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Morphological and densitometric research of the tibial bone in the post-natal development in domestic geese

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Investigated were morphological and densitometric parameters of the tihiotarsal bones in White Kołuda Geese over post-natal period as influenced by sex, age and bone area using dual-energy X-ray absorptiometry and peripheral quantitative computed tomography. The study was performed on 100 bones of geese obtained from both sexes on 1, 14, 28, 42 and 56 day of life. Body weight, bone weight, bone mineral density (BMD), bone mineral content (BMC), volumetric bone mineral density (vBMD), total bone mineral content (tBMC), cortical bone mineral density (CTR_DEN), cortical bone mineral content (CRT_CNT), trabecular bone mineral density (TRAB_DEN) and trabecular bone mineral content (TRAB_CNT) of tihiotarsal bone were determined.

vBMD in the proximal metaphyses generally increased with age in both sexes and in females was higher than in males (especially in mid-diaphysis). Similar trends were observed for tBMC in mid-

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diaphysis of bones. The tBMC in proximal metaphyses reached maximum in females as early as on day 28, whereas in males 2 weeks later (on day 42). A significant decrease in TRAB_DEN and TRAB_CNT was also observed in males in both sections of the bone on day 42 and 56, whereas TRAB_DEN in the mid-diaphysis attenuated between day 28 and 42. Most deformities of tibial bones in males were observed in week 6 (42 day). In females, tibiotarsal deformations and fractures were found in week 6 and 8 of age, which was slightly later than the critical developmental stage in males.

KEY WORDS: bone mineral content / bone mineral density / geese / mineralization / tihiotarsal bone

Skeleton diseases in birds contribute to serious problems concerning the modern poultry production. They include a shortened breeding period and rapid increase of muscle tissue weight that happens in too short time and is beyond the capability of the skeleton to adapt to heavy loading. The fast-growing body weight as well as lack of balance between the increase in muscle weight and the increase in bone mass may cause higher risk of bone deformations [Cooper *et al*. 2008, 2010] and fractures [Crespo *et al*. 1999, Cooper *et al*. 2010], including especially the tibiotarsal bone [Tatara *et al*. 2004a, Charuta *et al*. 2011].

The problems with the skeletal integrity and function are specific for domestic birds with very high production potential expressed by fast growth rate such as turkeys [Charuta *et al.* 2012b, 2012c], geese [Biesiada-Drzazga *et al*. 2009, Charuta *et al*. 2012d], ducks [Charuta *et al.* 2011, 2012a], chicken broilers [Barreiro *et al*. 2011, Olkowski *et al*. 2011, Shim *et al*. 2012, Charuta *et al*. 2013c], quails [Charuta *et al*. 2013b] and even ostriches [Horbańczuk *et al*. 2004, Cooper *et al*. 2008, 2010, Charuta 2013a], whose raising period may be shortened even to 6 months. Next to the shortened raising period, many other factors affect directly or indirectly the dynamics of ossification processes in farm birds, including genetic factors, nutrition, hormones, environmental conditions as well as age and sex [Tatara 2006, Tykałowski *et al*. 2010].

Tibial deformations are one of the significant problems related to the welfare of birds and profitability of production. Birds with deformed limbs have walking problems and their sitting on the bedding also causes malformations of thoracic muscles, affecting the quality of meat and the profitability of poultry production [Rath *et al*. 2000].

The aim of this study was to determine morphological and densitometric parameters characterising the osseous tissue in geese in the post-natal development, as influenced by age, sex and place in the bone, using a densitometer and peripheral quantitative computed tomography (pQCT) technique.

Material and methods

The study was performed on 100 birds, including 50 females and 50 males of White Kołuda Geese (W31). All geese were divided into 5 age-differentiated groups (n = 10 in each group of males and females) and slaughtered on day 1, 14, 28, 42, 56 of life. Left tibiotarsal bones were isolated from each bird, cleaned from soft tissues and kept frozen in plastic bags at the temperature between -25°C and -30°C.

