Mean values ($\bar{x}$) and ± SEM of densitometric and geometric parameters in the mid-diaphysis of tibiotarsal bones of males and females depending on age

Analysed feature/Age	Males ($\bar{x}$ ± SEM)	Females ($\bar{x}$ ± SEM)
Densitometric parameters		
vBMD (mg/cm^3)		
2 wk	637.64 ^{a} ± 12.59	588.40 ^{a} ± 17.15
4 wk	606.22 ^{a} ± 14.07	647.98 ^{b} ± 15.66
6 wk	539.12 ^{b} ± 14.07	538.77 ^{a} ± 19.18
BMC (mg/mm)		
2 wk	6.61 ^{a} ± 1.24	3.22 ^{a} ± 0.76
4 wk	22.03 ^{b} ± 1.38	21.59 ^{b} ± 0.69
6 wk	30.14 ^{c} ± 1.38	31.61 ^{c} ± 0.85
Geometric parameters		
SSI (mm^3)		
2 wk	5.53 ^{a} ± 2.35	2.09 ^{a} ± 1.89
4 wk	35.24 ^{b} ± 2.63	31.24 ^{b} ± 1.72
6 wk	62.42 ^{c} ± 2.63	69.46 ^{c} ± 2.11
PERI_C (mm)		
2 wk	11.37 ^{a} ± 0.41	8.26 ^{a} ± 0.35
4 wk	21.36 ^{b} ± 0.46	20.49 ^{b} ± 0.32
6 wk	26.34 ^{c} ± 0.46	27.13 ^{c} ± 0.39
ENDO_C (mm)		
2 wk	6.62 ^{a} ± 0.33	5.11 ^{a} ± 0.38
4 wk	13.40 ^{b} ± 0.37	12.32 ^{b} ± 0.35
6 wk	18.09 ^{c} ± 0.37	18.90 ^{c} ± 0.43
CRT_A (mm^2)		
2 wk	6.82 ^{a} ± 1.12	3.35 ^{a} ± 0.73
4 wk	22.01 ^{b} ± 1.25	21.30 ^{b} ± 0.67
6 wk	29.30 ^{c} ± 1.25	30.10 ^{c} ± 0.82
TRAB_A (mm^2)		
2 wk	4.66 ^{a} ± 0.69	2.45 ^{a} ± 0.58
4 wk	16.38 ^{b} ± 0.77	15.11 ^{b} ± 0.53
6 wk	24.96 ^{c} ± 0.77	26.42 ^{c} ± 0.65
TOT_A (mm^2)		
2 wk	10.0 ^{a} ± 1.6	5.45 ^{a} ± 1.3
4 wk	36.0 ^{b} ± 1.7	33.56 ^{b} ± 1.18
6 wk	55.0 ^{c} ± 1.7	58.69 ^{c} ± 1.45

$^{a, b, c}$ Means within a column with different superscripts are significantly different ($P \leq 0.05$).

Table 3

Mean values ($\bar{x}$) and ± SEM of densitometric and geometric parameters in the proximal metaphysis et particular ages depending on sex

Analysed feature	Sex	2 wk ($\bar{x}$ ± SEM)	4 wk ($\bar{x}$ ± SEM)	6 wk ($\bar{x}$ ± SEM)
Densitometric parameters				
vBMD (mg/cm^3)	males	227. 34 ± 5.46	217.47* ± 4.72	229.2 ± 7.06
	females	223.55 ± 6.11	239.45* ± 3.85	222.61 ± 7.06
BMC (mg/mm)	males	10.38* ± 0.5	28.11 ± 1.65	43.28 ± 2.46
	females	4.98* ± 0.56	24.36 ± 1.34	35.99 ± 2.46
Geometric parameters				
SSI (mm^3)	males	0.23* ± 0.14	2.76 ± 1.51	14.27 ± 4.32
	females	1.08* ± 0.16	6.55 ± 1.23	20.63 ± 4.32
PERI_C (mm)	males	23.2*± 0.5	40.23* ± 1.05	48.52 ± 1.82
	females	16.63 *± 0.54	35.61* ± 0.86	45.30 ± 1.82
ENDO_C (mm)	males	23.87* ± 0.48	39.97* ± 1.14	47.55 ± 1.99
	females	16.63 *± 0.54	34.89* ± 0.93	43.99 ± 1.99
CRT_A(mm^2)	males	0.28* ± 0.12	1.69* ± 0.69	7.02 ± 1.42
	females	1.30* ± 0.2	3.83* ± 0.56	8.97 ± 1.42
TRAB_A (mm^2)	males	20.49* ± 0.77	58.06* ± 2.86	85.80 ± 5.94
	females	9.94* ± 0.86	45.80* ± 2.34	74.31 ± 5.94
TOT_A (mm^2)	males	45.58 *± 1.71	128.99* ± 6.47	189.38 ± 13.26
	females	22.13* ± 1.91	101.89* ± 5.28	164.99 ± 13.26
Body weight (g)	males	395* ± 20.450	1580* ± 22.864	2550* ± 22.864
	females	339* ± 9.23	1341* ± 13.10	2263* ± 32.59
Bone weight (g)	males	4.0 * ± 0.371	13.3* ± 4.15	20.9 ± 0.41
	females	3.4 * ± 0.20	10.1* ± 0.07	20.31 ± 0.52

