

EVALUATION OF DENSITOMETRIC AND GEOMETRIC PARAMETERS OF TIBIOTARSAL BONES IN TURKEYS

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Abstract

The aim of the study was to analyse densitometric and geometric parameters of healthy and deformed tibiotarsal bones of male turkeys of heavy type with the use of computed tomography. The influence of a bone type (healthy or deformed) and the area of the measurements (the proximal metaphysis and middle of the diaphysis) on these parameters were studied. It was found that the diaphysis of the deformed tibiotarsal bones (45 g) had higher values of the studied parameters than healthy bones (36 g) and they were as follows: BMC - 60.04 mg/mm, vBMD - 549.11 mg/cm³, TRAB_A - 48.34 mm², CRT_A - 55.57 mm², PERI_C - 37.55 mm, ENDO_C - 25.42 mm, SSI - 151.19 mm³. The studied parameters were significantly lower in the proximal metaphyses of the deformed tibial bones and amounted to: BMC - 29.48 mg/mm, TRAB_A - 42.22 mm², CRT_A - 21.44 mm², PERI_C - 34.64 mm, ENDO_C - 28.06 mm, SSI - 44.87 mm³. On the basis of the conducted research, it was found that significant differences in the densitometric and geometric parameters between the proximal metaphysis and the middle of the diaphysis of the deformed bones may result in deformities and fractures of tibiotarsal bones.

Key words: turkey, tibiotarsal bones, leg deformities, computed tomography.

Meat type poultry suffer from many metabolic diseases of bones and disorders in the process of bone formation, which mainly attacks long bones: rickets (10), osteomalacia, and perosis (21). The most frequent abnormality - especially of cocks, is dyschondroplasia of the tibial bone (13, 16). It appears that clear clinical symptoms connected with metabolic diseases, such as growth disorders, rocking gait, lameness (12), resulting from this deformity, exist in all species of poultry. The most frequent cases were observed in turkeys (16), chicken broilers (5, 11), ducks (3), geese (2), and ratites (4, 6, 9).

The main aim of the presented study was to define the structure of the compact and spongy bone of healthy and deformed tibiotarsal bones of male turkeys suffering from noticeable limb problems (bowed pelvic limbs, unwilling moving) and to determine the relation between the body weight, the bone weight and its architectonic structure with the use of peripheral quantitative computed tomography (pQCT).

Material and Methods

The study, accepted by the Local Bioethical Committee for planned procedures on animals, was conducted on 9-week-old male turkeys of heavy type, Big 6. Densitometric and geometrical parameters of 10 healthy and 10 deformed tibiotarsal bones were analysed. It is essential to emphasise that the problem concerned approximately 20% of the flock and occurred in males. The birds were kept on deep bedding in a closed building, approximately 35 kg of the body weight per 1 m² of the floor with a permanent access to water and the standard fodder for farm animals.

The bones for the analysis were cleaned off the soft tissues and frozen at -25°C to -30°C for the time of the storing. Then, using a high resolution peripheral quantitative computed tomography (pQCT) XCT Research SA Plus (Stratec Medizintechnik GmbH, Germany), the analysis of the structure of the tibio-tarsal bones was conducted. The following densitometric and geometric parameters were determined:

vBMD - total volumetric bone mineral density (mg/cm^3).

BMC - total bone mineral content per 1 mm slice (mg/mm).

TRAB_A - trabecular area (mm^2). Cross sectional area of the trabecular area after the cortical and subcortical area has been peeled off.

CRT_A - cortical area in mm^2 . The area that is assigned to be pure cortical.

PERI_C - periosteal circumference in the „circular ring model” in mm.

ENDO_C endosteal circumference in the „circular ring model” in mm, (Fig. 1).

