

Computed tomographic analysis of tibiotarsal bone mineral density and content in turkeys as influenced by age and sex

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ABSTRACT: Changes in the volumetric bone mineral density (vBMD) and bone mineral content (BMC) of tibiotarsal bones of growing turkeys as affected by birds' age, sex, and within-the-bone location, respectively, were determined by computed tomography. The research was performed on 165 heavy-type BIG 6 turkeys reared between weeks (wk) 3–16 of age. The computed tomography (pQCT) measurement, conducted at 18 and 50% of the bone length, comprised a bone fragment which was 0.07 mm thick for the compact and the spongy substance collectively. It should be noted that the diaphyses of the tibiotarsal bones in turkeys (580 mg/cm³) had greater vBMD than the proximal metaphyses (300 mg/cm³), ($P \leq 0.001$). BMC was higher in metaphyses for both sexes. Significant differences between the BMC of the metaphyses and the diaphyses were observed in males and females at wk 3 ($P \leq 0.01$), 6 ($P \leq 0.05$), and 9 ($P \leq 0.001$), and at wk 3 ($P \leq 0.05$) and 12 ($P \leq 0.01$), respectively. vBMD in the diaphyses gradually attenuated with age for both sexes, from 688 mg/cm³ (wk 3) to 532 mg/cm³ (wk 16). vBMD of the metaphyses was constant in females, but in males it achieved maximum values of 350 mg/cm³ at wk 6 and 12 and minimum of 260 mg/cm³ at wk 9 and 16. Correlations between body weight (BW) and vBMD of the diaphyses were observed in males ($r = -0.85$, $P \leq 0.001$) and females ($r = -0.52$, $P \leq 0.01$). It can be concluded that vBMD loss in diaphyses diminished bone-breaking strength leading in investigated turkeys to deformities and bone fractures.

Keywords: volumetric bone mineral density; bone mineral content; tibiotarsal bone; turkey; mineralisation; computed tomography

The intensity of poultry production may lead to deformities in birds' bone density, especially in the tibiotarsal bones and bow legs (Peters et al., 2002; Cooper and Horbańczuk, 2004; Charuta et al., 2008; Cooper et al., 2008; Tykałowski et al., 2010). Clear clinical symptoms, such as growth disorders or lameness (Lynch et al., 1992) occur in different fowl species (Simsa and Ornan, 2007; Simsa et al., 2007), e.g. chicken broilers (Lynch et al., 1992; Lilburn, 1994), ducks (Charuta et al., 2011), and ratites (Horbańczuk et al., 2004; Cooper et al., 2010). Birds that suffer from the described condition are bow-legged and unwilling to move; in extreme cases, fractures of the diaphysis of the tibial bone may occur (Crespo

et al., 1999). Bone mechanical strength and resistance are related to increasing body weight during growth, high stocking rate (Jendral et al., 2008), lack of exercise (Crespo et al., 1999; Burs et al., 2008), and problems with mineralisation including changes in mineral density and content (Tatara et al., 2004).

Studying densitometric features of tibiotarsal bones in turkeys with the use of pQCT is one of the methods allowing determination of the birds' bone structure, including disorders of mineralisation. Korver et al. (2004) assessing the bone mineral density *in vivo* using pQCT found moderate correlations between vBMD and bone quality attributes such as bone strength and mineral content. The

authors claim that pQCT is an effective technique to measure vBMD in laying hens, which allows resolution of total vBMD as well as cortical and trabecular vBMD.

The bone tissue of fast-growing birds is particularly exposed to mechanical injuries. Thus, it is necessary to monitor growth and chemical composition of the tibiotarsal bones during breeding while feeding particular diets (Suchý et al., 2009; Ebeid et al., 2011). Healthy bones are, therefore, fundamental for proper functioning of a bird's mechano-skeletal system.

The objective of the present study was to determine the changes in the bone mineral density and bone mineral content of tibiotarsal bones of growing turkeys as affected by birds' age, sex, and within-the-bone location with the use of computed tomography.