During growth, the geese were fed with a standard mix (prepared by a commercial company BACUTIL Bedlno, Poland) containing 19.5% protein at the beginning of fattening (11.72 MJ/kg) and 15.5% protein at the end of fattening (12.14 MJ/kg). The weights of the bones were determined using the precise laboratory balance (AXIS AD 300, Poland) with the maximum range of 300 g and precision of 0.001g. Bone mineral density (BMD) and bone mineral content (BMC) of the bones from geese were determined using dual-energy X-ray absorptiometry (DEXA) and NORLAND EXCELL PLUS densitometer (FORT ATKINSON WI, USA). The collimated X-ray beam and the software intended to examine bones of small animals (Small Subject Scan, version 3.9.6) were used. Bones of one-day-old geese were scanned at the scanning resolution of 0.5 x 0.5 mm and scanning speed of 10 mm/s, while the bones obtained from remaining groups of the geese (14, 28, 42 and 56-day-old) were scanned using the scanning resolution of 1.5 x 1.5 mm and scanning speed of 30 mm/s. Bone mineral density was expressed in g/cm^2, whereas bone mineral content was expressed in grams. The measurements of BMD and BMC were performed for whole bone samples including trabecular and compact bone tissue compartments.

Using peripheral quantitative computed tomography (pQCT) and XCT Research SA Plus scanner (STRATEC MEDIZINTECHNIK GmbH, Pforzheim, Germany), densitometric parameters of the tibiotarsal bones were analysed. The determinations were performed at two locations of the bone: at 18% of bone length at proximal metaphysis and at 50% of bone length at mid-diaphysis. As a result of the measurements, total bone mineral content (tBMC - mg/mm per 1 mm slice), volumetric bone mineral density (vBMD – total bone mineral density, mg/cm^3), cortical bone mineral density (CRT_DEN – volumetric mineral density of the cortical bone, mg/cm^3) and cortical bone mineral content (CRT_CNT – the mineral content within 1 mm slice of the cortical bone, mg/mm) were determined. Furthermore, in the proximal metaphysis, trabecular bone mineral density (TRAB_DEN – volumetric mineral density of the trabecural bone, mg/cm^3) and trabecular bone mineral content (TRAB_CNT – the mineral content within 1 mm slice of the trabecular bone, mg/mm) were measured.

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).

The term "post-natal development" in the presented descriptions is a simplification and is conventional. The analysed birds for obvious reasons did not attain their maximum age due to the purposes for which they were raised.

Statistical

All data were presented as means ± SD. All the calculations were performed using the Statistica 9.0 software (StatSoft, Inc. Tulsa, USA). The statistical analysis was carried out using two-way and three-way ANOVA as well as the post-hoc T-Tukey's test. For all the comparisons, P-value <0.05 was considered as statistically significant. The analysed factors included: sex, age and place of the measurements of a bone.

The two-way ANOVA (for the body mass and the bone weight measurements) was performed in accordance with the following model:

$$Yijl = m + ai + bj + abij + eijl$$

where:

Yijl – value of the studied feature;

m – population mean;

ai – effect of the i sex (i = 1,2);

bj – j effect of the j age (j = 1st day,…,56th day);

abij – sex and age interaction effect;

eijl – random effect.

For the remaining features, the three-way ANOVA was used, with the calculations being performed using the following model:

$$Yijlp = m + ai + bj + cl + abij + acil + bcjl + abcijl + eijlp$$

where:

ai – effect of the i sex (i = 1,2);

bj – effect of the j age (j =1st day,…,56th day);

cl – effect of the l place in the bone;

abij, acil, bcjl, abcijl – effects of interactions between relevant factors;

eijlp – random effect.