*Means within a column are significantly different (P≤0.05).

Table 4

Mean values ($\bar{x}$) and ± SEM of densitometric and geometric parameters in the mid-diaphysis et particular ages depending on sex

Analysed feature	Sex	2 wk ($\bar{x}$ ± SEM)	4 wk ($\bar{x}$ ± SEM)	6 wk ($\bar{x}$ ± SEM)
Densitometric parameters				
vBMD (mg/cm^3)	males	637.64* ± 12.89	606.22 ± 20.03	539.12 ± 15.86
	females	588.4* ± 12.89	647.98 ± 16.35	538.77 ± 15.86
BMC (mg/mm)	males	6.61* ± 0.30	22.03 ± 0.79	30.14 ± 1.9
	females	3.22* ± 0.30	21.59 ± 0.64	31.61 ± 1.9
Geometric parameters				
SSI (mm^3)	males	5.53* ± 0.27	35.24 ± 1.47	62.42 ± 4.13
	females	2.09* ± 0.27	31.24 ± 1.20	69.46 ± 4.13
PERI_C (mm)	males	11.37* ± 0.25	21.36 ± 0.43	26.34 ± 0.56
	females	8.26* ± 0.25	20.49 ± 0.35	27.13 ± 0.56
ENDO_C (mm)	males	6.62* ± 0.20	13.40 ± 0.48	18.09 ± 0.46
	females	5.11* ± 0.20	12.32 ± 0.39	18.90 ± 0.46
CRT_A (mm^2)	males	6.82* ± 317	22.01 ± 0.77	29.0 ± 1.71
	females	3.35* ± 0.31	21.30 ± 0.63	30.10 ± 1.71
TRAB_A (mm^2)	males	4.66* ± 0.19	16.38 ± 0.65	24.96 ± 1.09
	females	2.45* ± 0.19	15.11 ± 0.53	26.42 ± 1.09
TOT_A (mm^2)	males	10.36* ± 0.43	36.40 ± 1.44	55.45 ± 2.43
	females	5.45* ± 0.43	33.56 ± 1.18	58.69 ± 2.43

*Means within a column are significantly different (P≤0.05).

Pearson's correlation coefficient analysis showed that there was no correlation between vBMD and the other densitometric and geometric parameters in the proximal metaphysis. CRT_A is an exception. This trait displayed a positive correlation, r = 0.26, at ($P \leq 0.05$), (Table 5).

In the group of females, on the other hand, vBMD at the mid-diaphysis increased between 2 wk and 4 wk by app. 60 mg/cm^3, ($P \leq 0.05$), but between 4 wk and 6 wk vBMD unfavorably decreased by as much as 109 mg/cm^3, at $P \leq 0.05$ (Table 2).

Another investigated densitometric parameter in the tibia of broiler chickens was BMC.

BMC in both sexes was higher in the proximal metaphyses than in the diaphyses. Both in males and in females BMC in tibial proximal metaphyses increased during the entire posthatching development period. The largest increase of BMC was observed between 2 weeks and 4 weeks. In this period, BMC increase could be observed for both sexes: app. 17 mg/mm for males and app. 19 mg/mm for females ($P \leq 0.05$), (Table 1).