MATERIAL AND METHODS

The research was conducted on 165 heavy-type BIG 6 turkeys reared according to production scheme recommended by Kartzfehn (Moorgut Kartzfehn von Kameke GmbH & Co. KG, Bösen, Germany) in wk 3–21 of age. Birds were divided into 11 age groups: 3, 6, 9, 12, and 16 wk of life (both sexes) and group of wk 21 that was composed only of males. The study was conducted on 15 males and females from each age group which were kept separately on bedding under standard turkey farming conditions with free access to feed and water. Five types of mixed feeds (Bacutil Sp. z.o.o., Bedlno Radzyńskie, Poland) with different levels of protein and energy were used (Table 1). Since according to the production scheme females were kept till wk 16 of age and then they were slaughtered, the comparison of vBMD and BMC was done until this period (16 wk). The body weight (BW) and bone weight were also determined, as shown in Table 2.

The growth rate during rearing period in turkey females and males varied from 29 to 35 and 93 to 125 g per day in 3- and 16-week-old birds, respectively. The feed intake per kg of body weight gain in females and males ranged 1.37–1.39 kg of feed (3 wk) and 2.88–2.56 kg of feed (16 wk). In turn, the mortality rate was very low (ca. 2%) during the whole period of rearing. The density per m² with respect to age and sex was as follows: 9–10 birds till wk 5 for both sexes, and 3.5 females and 2.5 males in wk 16. The lighting cycle is shown in Table 3.

After turkey's slaughtering the left tibiotarsal bones determined for analysis were cleaned off the soft tissues and frozen for storing at –25 to –30°C. To record all the activities, an X-ray plate graph of the isolated bones with an aluminium trochlea was taken to make an image of the degree of bone mineralisation.

Using a high resolution peripheral quantitative computed tomography (pQCT) XCT Research SA Plus (Stratec Medizintechnik GmbH, Pforzheim, Germany), the analysis of *in situ* structure of the proximal metaphysis and the middle of the diaphysis of the tibiotarsal bones was performed. The taken measurements of bones are described below.

Volumetric bone mineral density (vBMD) *in situ* – mean density (mg/cm³) at 18% of the length of the metaphysis and 50% of the length of the diaphysis of the tibiotarsal bone, combined of the compact and the cancellous bone.

Bone mineral content (BMC) *in situ* – bone mineral content (mean) per 1 mm slice (mg/mm) was attained. Mineral content deduced of the total bone (within a 1 mm slice). The measurements were conducted at 18% of the length of the metaphysis and 50% of the length of the diaphysis of the tibiotarsal bone, collectively for the compact and the cancellous bone. The pQCT measurement, conducted at 18 and 50% of the bone length, comprised a bone fragment which was 0.07 mm thick for the compact and the spongy substance collectively.

Table 1. Mixed feeds with differentiated content of energy, protein, Ca, P, and vitamin D₃ used in turkeys' diet

	Age (week)/sex	Energy (MJ/kg)	Protein (%)	Ca (%)	P (%)	Vitamin D ₃ /kg mixed feeds (j.m.)
Starter 1	0–3	11.63	27.5	1.40	1.00	4500
Starter 2	4–6	12.05	25.5	1.30	1.00	4500
Grower 1	7–9	12.39	23.0	1.20	0.80	4000
Grower 2	10–12	12.76	21.5	1.20	0.80	4000
Grower 3	13–16	13.18	18.5	1.20	0.80	3500
Finisher	17–21, males only	13.35	17.0	1.10	0.75	3500

Table 2. Means and standard error of the mean of body weight (BW) and tibiotarsal bone weight in turkeys as influenced by age and sex

Age (weeks)	Males (mean $\pm$ SEM)		Females (mean $\pm$ SEM)	
	BW (g)	bone weight (g)	BW (g)	bone weight (g)
3	740** $\pm$ 25.5	4.43** $\pm$ 0.4	612** $\pm$ 11.8	7.45** $\pm$ 0.54
6	2 714** $\pm$ 48.8	15.17* $\pm$ 0.84	2 278** $\pm$ 13.4	16.12** $\pm$ 1.03
9	5 100** $\pm$ 209.8	45.19** $\pm$ 2.58	4 162** $\pm$ 240.3	29.02** $\pm$ 1.66
12	9 344** $\pm$ 71.6	60.25** $\pm$ 1.53	6 954** $\pm$ 90.6	48.86** $\pm$ 2.97
16	14 196** $\pm$ 217.8	78.48* $\pm$ 6.36	10 435** $\pm$ 217	71.88* $\pm$ 4.77