Strength strain index (SSI) was also calculated. SSI and the coefficient of the mechanical resistance was analysed of the diaphysis of the tibiotarsal bone (moment of resistance) and expressed in mm^3 . The method of specifying a predicted SSI is based on the assumption that the resistance of long bones is dependent on the qualities of the compact bone tissue, particularly on its density and location on the circle of the cross section. First, cross-sectional bending or

twisting moment of inertia (CSMI) is specified, characterising geometric shape of the cross-section in relation to the three-dimensional coordinate system crossing a geometric middle of the bone. The calculation includes specifying the total voxel area (the smallest element of the space, the equivalent of a pixel in a two-dimensional graphics) of a layer of a compact tissue multiplied by a squared distance from a selected axis (Fig. 2). The programme to calculate SSI (the moment of resistance) applies a tomographically specified vBMD and the bone radius (Fig. 3). In the presented study, the bending factor of bones was calculated on the basis of the moment of inertia (xCSMI).

Tomographic analysis of the tibiotarsal bone was performed in the middle (50%) of the bone length and at 18% of the proximal metaphysis, at the voxel of 0.07 mm and the scanning speed of 4 mm/min. The area for the 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 at 0.9 cm^{-1} .

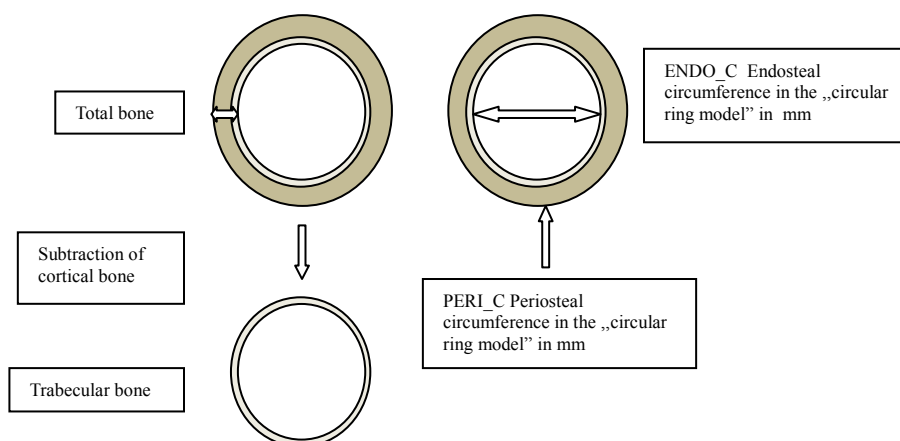


Fig. 1. Geometric parameters of bones presented graphically.

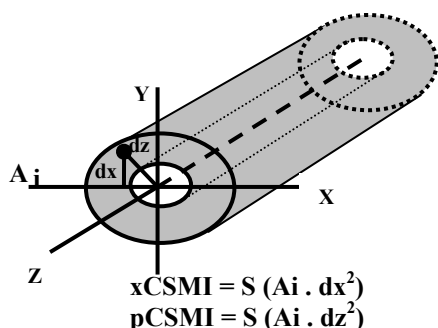


Fig. 2. Schematic presentation of the cross-sectional moments of inertia related to bending (xCSMI) or twisting (pCSMI) computed as a sum of areas of given voxels (A_i) and squared distance from a given axis (dx , dz); the formula for calculating SSI (14, 15).

$$SSI = \sqrt[3]{\frac{\sum_{n=1}^n \left[A \cdot d^2 \cdot \frac{vBMD}{avBMD} \right]}{EcMx}}$$

Fig. 3. The formula to calculate the indicator of mechanical resistance (SSI).

A – area of the sector (voxel), d – distance from the geometric centre, vBMD –volumetric mineral density of the sector (voxel), avBMD – density according to physiological standards ($1,200 \text{ mg}/\text{cm}^3$), EcMx – maximal radius along the X axis.

Statistical analysis. The conformity with the normal distribution was studied. The features that diverged from the normal distribution (TRAB_A and CRT_A) were transformed to make it similar to the normal distribution. A transformation of $y = \ln(x+1)$ type was used. Two-way analysis ANOVA was used. The studied features were: types of bones (healthy and deformed) and the area of the bone (the proximal metaphysis and middle of the diaphysis). The analysis was conducted in accordance with the model:

$$y_{ijl} = m + a_i + b_j + ab_{ij} + e_{ijl}.$$

where: y_{ijl} - value of the studied feature, m - population average, a_i - effect of the level of A factor, b_j - effect of the level of B factor, ab_{ij} - effect of interaction between i and j , e_{ijl} - random error.