Results and discussion

The mean body weight, bone weight and densitometric parameters, as influenced by sex, age and location on the bone are presented in Tables 1-3. Significant differences in body weight between males and females were found only in 42-day-old birds; males were heavier by 450 g as compared to females (P<0.05, Tab. 1). BMD and BMC of tibiotarsal bone in both sexes increased gradually with age and differences were observed between day 1 and 14, and 14 and 28 d (P<0.05, Tab. 2). The values of BMD and BMC on day 1 and 14 were also significantly different from those obtained on day 28, 42 and 56 (P<0.05, Tab. 2).

The vBMD in the proximal metaphyses generally increased with age in both sexes, and in females presented a higher value as compared to males. Significantly higher values of vBMD in the mid-diaphysis of males were shown on day 14, 42 and 56 as compared to 28-day and in case of females on day 28, 42 and 56 in comparison with 1- and 14-day old geese (P<0.05, Tab. 3). vBMD in proximal metaphysis of the tibiotarsal bone of 42-day-old males was lower by 79 mg/cm^3 as compared to 28-day-old birds. vBMD in the mid-diaphysis area was significantly higher in 14-day-old

Table 1. Body weight and bone weight in male and female geese as related to age 1, 14, 28, 42 and 56 days of life

Age	Body weight (g)		Bone weight (g)	
	males	females	males	females
1 day	72^{aA}±5.83	65^{aA}±5.91	0.83^{aA}±0.05	0.74^{aA}±0.07
14 days	731^{aB}±58.18	708^{aB}±56.69	7.78^{aB}±0.71	7.38^{aB}±0.66
28 days	2220^{aC}±199.80	2130^{aC}±191.91	24.16^{bC}±2.17	22.00^{aC}±1.54
42 days	3760^{bD}±300.55	3310^{aD}±264.23	34.06^{bD}±2.38	32.35^{aD}±2.26
56 days	4450^{aE}±400.52	4165^{aE}±333.20	34.85^{aD}±2.41	33.58^{aD}±2.35

^{ab}Means in rows (males *versus* females) marked with different superscripts differ significantly at P<0.05.
$^{AB...}$Means in each column marked with different superscripts differ significantly at P<0.05.

Table. 2. Bone mineral density (BMD) and bone mineral content (BMC) of the tibiotarsal bone in male and female geese at the age of 1, 14, 28, 42 and 56 days of life using a densitometer

Age	BMD (g/cm^2)		BMC (g)	
	males	females	males	females
1 day	0.01^{A}±0.01	0.01^{A}±0.01	0.01^{A}±0.01	0.01^{A}±0.01
14 days	0.10^{B}±0.72	0.10^{B}±0.07	0.83^{B}±0.06	0.90^{B}±0.07
28 days	0.19^{C}±0.01	0.20^{C}±0.01	2.71^{C}±0.21	2.84^{C}±0.22
42 days	0.20^{C}±0.01	0.21^{C}±0.01	3.25^{C}±0.26	3.62^{C}±0.28
56 days	0.20^{C}±0.04	0.20^{C}±0.01	3.51^{C}±0.28	3.15^{C}±0.23

^{ABC}Means in each column marked with different superscripts differ significantly at P<0.05.

males as compared to females. Opposite difference was found comparing vBMD of mid-diaphysis in 28 day-old males and females.

The tBMC in proximal metaphyses reached the maximum value in females as early as on day 28, whereas in males 2 weeks later (on day 42). tBMC values in proximal metaphysis were significantly higher in days 28, 42 and 56 as compared to age of day 1 and 14 both in males and females (P<0.05). Total bone mineral content values in proximal metaphysis were significantly higher on day 14 in both sexes as compared to this parameter in 1-day-old birds (P<0.05). Values of tBMC in mid-diaphysis of tibiotarsus were significantly higher in 28, 42 and 56 day old males and females when compared to the groups of the age of 1 and 14 days (P<0.05, Tab. 3).