*means within a row are different ($P \leq 0.05$)**means within a row are different ($P \leq 0.01$)

Table 3. Lighting cycle during the rearing of turkeys as related to the bird's age

Lighting cycle	Lighting time (daily light + artificial light) (h)	Required intensity of lighting (Lux)
1 st day	20	80–100
2 nd day	19	80–100
3 rd day	18	80–100
4 th day	17	80–100
5 th day	16	80–100
6 th day	15	80–100
7 th day	14	80–100
8 th day	14	30–40
Until 12 th day	14	20
From 15 th week	14–18	20

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

Statistical analysis

The data were transformed ($\ln(x+1)$) to obtain normal distribution. The statistical analysis for comparison of age-specific influence on bone traits was done using the GLM Procedure of SAS (Statistical Analysis System, Version 9.2., 2008) adjusted by Tukey-Kramer method for multiple comparison for

the SAS model. Analyses were performed as a full random design, using the MIXED Procedure of SAS. The following overall linear model for comparison of the analyzed traits was used:

$$Y_{ijk} = \mu + S_i + A_j + (SA)_{ij} + G_k + (SG)_{ik} + (AG)_{jk} + (SAG)_{ijk} + e_{ijk}$$

where:

S_i = effect of the i^{th} section

A_j = effect of the j^{th} age

G_k = effect of the k^{th} gender

e_{ijk} = usual error term

vBMD and BMC values of 21-week-old males, in which the influence of age, sex, and interaction between these two factors were analyzed, were not used in the statistical model. The values given there are only informative. The relations between the body weight, bone weight, and vBMD and BMC of the proximal metaphysis and the middle of the diaphysis of tibiotarsal bones were determined using Pearson's correlation coefficient.

This research was approved by the Local Committee for Ethics in Experimentation on Animals (document No. 55/2008).

RESULTS AND DISCUSSION

Means for BMC and vBMD in the bone tissue *in situ* of the proximal metaphysis and diaphysis of the tibiotarsal bones of turkeys as influenced by age and sex are presented in Tables 4 and 5. BMC both in the proximal metaphysis and the diaphysis of the tibiotarsal bones in 3-week-old females occurred higher than in 3-week-old males ($P \leq 0.001$), whereas at the age of 16 wk it was attenuated (Table 4). Additionally, BMC in the proximal metaphysis attenuated only in females between wk 12 and 16 ($P \leq 0.05$) (Table 4). BMC of the proximal metaphysis and the middle of tibial bones was significantly related to age (Table 4).

Differences in BMC between the bone sections at particular ages were observed in males in wk 3 ($P \leq 0.01$), 6 ($P \leq 0.05$), and 9 ($P \leq 0.001$). At the age of wk 9 BMC differences between bone sections were the highest in males ($P \leq 0.001$). In proximal metaphyses, BMC amounted to 75.36 mg/mm, whereas in the middle of the diaphyses it was 40.7 mg/mm ($P \leq 0.01$). Such a big difference in BMC between the bone sections may be the cause of bone deformities in wk 9 in males (Figure 1), whereas in the group of females BMC differences between the middle of the diaphysis and the proximal metaphysis were observed only in wk 3 ($P \leq 0.05$) and 12 ($P \leq 0.01$) (Table 5).

In the middle of tibial bones BMC gradually increased with the age of turkeys, reaching 90 mg/mm (wk 16) in males and 70 mg/mm (wk 16) in females at slaughter maturity (Table 4).