Analysis of Pearson's correlation coefficient showed that BMC in both bone sections displayed positive correlation with all the geometric and densitometric parameters (except for vBMD at the mid-diaphysis, which was r = - 0.23 but the difference was not statistically significant). The strongest correlations were observed between BMC and TRAB_A, and TOT_A in the proximal metaphysis, they were (r = 0.98) and (r = 0.98) respectively, $P \leq 0.01$, and between BMC and CRT_A at the mid-diaphysis (r = 0.99), $P \leq 0.05$.

When analyzing the SSI values in both bone sections, it was concluded that they were significantly lower in the proximal metaphysis in relation to diaphysis. The proximal metaphyses of males were characterized by lower values of SSI compared to females, e.g. in the group of 4 wk males, the SSI value amounted to 2.76 mm^3, while in females at

Table 5

Pearson's correlation coefficient for densitometric and geometric parameters, body weight and bone weight in particular bone sections

Proximal metaphysis										
	vBMD	BMC	SSJ	PERI_C	ENDO_C	CRT_A	TRAB_A	TOT_A	Body weight	Bone weight
vBMD	1	0.03	0.26	-0.04	-0.06	0.26	-0.10	-0.12	0.01	-0.03
BMC	ns	1	0.50	0.98	0.97	0.55	0.98	0.98	0.94	0.91
SSI	ns	**	1	0.47	0.43	0.97	0.46	0.44	0.63	0.64
PERI_C	ns	**	**	1	0.99	0.51	0.98	0.98	0.94	0.91
ENDO_C	ns	**	**	**	1	0.47	0.98	0.98	0.92	0.90
CRT_A	*	**	**	**	**	1	0.50	0.50	0.69	0.69
TRAB_A	ns	**	**	**	**	**	1	1.0	0.93	0.92
TOT_A	ns	**	**	**	**	**	**	1	0.93	0.91
Body weight	ns	**	**	**	**	**	**	**	1	0.98
Bone weight	ns	**	**	**	**	**	**	**	**	1
Middle of the diaphysis										
	vBMD	BMC	SSJ	PERI_C	ENDO_C	CRT_A	TRAB_A	TOT_A	Body weigh	Bone weight
vBMD	1	-0.23	-0.40	-0.33	-0.48	-0.23	-0.40	-0.40	-0.43	-0.46
BMC	ns	1	0.96	0.98	0.94	0.99	0.98	0.98	0.94	0.93
SSI	**	**	1	0.96	0.96	0.96	0.98	0.98	0.95	0.96
PERI_C	*	**	**	1	0.98	0.98	0.98	0.98	0.96	0.95
ENDO_C	**	**	**	**	1	0.94	0.98	0.98	0.97	0.96
CRT_A	ns	**	**	**	**	1	0.97	0.97	0.94	0.92
TRAB_A	**	**	**	**	**	**	1	1.0	0.97	0.97
TOT_A	**	**	**	**	**	**	**	1	0.97	0.97
Body weigh	**	**	**	**	**	**	**	**	1	0.98
Bone weight	**	**	**	**	**	**	**	**	**	1

**Correlation is significant at the $P \leq 0.01$. *Correlation is significant at the $P \leq 0.05$. ns = not significant.

the same age it was higher by 3.79 mm^3, at ($P \leq 0.05$), (Table 1). Table 2 presents the SSI values at mid-diaphyses. It was concluded that in both gender groups this indicator increased during the post-natal development at ($P \leq 0.05$).

Pearson's correlation coefficient allowed for determining a correlation in proximal metaphysis and mid-diaphysis between SSI and CRT_A, which amounted r = 0.97 for proximal metaphysis and r = 0.96 for mid-diaphysis, at $P \leq 0.01$. In mid-diaphysis, strong correlations between SSI and TRAB_A (r = 0.98) and TOT_A (r = 0.98) were observed at $P \leq 0.01$. The analysis of geometric values of both sections of tibial bone revealed that the average PERI_C and ENDO_C values were greater in diaphysis in males at $P \leq 0.05$ (Table 3).

In the proximal metaphysis, both PERI_C and ENDO_C during the posthatching development in both sexes increased between 2 and 6 weeks, at ($P \leq 0.05$). Similar correlations were observed in mid-diaphyses of tibiae (Tables 1, 2).