T-Tukey's test was applied to compare the averages at $P \leq 0.05$ and $P \leq 0.01$. The relations between the studied features, the body weight and the bone weight were also analysed. Furthermore, relationships between the examined features and body and bone weight were tested, with the help of Pearson's correlation coefficient. Statistica (9.0) programme was used.

Results

The body weight of 9-week-old male turkeys with problems with the pelvic limbs ranged from 4,100 g to about 5,700 g, whereas, the males without signs of pelvic limb problems had significantly lower body weight ranging from 2,000 g to 3,600 g. It is worth emphasising that the deformed tibial bones of the studied turkeys were almost twice heavier ($\bar{x}$ 45 g) than

those of the healthy individuals ($\bar{x}$ 36 g).

The analysis of variance showed a significant relationship between the area of the studied bone (proximal metaphysis and middle of the diaphysis) and the type of bone (healthy and deformed) for every analysed feature (Table 1).

T-Tukey's test used to compare the averages showed statistically higher values of vBMD in the middle of the diaphysis of the tibiotarsal bone than in the proximal metaphysis both in healthy and deformed bones (Table 1). The correlation coefficient ($r = 0.63$, $P \leq 0.05$) showed that the vBMD increased in the middle of the diaphysis of deformed bones with the increase of the body (Table 2).

BMC in the middle of the diaphysis of the deformed bones was also significantly statistically higher than in the proximal metaphysis ($P \leq 0.01$). It amounted to 60.04 mg/mm and 29.48 mg/mm, respectively (Table 1). Pearson's correlation coefficient between BMC of the middle diaphysis and the weight of the deformed bones was $r = 0.68$, at $P \leq 0.05$ (Table 2).

It was stated that TRAB_A in the proximal metaphyses of the deformed tibial bones had statistically significant lower values than in healthy bones, only 42.22 mm² ($P \leq 0.01$) (Table 1). The correlation coefficient between TRAB_A in the proximal metaphyses of the deformed bones and the body weight was $r = 0.66$, $P \leq 0.05$ (Table 2). In healthy bones, Pearson's correlation coefficient was $r = 0.70$ for the body weight and $r = 0.79$ for the bone weight ($P \leq 0.05$). The interaction among TRAB_A, body weight, and bone weight in the middle of the diaphysis appeared significant in ill individuals ($r = 0.76$, $r = 0.72$, $P \leq 0.05$).

Table 1

Mean values ($\bar{x}$) and standard deviation $\pm$ SD of examined features of health (n=10) and deformed bones (n=10), depending on the place of the bones in 9-week-old turkeys males

Parameters	Type of bones	Proximal metaphysis ($\bar{x}$) $\pm$ SD	Middle of the diaphysis	Average ($\bar{x}$) $\pm$ SD
BMC mg/mm	Deformed	29.48 ^{Bb} $\pm$ 21.84 ^{**}	60.04 ^{Aa} $\pm$ 19.20 ^{**}	44.76 ^a $\pm$ 20.43
	Healthy	47.46 ^{Aa} $\pm$ 13.16 ^{**}	42.73 ^{Bb} $\pm$ 12.07 ^{**}	45.10 ^a $\pm$ 14.89
vBMD mg/cm ³	Deformed	369.73 ^a $\pm$ 47.44 ^{**}	549.11 ^a $\pm$ 85.49 ^{**}	459.42 ^a $\pm$ 63.87
	Healthy	345.38 ^a $\pm$ 16.16 ^{**}	499.64 ^a $\pm$ 58.9 ^{**}	422.51 ^a $\pm$ 41.98
TRAB_A mm ²	Deformed	42.22 ^{Bb} $\pm$ 53.32	48.34 ^a $\pm$ 10.69	45.28 ^a $\pm$ 24.75
	Healthy	74.92 ^{Aa} $\pm$ 22.67 ^{**}	36.48 ^a $\pm$ 9.02 ^{**}	55.70 ^a $\pm$ 12.54
CRT_A mm ²	Deformed	21.44 ^{Bb} $\pm$ 10.58 ^{**}	55.57 ^a $\pm$ 17.11 ^{**}	38.50 ^a $\pm$ 13.87
	Healthy	36.19 ^{Aa} $\pm$ 12.30	40.34 ^a $\pm$ 10.45	38.26 ^a $\pm$ 11.81
PERI_C mm	Deformed	34.64 ^b $\pm$ 11.78	37.55 ^a $\pm$ 3.96	36.10 ^a $\pm$ 6.89
	Healthy	47.12 ^a $\pm$ 6.89 ^{**}	31.96 ^a $\pm$ 4.04 ^{**}	39.54 ^a $\pm$ 6.71
ENDO_C mm	Deformed	28.06 ^b $\pm$ 12.53	25.42 ^a $\pm$ 2.57	26.74 ^b $\pm$ 6.31
	Healthy	39.06 ^a $\pm$ 7.08 [*]	22.62 ^a $\pm$ 2.92 [*]	30.84 ^a $\pm$ 5.49
SSI mm ³	Deformed	44.87 ^{Bb} $\pm$ 54.87 ^{**}	151.19 ^a $\pm$ 45.96 ^{**}	98.03 ^b $\pm$ 49.25
	Healthy	111.45 ^{Aa} $\pm$ 64.15	123.51 ^a $\pm$ 43.44	117.48 ^a $\pm$ 68.31