Differences in BMC in the middle of the diaphyses were observed in females age groups between

Table 4. Means and standard error of the mean of BMC and BMD in sections within bones: proximal metaphyses and in the middle of the diaphyses of tibiotarsal bones in turkeys as influenced by age and sex

Section within bones	Item	Age (weeks)	Mean $\pm$ SEM		
			males	females	pooled
Proximal metaphysis	BMC (mg/mm)	3	11.72 ^{aA-C***} $\pm$ 0.72	22.58 ^{A***} $\pm$ 2.17	17.151 $\pm$ 1.51
		6	29.86 ^{AD} $\pm$ 1.52	27.67 ^B $\pm$ 2.32	28.76 $\pm$ 1.38
		9	75.36 ^{BD} $\pm$ 4.65	55.90 ^{AB} $\pm$ 2.75	65.92 $\pm$ 3.27
		12	83.88 ^C $\pm$ 1.54	95.51 ^{aAB} $\pm$ 6.68	89.49 $\pm$ 3.44
		16	86.29 ^a $\pm$ 3.86	82.69 ^a $\pm$ 4.57	84.49 $\pm$ 2.96
	vBMD (mg/cm ³)	3	270.34 ^a $\pm$ 10.80	294.02 $\pm$ 6.69	282.00 $\pm$ 6.62
		6	343.44 ^{aA} $\pm$ 11.78	304.13 $\pm$ 23.37	323.78 $\pm$ 13.37
		9	261.05 ^A $\pm$ 10.04	295.15 $\pm$ 3.88	277.51 $\pm$ 6.31
		12	352.80 ^B $\pm$ 4.43	317.28 $\pm$ 7.00	335.65 $\pm$ 5.23
		16	259.50 ^{B**} $\pm$ 12.32	307.38 ^{**} $\pm$ 10.53	283.44 $\pm$ 9.05
Middle of diaphysis	BMC (mg/mm)	3	7.83 ^{A-D***} $\pm$ 0.41	16.38 ^{AB***} $\pm$ 1.37	12.15 $\pm$ 1.05
		6	20.89 ^{AEF} $\pm$ 1.42	22.23 ^{CD} $\pm$ 0.41	21.55 $\pm$ 0.74
		9	40.78 ^{BE} $\pm$ 2.06	47.16 ^{AC} $\pm$ 3.09	43.86 $\pm$ 1.91
		12	74.85 ^{CF} $\pm$ 1.13	67.61 ^{BD} $\pm$ 2.13	71.35 $\pm$ 1.35
		16	91.63 ^D $\pm$ 1.92	68.86 $\pm$ 3.02	80.25 $\pm$ 2.70
	vBMD (mg/cm ³)	3	690.48 ^a $\pm$ 14.10	686.82 ^A $\pm$ 16.02	688.65 $\pm$ 10.49
		6	664.43 ^A $\pm$ 13.81	627.12 $\pm$ 29.80	645.77 $\pm$ 16.51
		9	583.42 ^{aA} $\pm$ 5.04	526.73 ^A $\pm$ 14.70	556.05 $\pm$ 9.15
		12	566.40 $\pm$ 5.01	537.89 $\pm$ 12.05	552.63 $\pm$ 6.80
		16	540.05 $\pm$ 4.88	524.76 $\pm$ 10.50	532.40 $\pm$ 5.86

BMC = bone mineral content, BMD = bone mineral density, vBMD = volumetric bone mineral density

^{a,b}means within a column with the same superscripts are different ($P \leq 0.05$)

^{A-F}means within a column with the same superscripts are different ($P \leq 0.01$)

means within a row are different: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

wk 3 and 9 as well as between wk 6 and 9 ($P \leq 0.01$) (Table 4), and between sex groups at 3 wk of age ($P \leq 0.001$) (Table 4). Similarly, differences in BMC of the proximal metaphysis were also observed between males and females in wk 3 ($P \leq 0.001$) (Table 4).

Pearson's coefficients of simple correlations showed that BMC in proximal metaphysis of the tibiotarsal bones was positively correlated with BW of males ($r = 0.89$, $P \leq 0.001$) and females ($r = 0.76$, $P \leq 0.001$) (Table 6). The correlation coefficient in the middle of tibial bones also showed the increase of BMC and amounted to $r = 0.96$ ($P \leq 0.001$) for males and $r = 0.85$ ($P \leq 0.001$) for females, respectively. When BW increased, BMC went up in the diaphyses of the tibial bones as well (Tables 2 and 6).