When analyzing the CRT_A of the surface of compact osseous tissue it was concluded that the proximal metaphysis of the tibiotarsal bone, its surface increases during the development in both sexes, however statistically significant differences were observed in both sexes only between 4 and 6 wk ($P \leq 0.05$). Statistically significant differences between males and females at the proximal metaphysis were observed only at 2 weeks ($P \leq 0.05$). In metaphysis, CRT_A also increases during the development in both sexes – statistically significant differences were observed between sexes ($P \leq 0.05$), (Table 2).

The surface of trabecular bone TRAB_A in the proximal metaphysis was significantly greater than the CRT_A surface, e.g. in six-week-old males, TRAB_A was 85 mm^2, and CRT_A only 7 mm^2. In the diaphysis, the proportions were different as CRT_A was greater than TRAB_A on the bone cross-section. For six-week-old males, the values of bone surface amount to respectively: 29.30 mm^2 i 24.96 mm^2 (Table 2).

The Pearson's correlation coefficient in the case of CRT_A showed positive correlation in the proximal metaphysis of bones with SSI (r = 0.97) and with BMC (r = 0.55), at $P \leq 0.01$, in the metaphysis of tibial bones, the correlation between these characteristics amounted to: r = 0.96 and r = 0.99, respectively at $P \leq 0.01$, (Table 5).

The total bone surface, TOT_A, was also calculated for diaphysis and metaphysis of tibial bones. It was concluded that during the entire post-natal development in both sexes, TOT_A increased ($P \leq 0.05$). The differences between sexes in TOT_A, were noted in the proximal diaphysis in weeks 2 and 4, and in the mid-metaphyses, only in week 2 ($P \leq 0.05$). The total bone surface TOT_A was positively correlated with BMC, CRT_A and TRAB_A at diaphyses and metaphyses of tibial bones (Table 5).

In males, during the posthatching development, a loss of relative bone weight was observed from 1.03% to 0.79% (Table 6).

Table 6

Relative bone weight in chicken broilers

Relative bone weight		
	Males	Females
2 wk	1.03%	0.97%
4 wk	0.84%	0.75%
6 wk	0.79%	0.89%

Discussion

Sexual dimorphism is very common in birds. It is visible as morphological differences and it stems from different physiological purpose of both sexes. According to the most recent studies (TATARA *et al*. 2012a), it is manifested by hormonal and metabolic differentiation resulting in different amounts of muscle tissue, its amino-acid composition, body mass and skeletal properties.

The result of our own research on body mass in Ross 308 at the second week of life, when the observation was initiated, indicate a significant sexual dimorphism expressed in different body mass of males and females. Body weight of males at the second week of life was higher by 55 g compared to females ($P \leq 0.05$).

The results obtained in our research also indicate sexual dimorphism related to bone mass. In the second week, the females had lighter bones than males by 0.6 g ($P \leq 0.05$). It was also confirmed by other (ROSE *et al*. 1996). The author concluded that the tibial bones of female broilers are less prone to deformation than male bones, which was confirmed by our research.

Tibiotarsal bone was used in the study. The choice of this bone was deliberate. Other researchers investigating this bone (TATARA *et al*. 2005) showed significantly higher values of mechanical and geometrical parameters and higher mineralization than in other bones of the pelvic limb. For that reason the tibiotarsal bone proved to be have greater mechanical resistance and for that reason it was more fit for research than the femur.

Our own research also indicates that between the second and fourth week of life, the broilers (Ross 308) during posthatching period in males, experienced the highest increase of body mass. Also during that period, in males, in both examined bone sections, where vBMD increase was not observed, the values of this parameter decreased.

So the values of vBMD in the fourth week in males, were too low in order to maintain the high body mass without any harm to the tibial architecture. Both in the proximal metaphysis and in mid-diaphysis, lower values of vBMD during weeks 4 in males resulted in bends and even breaks of tibiotarsal bone. The relative tibial mass, decreasing during the development in males caused further bone deformation. Deformations and breaks were located on the border of the proximal metaphysis and diaphysis in males. High circuits of ENDO_C as well as low values of the predicted SSI in proximal metaphysis amounting only to 2.76 mm^3 (two times higher in females) also influenced the weakness of the shin in males.