CRT_DEN of proximal metaphysis and mid-diaphysis reached significantly higher value in 56-day-old males and females when compared to 42-day-old sex-matched geese and the values obtained in both these groups occurred significantly higher when compared to 1, 14 and 28-day-old groups of birds (P<0.05). Significantly higher

Table 3. Volumetric bone mineral density (vBMD), total bone mineral content (tBMC), cortical bone mineral density (CRT_DEN), cortical bone mineral content (CRT_CNT), trabecular bone mineral density (TRAB_DEN) and trabecular bone mineral content (TRAB_CNT) of proximal metaphysis and mid-diaphysis of the tibiotarsal bone in male and female geese at the age of 1, 14, 28, 42 and 56 days of life using computed tomography

Parameter	Age	Place of measurement			
		proximal metaphysis		mid-diaphysis	
		males	females	males	females
vBMD (mg/cm^3)	1 day	120.8^{Aa*}±9.17	144.7$^{Aa\#}$±12.0	398.8^{Aa*}±31.9	404.7$^{Aa\#}$±32.3
	14 days	275.3^{Ba*}±22.5	263.2$^{Ba\#}$±20.1	545.8^{Bb*}±43.6	483.0$^{Aa\#}$±38.6
	28 days	374.9^{Ca*}±30.1	351.0$^{Ca\#}$±28.1	451.7^{Ab*}±36.1	539.3$^{Ba\#}$±43.1
	42 days	295.9^{Ba*}±24.2	329.7$^{Cb\#}$±26.3	535.6^{Ba*}±42.8	538.2$^{Ba\#}$±43.1
	56 days	424.7^{Ca*}±34.2	453.8$^{Db\#}$±39.4	530.3^{Ba*}±42.3	585.9$^{Ba\#}$±46.5
tBMC (mg/mm)	1 day	1.16^{A}±0.12	1.29^{A}±0.10	1.50^{A}±0.10	1.70^{A} ±0.14
	14 days	19.39^{B}±1.59	18.65^{B}±1.49	10.18^{A}±0.98	7.91^{A} ±0.63
	28 days	37.66^{Ca}±3.09	48.06^{Cb}±3.84	32.05^{B}±2.63	21.49^{B}±1.72
	42 days	46.15^{C}±3.59	43.13^{C}±3.32	34.63^{B}±2.67	30.63^{B}±2.45
	56 days	34.34^{C}±2.75	37.94^{C}±3.00	32.05^{B}±2.46	39.72^{B}±3.01
CRT_DEN (mg/cm^3)	1 day	625.2^{A*}±49.7	636.9$^{A\#}$±49.9	683.6^{A*}±54.9	699.5$^{A\#}$±55.7
	14 days	613.9^{A*}±48.8	642.2$^{A\#}$±50.1	813.4^{B*}±66.0	812.0$^{B\#}$±65.0
	28 days	637.5^{A*}±50.0	625.0$^{A\#}$±48.9	776.0^{B*}±62.1	959.1$^{B\#}$±76.8
	42 days	773.8^{B*}±60.1	754.8$^{B\#}$±60.1	1038.1^{C*}±83.0	1047.2$^{C\#}$±82.8
	56 days	989.0^{C*}±78.9	987.53$^{C\#}$±86.2	1087.4^{C*}±86.9	1079.9$^{C\#}$±87.3
CRT_CNT (mg/mm)	1 day	0.31^{A}±0.02	0.19^{A}±0.03	1.48^{A}±0.12	1.07^{A}±0.06
	14 days	0.54^{A}±0.03	0.30^{A}±0.04	6.74^{A}±0.42	9.13^{AB}±0.64
	28 days	7.63^{B}±0.34	7.40^{B}±0.41	20.08^{Ba}±1.46	15.28^{Bb}±1.02
	42 days	25.40^{C}±2.77	28.76^{C}±2.66	28.42^{BCa}±2.44	15.90^{Bb}±1.72
	56 days	36.63^{Da}±3.95	30.73^{Cb}±2.72	36.63^{Ca}±2.31	30.77^{Cb}±2.71
TRAB_DEN (mg/cm^3)	1 day	120.8^{A}±9.7	100.5^{A} ±8.1	–	0.58^{A} ±0.05
	14 days	229.9^{Ca*}±20.99	140.7$^{Bb\#}$±11.3	182.0^{A*} ±14.0	174.3$^{B\#}$±13.9
	28 days	197.1^{Ba*}±15.8	64.94$^{Ab\#}$±5.21	292.4^{Ba*}±23.3	211.9$^{Bb\#}$±16.9
	42 days	43.20^{A*}±3.46	34.43$^{A\#}$±2.77	18.45^{Ca*}±1.24	57.53$^{Ab\#}$±4.62
	56 days	16.25^{A} ±1.46	25.75^{A}±2.08	10.38^{Da} ±0.83	22.45^{Ab}±2.05
TRAB_CNT (mg/mm)	1 day	–	–	–	–
	14 days	5.72^{B} ±0.342	5.98^{B} ±0.47	1.87^{B}±0.15	0.94^{A}±0.073
	28 days	12.91^{C}±1.033	12.96^{C}±1.04	6.58^{C}±0.53	1.15^{B}±0.087
	42 days	1.31^{A}±0.105	3.39^{A}±0.27	1.30^{A}±0.10	0.98^{A}±0.078
	56 days	0.28^{A}±0.022	2.11^{A}±0.06	0.43^{A}±0.03	0.76^{A}±0.06