It was observed that when BMC increased in the diaphyses of tibiotarsal bones, vBMD in the middle of diaphyses decreased, both for males and females (Table 4). The correlation coefficient be-

tween vBMD in the middle of the diaphyses and BMC was $r = -0.52$ ($P \leq 0.01$) for females and $r = -0.85$ ($P \leq 0.001$) for males, respectively (Table 6).

vBMD depended significantly on the location within the bone and the birds' age. Significantly ($P \leq 0.001$) higher vBMD was found in diaphyses of the tibial bones. vBMD lowered gradually with age in the middle of the diaphyses (Table 4).

vBMD in the proximal metaphyses of the tibiotarsal bones in 3-week-old turkeys (σ^7 pooled) amounted to 282 mg/cm³, whereas in the diaphyses it was much higher and it reached to 689 mg/cm³ ($P \leq 0.001$). vBMD in the proximal metaphyses was significantly ($P \leq 0.01$) lower (two-fold) than in the diaphyses of bones (Table 4). vBMD in females was similar for all age groups in the metaphyses (Table 4), whereas in males the vBMD achieved maximum at wk 6 and 12 of age (approximately 350 mg/cm³) and minimum at wk 9 and 16 (approximately 260 mg/cm³). Differences in vBMD in

Table 5. Comparison of differences between bone sections for particular ages (contract with Table 4)

Sex	Age (weeks)	Section within bone	
		proximal metaphyses	middle of the diaphyses
BMC (mg/mm)			
Males	3	**	**
	6	*	*
	9	***	***
	12	ns	ns
	16	ns	ns
Females	3	*	*
	6	ns	ns
	9	ns	ns
	12	**	**
	16	ns	ns
vBMD (mg/cm³)			
Males	3	***	***
	6	***	***
	9	***	***
	12	***	***
	16	***	***
Females	3	***	***
	6	***	***
	9	***	***
	12	***	***
	16	***	***

BMC = bone mineral content, vBMD = volumetric bone mineral density, ns = not significant
means within a row are different: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

the proximal metaphyses were observed in males between wk 6 and 9 ($P \leq 0.01$) (Table 4). It should be noted that the problem of bone deformities was observed in wk 9 mainly in males (30%) until the cessation of the fattening period (Figure 1).

The vBMD in the middle of the diaphyses of the tibial bone during the first 3 wk in both sexes had the biggest bone mass – near to 700 mg/cm^3 (♂♀) and later gradually attenuated with the age both in males and females to around 530 mg/cm^3 (16 wk). Differences in vBMD of the middle of diaphyses were observed in males between wk 3–9 ($P \leq 0.05$) and 6–9 ($P \leq 0.01$) (Table 4). Pearson's correlation coefficient between the body weight and vBMD of diaphyses amounted to $r = -0.52$ ($P \leq 0.01$) for females and $r = -0.85$ ($P \leq 0.001$) for males. It was proved that vBMD of the diaphyses dropped in relation to the increasing weight of tibial bones for both sexes (Table 6). On the basis of the analysis of

the correlation coefficient, it was also observed that when BMC increased in the metaphyses, vBMD dropped in the diaphyses ($r = -0.68$, $P \leq 0.001$) (Table 6).

It was for the first time when vBMD and BMC in the proximal metaphysis and middle of tibial bones were analyzed and described systematically during the entire developmental period of the turkeys' production life, i.e. from wk 3 to slaughter age using computed tomography.

Currently there has been a little information in available literature discussing the computed tomographic analysis of tibiotarsal bone mineral density and content in poultry. Until now Tatara et al. (2005) have analyzed mechanical parameters of the tibial and femoral bones but only in one period: in 19-week-old female turkeys with the use of computed tomography, and stated that the parameters and mineral density were higher in tibial than in the femoral bones. In the previous research Tatara et al. (2004) studying the internal microstructure of femoral and tibial bones in healthy vs. sick turkeys with a computed tomography reported that in the former BMD of the trabecular bone was higher than in the latter, whereas the cortical bone density of the femoral bone in both groups of birds was similar. Thus, disorders of microstructure occurred merely in the trabecular bone.