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

^{A, B} Means within a column with different superscripts are significantly different ($P \leq 0.01$)

^{**} Means within a row are significantly different ($P \leq 0.01$)

Table 2

Pearson's correlation coefficient between the examined features, with importance of correlation marked

		BW	Bone weight	BMC	vBMD	TRAB_A	CRT_A	PERI_C	ENDO_C	SSI
Healthy bones proximal metaphysis	BW	1.00								
	Bone weight	0.51								
	BMC	0.69*	0.79*							
	vBMD	0.35	-0.31	-0.32						
	TRAB_A	0.70*	0.79*	0.54	0.11					
	CRT_A	0.16	-0.30	-0.01	-0.00	0.51				
	PERI_C	0.56	0.54	0.81*	-0.38	0.75*	0.50			
	ENDO_C	0.45	0.61	0.76*	-0.55	0.66*	0.42	0.95		
	SSI	0.11	-0.11	0.11	-0.21	0.50	0.96*	0.62	0.55	1.00
Deformed bones, proximal metaphysis	BW	1.00								
	Bone weight	0.54								
	BMC	0.72*	0.44							
	vBMD	0.41	0.65*	0.76*						
	TRAB_A	0.66*	0.19	0.75*	0.35					
	CRT_A	0.39	0.32	0.79*	0.76*	0.51				
	PERI_C	0.37	-0.13	0.54	-0.06	0.57	0.25			
	ENDO_C	0.28	-0.13	0.07	-0.50	0.22	-0.29	0.78*		
	SSI	0.29	0.35	0.76*	0.82*	0.42	0.94*	0.17	-0.41	
Healthy bones, middle of diaphysis	BW	1.00								
	Bone weight	0.43								
	BMC	0.67*	0.31							
	vBMD	0.14	0.12	0.33						
	TRAB_A	0.62	0.82*	0.59	0.14					
	CRT_A	-0.44	0.29	-0.04	0.47	0.06				
	PERI_C	-0.47	0.04	-0.22	0.22	-0.11	0.87*			
	ENDO_C	-0.32	0.14	-0.25	0.14	-0.04	0.76*	0.94*		
	SSI	-0.53	0.08	-0.21	0.34	-0.13	0.94*	0.95*	0.88*	1.00
Deformed bones, middle of diaphysis	BW	1.00								
	Bone weight	0.33								
	BMC	0.55	0.68*							
	vBMD	0.63*	0.22	0.49						
	TRAB_A	0.76*	0.72*	0.83*	0.64*					
	CRT_A	0.63*	0.71*	0.99*	0.53	0.86*				
	PERI_C	0.46	0.40	0.84*	0.17	0.60	0.81*			
	ENDO_C	0.22	0.33	0.40	-0.47	0.24	0.38	0.64*		
	SSI	0.67*	0.64*	0.97*	0.58	0.89*	0.97*	0.80*	0.38	

* $P \leq 0.05$

Deformed tibial bones had lower CRT_A in proximal metaphyses in relation to healthy bones (Table 1). CRT_A of the middle diaphysis increased in deformed bones when the bone weight increased, as well ($r = 0.71$, $P \leq 0.05$). The influence of the body weight on CRT_A was also observed (Table 2).