$^{AB...}$Means in columns marked with different superscripts differ significantly at P<0.05.
^{ab}Means in rows marked with different superscripts show significantly different values between males and females at particular place of measurement for P<0.05.
*Identical superscript symbols in rows indicate significant differences in males between the place of measurement in the bone (proximal metaphysis *versus* mid-diaphysis) at P<0.05.
$^{\#}$Identical superscript symbols in rows indicate significant differences in females between the place of measurement in the bone (proximal metaphysis *versus* mid-diaphysis) at P<0.05.

values of CRT_DEN were found in mid-diaphysis when compared to metaphysis both in males and females at all the investigated developmental stages (P<0.05, Tab. 3).

The lowest values of CRT_CNT in post-natal development of geese were observed on day 1 and 14 in metaphyses of both sexes. On day 28, there was a significant growth in the value of the parameter for males and females, both in metaphyses and diaphyses (P<0.05, Tab. 3). CRT_CNT was significantly higher in proximal metaphysis of tibiotarsus in 56-day old males when compared to the group at the age of 42-days. The CRT_CNT was significantly higher in both these groups of males when compared to the parameter obtained in all the other age-differentiated groups of (P<0.05). The CRT_CNT reached significantly higher values in mid-diaphysis of tibiotarsus in 56-, 42- and 28-day-old males when compared to birds at the age of 1 and 14 days (P<0.05). Significantly higher value of CRT_CNT of mid-diaphysis stated in 56-days old of males when compared to 28 days (P<0.05). CRT_CNT reached significantly higher value in mid-diaphysis of tibiotarsus in 56-day old females when compared to all the remaining sex-matched groups (P<0.05, Tab. 3).

TRAB_DEN of proximal metaphysis reached higher value on day 14 on both sexes (P<0.05). In males, the highest value of TRAB_DEN was reached in mid-diaphysis of 28 day and the differences between groups were found significant (P<0.05). In females, significantly higher values of TRAB_DEN in mid-diaphysis were stated in 28- and 14-day-old birds when compared to all the other age-differentiated groups (P<0.05). At the age of 28 days, TRAB_DEN of mid-diaphysis was significantly higher in males than in females, while opposite results were obtained at the age of 42 and 56 days (P<0.05). In 14-days-old males, TRAB_DEN of proximal epiphysis was significantly higher than in mid-diaphysis, while opposite results were obtained in 28-day-old and 42-day-old males (P<0.05). In 14- and 28-day-old and 42-day-old females, TRAB_DEN values of mid-diaphysis were significantly higher than those of proximal metaphysic (Tab. 3).