It should be noted that BMC and vBMD and mineralisation changes of tibial bones in poultry depend on different factors, e.g. housing system, type of breeds, and growth rate of birds. Burs et al. (2008) in the research on the tibiotarsal bone strength of turkeys of the heavy type showed significant differences between mechanical strength of the tibia and the system of keeping birds. According to them, in order to increase the strength of the tibial bone of turkeys, it is advisable to keep birds in a house with access to the range area. Active turkeys had stronger bones. Thus, exercise positively influenced the process of strengthening of bones in birds. Jendral et al. (2008) found that reduction of the physical exercise of hens led to loss of the bone weight and increased the tendency of bones to fractures.

Talaty et al. (2009a, 2009b) confirmed the mineralisation changes of bones and the differences in the features of the skeleton between various lines of meat-type chickens. In turn, Williams et al. (2003) emphasised that the growth rate of bones is strictly associated with their mineral content and density. They showed that tibial bones of slow growing birds



Figure 1. Tibiotarsal bones of 9-week-old turkeys. Healthy tibiotarsal bone of 9-week-old males (1), deformed tibiotarsal bones of 9-week-old female (2) and 9-week males (3–5). The bone shade is different due to different saturation. Mineralised bone tissue absorbs more X-rays and shows as a shade (light area) contrasting with other parts of the bone (dark area). Visible lower saturation of the bone shade in the metaphyses (dark area)

Table 6. Pearson's correlation coefficient (upper part – correlation coefficients, lower part – correlation significance, *P*)

Sex	Trait	BW	Bone weight	BMC (proximal metaphyses)	vBMD (proximal metaphyses)	BMC (middle of diaphyses)	vBMD (middle of diaphyses)
Females	BW		0.93	0.76	0.13	0.85	–0.52
	bone weight	***		0.70	0.11	0.85	–0.50
	BMC (proximal metaphyses)	***	***		0.22	0.87	–0.52
	vBMD (proximal metaphyses)	ns	ns	ns		0.05	0.04
	BMC (middle of diaphyses)	***	***	***	ns		–0.52
	vBMD (middle of diaphyses)	**	**	**	ns	**	
Males	BW		0.92	0.89	0.05	0.96	–0.85
	bone weight	***		0.89	0.02	0.89	–0.83
	BMC (proximal metaphyses)	***	***		0.04	0.92	–0.85
	vBMD (proximal metaphyses)	ns	ns	ns		0.01	0.12
	BMC (middle of diaphyses)	***	***	***	ns		–0.85
	vBMD (middle of diaphyses)	***	***	***	ns	***	
Males + females	BW		0.92	0.85	0.06	0.94	–0.66
	bone weight	***		0.82	0.34	0.88	–0.64
	BMC (proximal metaphyses)	***	***		0.10	0.89	–0.68
	vBMD (proximal metaphyses)	ns	ns	ns		0.08	0.04
	BMC (middle of diaphyses)	***	***	***			–0.64
	vBMD (middle of diaphyses)	***	***	***			

BW = body weight, BMC = bone mineral content, vBMD = volumetric bone mineral density, ns = not significant differences: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

had higher mineral content and lower bone porosity than those of fast growing birds.

CONCLUSION

We conclude that the diaphyses of the tibiotarsal bones in the investigated turkeys had greater vBMD than the proximal metaphyses ($P \leq 0.001$). BMC was higher in metaphyses for both sexes. vBMD in the diaphyses gradually attenuated with age for both sexes, whereas in the metaphyses it was constant in females, but in males it achieved maximum value at wk 6 and 12 and minimum at wk 9 and 16. The authors suggest that computed tomography may be a suitable method to evaluate the changes in vBMD and BMC in tibiotarsal bones allowing determination of the bone structure as related to bird's age and sex. It may be a useful tool for detection of some disorders in mineralisation process during the growth period in turkey flocks.

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