Based on the presented results (Table 5) it was concluded that in both bone sections, the parameters (TRAB_A, BMC, CRT_A, PERI_C, ENDO_C and SSI) were strongly correlated. The densitometric parameters in the proximal metaphysis of the tibial bone did not show this correlation in relation to other parameters. It can be concluded that it is independent to other parameters. CRT_A was an exception which was correlated with vBMD (r = 0.26) at ($P \leq 0.05$). When analyzing the correlation coefficients it was concluded that the BMC parameter significantly affected the remaining densitometric and geometric parameters ($P \leq 0.01$). It was also concluded that along with the increase of body mass in chickens, the values of the following parameters increased: BMC, SSI, PERI_C, ENDO_C, CRT_A, TRAB_A, TOT_A.

When analyzing the proximal metaphysis of tibiotarsal bone, it was concluded that in this section, all densitometric and geometric parameters increased gradually with the poultry age. Densitometric parameters (vBMD, BMC) in males at the sixth week, were (slightly) higher than in females. It is also worth emphasizing that BMC in proximal metaphysis of tibial bones in both sexes was higher compared to the diaphysis. Among the geometric parameters in the 6^{th} week, TRAB_A, TOT_A and PERI_C were higher in males, while SSI and CRT_A were higher in males (Table 1).

The next examined bone section in broiler chickens was the mid-diaphysis of the tibial bone. It was shown that the densitometric and geometric parameters during the second week are higher in males. The same parameters were most often higher in females during the 6^{th} week (with the exception of vBMD). Most of the parameters, both densitometric and geometric (BMC, SSI, PERI_C, ENDO_C, CRT_A, TRAB_A, TOT_A), showed a tendency to increase with age. The vBMD (volumetric bone mineral density *in situ*) is the only parameter that did not show any correlation with age. It was also concluded that vBMD was significantly higher in diaphysis and it amounted to approximately 600 mg/cm^3, while the proximal metaphysis of the tibial bone was characterized by a significantly lower bone density, only approximately 230 mg/cm^3 (Table 1, 2). It is worth emphasizing that vBMD, as well as BMC are key parameters in bone mineralization, however we did not observe any positive correlations between them. Loss of vBMD can suggest insufficiency of minerals in diet or disorders of their absorption. Bones with mineral deficiency are more susceptible to bending and breaking. Numerous authors investigated the dependencies between body mass of animals, bone mass and values of geometric and densitometric parameters of bones.

KRUPSKI and TATARA (2007) showed that the values of densitometric and mechanical bone parameters in mid-tibia increased with age of birds. In examined turkeys, during the development of tibiotarsal bone, no drops in vBMD values were observed.

ONYANGO *et al*. (2003) conducted research on the influence of calcium and phosphorus on the skeleton and metabolism. It was showed that BMC and vBMD parameters increase with the increasing amount of Ca and P in the organism. Such dependencies were also obtained by ROWLAND (1968). These studies showed high correlation between the densitometric parameters (BMC and vBMD) and the use of diet containing substantial amounts of Ca and P. These data suggest that densitometric parameters (vBMD, BMC) were very sensitive to calcium and phosphorus insufficiencies in the diet of poultry. As a result, the high concentration of vBMD and BMC can prevent bone diseases, including tibiotarsal bone. We did not, however, observe any correlation between densitometric parameters (Table 5).

Pearson's correlation coefficient in our own research showed that BMC values in proximal metaphysis and diaphysis were positively correlated with body weight BW, body mass and densitometric and geometric parameters at $P \leq 0.01$. The study of TYMCZYNY *et al*. (2012), which were conducted on pig mandibule revealed that BMC was positively correlated with all densitometric and geometric parameters as well as mass and mandibule length. However, as far as vBMD is concerned, the researchers observed no statistically significant correlations with body weight, mandibule weight, mandibule length, bone volume or cortical bone density. There is a positive correlation between vBMD and cortical bone density, bone mineral density, bone mineral content, maximum elastic strength, ultimate strength. In pig, the densitometric and geometric parameters were investigated in the femur and tibial bones (TATARA *et al*. 2012b). In these studies, the obtained results did not show statistically significant differences in weight, length, volumetric mineral bone density of the trabecular and cortical bone, vertical and horizontal diameters of the mid-diaphysis, cross-sectional area, secondo moment of interia, mean relative wall thickness, cortical index, maximum elastic strength and ultimate strength of femur and tibia in males ($P \leq 0.05$). Also in swine the analysis of bone mineral content (BMC) in L1-L4 vertebrae showed positive correlation of this parameter with bone weight (TATARA *et al.* 2007).