TRAB_CNT of proximal metaphysis and mid-diaphysis in both sexes reached highest values at the age of 28 days of life (P<0.05).

The problems with skeleton observed in farm birds are characteristic for pelvic limbs, which carry the entire body weight. A number of research tests made it possible to determine that both the tibiotarsal and the tarsometatarsal bone may serve as a model bone in studies on the quality of skeleton of farm birds [Tatara *et al*. 2005, Charuta *et al*. 2013d]. As deformations and fractures of tibiotarsal bones occurred frequently in geese kept for meat, the values of densitometric parameters characterising the bone in post-natal period were analysed in this study. The influence of such factors as age and sex on the investigated properties of tibiotarsus was considered. It must be stressed that no research of a similar scope has so far been carried out and no satisfactory information has been acquired on skeletal growth processes in geese, especially during postnatal period of systemic development. Thus, the current study provides basic physiological data on densitometric properties of tibiotarsal bone.

During the post-natal development, bones undergo structural remodelling process, the aim of which is to adapt the bone structure in such way that it can resist gravity forces and acting forces and play specific mechanical functions [Komosa *et al*. 2013]. Adequate BMC, BMD and vBMD values, in particular at the metaphyses of tibiotarsal bones, are responsible for mechanical resistance in the period of fast gain of body weight. It is worth stressing that metabolic processes are the most intensive in trabecular bone of proximal metaphyses [Czerwiński 1994, Tatara *et al*. 2004b]. The investigated geese were subjected to intensive rearing, as a result of which they attained slaughter maturity at week 8 of life. Due to the fast growth and high increase in body weight, the skeleton of geese was especially at risk of deformations and fractures.

It was observed that in male geese in the post-natal development vBMD values decreased in metaphyses (week 6) and in diaphyses (week 4) of tibiotarsal bones. The decrease in the vBMD values in males occurred when body weight increased at the fastest rate – between week 4 and 6. In that period body weight of males increased by 1550 g. In females, in turn, deformations were observed in the 6 and 8 week of life, that was slightly later than in males. Moreover, there were slightly less frequent tibial deformations in females than in males. The analysis of ten bones showed tibial deformations in two 6-week-old males (20% of investigated birds), while only one deformed bone out of the ten analysed ones belonged to the females (10% of the investigated birds).

The decrease in vBMD values in the post-natal period occurred also in other males of avian fowls. For instance, in 4-week-old male ducks in the post-natal period, the decrease of vBMD values at proximal metaphysis of tibiotarsal bone was observed [Charuta *et al*. 2011]. In turkeys, in turn, the decrease in BMD values at proximal metaphyses and diaphyses occurred in 9th week of life and was more frequent in males than in females [Charuta *et al*. 2012b,c]. On the other hand, Krupski and Tatara [2007] did not show any decrease in vBMD values in turkeys in post-natal development. In male broiler chickens vBMD values decreased in week 4 of life at the two analysed sections of the bone [Charuta *et al*. 2013c].

In the earlier study by Charuta *et al*. [2012d], the osseous structure of tibia in geese reared semi-intensively (up to the week 16) was analysed using the Trabecula® software. It was found that similarly to geese kept until week 8, in 6-week-old males, the decrease in the number, volume and density of radiologically identified trabeculae was obtained at the proximal metaphyses of tibia. It is worth underlining that these parameters contribute reasonably to bone strength. The use of two different methods of tibia examination has proven that the critical moment in which the strength of tibia in males decreased considerably was week 6 of life.

In conclusion, the present study showed differences in morphological and densitometric parameters of tibiotarsal bones in geese during post-natal development in relation to sex, age and bone section. Attenuating values of densitometric parameters during the postnatal development were one of the causes of deformities and fractures of tibial bones in geese, in males between day 28 and 42 of life, whereas in females two weeks later, between day 42 and 56.

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