PERI_C of healthy tibial bones was statistically significantly higher ($P \leq 0.01$), whereas PERI_C of deformed tibial bones did not show any statistically significant differences in the studied areas. The coefficient of the mechanical resistance (SSI) in the middle of deformed bones was 151.19 mm^3 , whereas in proximal metaphyses was only 44.87 mm^3 (Table 1). Pearson's correlation coefficient for SSI of deformed bones in the middle of the diaphysis was $r = 0.67$ and

0.64, at $P \leq 0.05$ in relation to BW and the bone weight and it proved that there was a correlation between these features (Table 2). Deformed bones were more prone to fractures in proximal metaphyses in relation to healthy bones. The mechanical resistance coefficient was 44.87 mm^3 for deformed bones and 111.45 mm^3 for healthy bones ($P \leq 0.01$).

Discussion

The tibia is the bone that is most frequently examined in poultry, including turkeys. In the process of bone formation in growing birds, the phenomenon of

modelling is often described. It is connected with the fact that the working strength stimulates the process of bone formation, modifying its shape and weight, and forming it (7). Thus, achieving so called bone peak mass is strongly dependent on loading and physical activity of a growing animal. According to Haapasalo *et al.* (8), the growth process is an important period for a healthy bone geometry formation, *i.e.* the spatial conformation.

The conducted research proved that deformed, bowed tibial bones of 9-week-old turkeys, which were loaded with a higher body weight in relation to healthy bones, had a bigger content of the compact and cancellous bone in the diaphysis than the diaphysis of the healthy bones. Thus, BMC, vBMD, TRAB_A, and CRT_A values were higher in the diaphysis. The diaphysis of the deformed bones was more resistant to fractures than the diaphysis of the healthy bones. The coefficient of mechanical resistance was higher in the deformed tibial diaphysis. Whereas, metaphyses of the deformed tibial bones had lower BMC, TRAB_A, and CRT_A than metaphyses of the healthy bones. Deformed tibial bones in proximal metaphyses were more prone to fractures.

Due to the high frequency of occurrence of tibial deformities, a number of experiments were conducted aiming at the recognition of diseases. Burs *et al.* (1) stated that the process of mineralisation of tibial bones may have a positive influence on a bigger freedom of movement of young turkeys.

Talaty *et al.* (17, 18) also conducted research on the resistance of tibial bones. The authors analysed changes in mineralisation of the tibial bones of chicken broilers, cocks, and hens, aged 2-8 weeks, using dual X-ray absorptometry (DEXA). They found that the bone mineral density was the biggest in 4-week-old chicken broilers and slightly higher in cocks.

Charuta *et al.* (3) analysed changes in the structure of the spongy substance of the tibiotarsal bones in ducks in the postnatal development using the Trabecula programme for studying the bone tissue. It was stated that during the development (from the hatching to the slaughter maturity) 6-week-old ducks had the lowest number of trabeculae and the lowest density of trabeculation, which showed that remodelling of the cancellous bone tissue caused by the increase in the body weight took place. At that age, ducks suffered from the highest number of fractures of the tibial bones and locomotor disorders.

Tatara *et al.* (19) also analysed mechanical and geometrical parameters of the tibial bones of turkeys coming from two groups of 17-week-old birds: healthy and those, which had limb problems. The achieved results showed that turkeys with deformed limbs and locomotor disorders had lower mineral density of the femoral and tibial bones. Tatara *et al.* (20) also analysed mechanical and geometrical parameters of the femoral and tibial bones of healthy turkeys with the use of computed tomography and stated that the tibiotarsal bones had bigger mineral density than the femoral bones.

Summing up, the conducted research showed that mineralisation disorders and modelling occur in the

tibiotarsal bones and are influenced by a high body weight during the development of turkeys. It is the result of bone adaptation of the birds to high body weight achieved in a short period.

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