In other studies, TATARA *et al.* (2011) analyzed the correlations between densitometric, geometric and morphometric parameters of the femur bone in 5 month old Polish Merino sheep. The obtained results showed significant correlations between morphometric, densitometric, and mechanical parameters of the femur. Statistically significant correlations between the vBMD of cortical bone, maximum elastic strength (Wy) ($r = 0.61$) and ultimate strength (Wf) ($r = 0.57$) indicate the highest value of this densitometric parameter for mechanical strength prediction of bones *in vivo*. In other species of experimental animals such as rats, examinations of the femur revealed that the strength and stiffness of the integrated diaphysis were found to depend on both cross-sectional interia and body weight and not on bone mineral density (FERRETTI *at al*. 1993a; 1993b). As a result of the differences obtained in the previous studies on turkeys, pigs, sheep and rats as well as on our own research, we can conclude that the processes of modeling and restructuring of bones are species-specific.

However, when we analyze the correlation between the parameters of the tibiotarsal bone in its separate sections, as well as the values of different parameters obtained during the course of post-hatching development, use of the tibia as a model bone for studies on the skeleton quality in poultry can be acknowledged. It is also worth emphasizing that in order to better understand the values of densitometric, geometric and durability parameters it is necessary to conduct similar analyses in several bone locations because, as showed in our studies, the values of these parameters in different bone sections are variable.

References

BOND P.L., SULLIVANT T.W., DOUGLAS J.H., ROBESON L.G. 1991. Influence of age, sex, and method of rearing on tibia length and mineral deposition in broilers. Poult. Sci. **70**: 1936-1942.

CHARUTA A., COOPER R.G. 2012a. 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., COOPER R.G., PIERZCHAŁA M., HORBAŃCZUK J.O. 2012b. Computed tomographic analysis of tibiotarsal bone mineral density and content in postnatal turkeys (*Meleagris gallopavo*) as influenced by age and sex. Czech J. Anim. Sci. **57**: 572-578.

CHARUTA A., DZIERZĘCKA M., BIESIADA-DRZAZGA B. 2012c. Evaluation of densitometric and geometric parameters of tibiotarsal bones in turkeys. Bull. Vet. Inst. Pulawy **56**: 379-384.

CHARUTA A., DZIERZĘCKA M., CZERWIŃSKI E., COOPER R.G., HORBAŃCZUK J.O. 2012d. 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., BIESIADA-DRZAZGA B., DZIAŁA-SZCZEPAŃCZYK E., COOPER R.G. 2013. Age- and sex-related changes in mineral density and mineral content of the tibiotarsal bone in quails during post-hatching development. Kafkas Univ. Vet. Fak. Derg. **19**: 31-36.

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 tibio-tarsal bones of the Peking domestic duck (*Anas platyrhynchos var. domestica*). Poult. Sci. **90**: 830-835.

CHARUTA A., MAJCHRZAK T., CZERWIŃSKI E., COOPER R.G. 2008. Spongious matrix of the tibio-tarsal bone of ostriches (Struthio camelus)- a digital analysis. Bull. Vet. Inst. Pulawy. **52**: 285-289

COOK M.E. 2000. Skeletal Deformities and Their Causes. Poult. Sci. **79**: 982-984.

COOPER R.G., HORBAŃCZUK J.O., NARANOWICZ H., MALISZEWSKA E. 2008. Sex-based comparison of limb segmentation in ostriches aged 14 months with and with-out tibiotarsal rotation. J. South African Vet. Assoc. **79**: 142-144.

CRESPO R., STOVER S.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. Poult. Sci. **79**: 602-608.

FERRETTI J.L., CAPOZZA R.F., MONDELO N., MONTUORI E., ZANCHETTA J.R. 1993a. Determination of femur structural properties by geometric and material variables as a function of body weight in rats. Evidence of a sexual dimorphism. Bone **14**: 265-270.

FERRETTI J.L., CAPOZZA R.F., MONDELA M., ZANCHETTA J.R. 1993b. Interrelationships between densitometric, geometric, and mechanical properties of rat femora: inferences concerning mechanical regulation of bone modeling. J. Bone Miner. Res. **8**: 1389-96.

HORBAŃCZUK J.O., HUCHZERMEYER F., PARADA R., PŁAZA K. 2004. Four-legged ostrich (*Struthio camelus*) chick. Vet. Rec. **154**: 736.

JENDRAL M.J., KORVER D.R., CHURCH J.S., FEDDES J.J.R. 2008. Bone mineral density and breaking strength of white leghorns housed in conventional, modified, and commercially available colony battery cages. Poult. Sci. **87**: 828-837.

KRUPSKI W., TATARA M. R. 2007. Interrelationships between densitometric, morphometric, and mechanical properties of the tibia in turkeys. Bull. Vet. Inst. Pulawy **51**: 621-626.

OLKOWSKI A.A., LAARVELD B., WOJNAROWICZ C., CHIRINO-TREJO M., CHAPMAN D., WYSOKIŃSKI T.W., QUARONI L. 2011. Biochemical and physiological weaknesses associated with the pathogenesis of femoral bone degeneration in broiler chickens. Avian Pathol. **40**: 639-650.

ONYANGO E.M., HESTER P.Y., STROSHINE R., ADOELA O. 2003. Bone densitometry as an indicator or percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. Poult. Sci. **82**: 1787-1791.

RATH N.C., BALOG J.M., HUFF W.E., HUFF R.G., KULKARNI G.B., TIERCE J.F. 1999. Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. Poult. Sci. 1999. **78**: 1232-1239.

RATH N.C., HUFF G.R., HUFF W.E., BALOG J.M. 2000. Factors Regulating Bone Maturity and Strenght in Poultry. Poult. Sci. **79**: 1024-1032.

ROSE N., CONSTANTIN P., LETERRIER C. 1996. Sex differences in bone growth of broiler chickens. Growth Dev. Aging. **60**: 49-59.

ROWLAND L.O., HARMS R.H., WILSON H.R., AHMED E M., WALDROUP P.W., FRY J.L.1968. Influence of various dietary factors on bone fragility of caged layers. Poult. Sci. **47**: 507–511.

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. Poult. Sci. **91**:1790-1795.

STOFANIKOWA J., SALY J., MOLNAR L., SESZTAKOVA E., BILEK J. 2011. The influence of dietary zinc content on mechanical properties of chicken tibiotarsal bone. Acta Vet., Beograd **61**: 531-541.

TALATY P.N., KATANABF M.N., HESTER P.Y. 2010. Bone mineralization in male commercial broilers and its relationship to gait score. Poult. Sci. **89**: 342-348.

TATARA M.R., BRODZKI A., PYZ-ŁUKASIK R., PASTERNAK K., SZPETNAR M. 2012a. Sex-related differences in skeletal muscle amino acid concentrations in 20 week old turkeys. J. Poult. Sci. **49**:219-223.

TATARA M.R., KRUPSKI W., TYMCZYNA B., ŁUSZCZEWSKA-SIERAKOWSKA I. 2012b. Biochemical bone metabolizm 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., TYMCZYNA B., MAJCHER P., ROSENBEIGER P., NIEDZIELA D. 2011. 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., SIERANT-ROŻMIEJ N., KRUPSKI W., MAJCHER P., ŚLIWA E., KOWALIK S., STUDZIŃSKI T. 2005. Quantitative computer tomography for the assessment of mineralization of the femur and tibia in turkeys. Medycyna Wet. **61**: 225-228.

TATARA M.R., ŚLIWA E., KRUPSKI W., WORZAKOWSKA M.N., STUDZIŃSKI T. 2007. Interrelationships between morphometric, densitometric and mechanical properties of lumbar vertebrae in pigs. J. Pre-Clin. Clin. Res. **1**: 165-170.

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.

WILLIAMS B., WADDINGTON D., MURRAY D.H., FARQUHARSON C. 2004. Bone strength during growth: influence of growth rate on cortical porosity and mineralization. Calcif. Tissue Int.**74**: 236-45.