Age- and Sex-Related Changes in Mineral Density and Mineral Content of the Tibiotarsal Bone in Quails During Post-hatching Development

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Summary

For the first time computed tomography has been used to analyse tibiotarsal bones volumetric mineral density (vBMD) and mineral content (BMC) in quails during their post-hatching development. The analysis was performed including eighty male and female quails aged 2, 4, 6 and 8 weeks, respectively. Statistical calculations were performed using two-way ANOVA. It was found that vBMD generally in the middle of the diaphyses in the tibiotarsal bones in quails was two-fold higher vs. the metaphyses. Decrease of vBMD and BMC in the metaphyses of the tibiotarsal bones in males occurred between the 4 wk and 6 wk of the post-hatching development; the lowest values of vBMD (217 mg/ccm) and BMC (3.68 mg/mm) were observed in 6 wk males. In turn, in females, the decrease of vBMD in the metaphyses occurred in the 4th and 8th wk. Pearson's correlation coefficient between the metaphyseal vBMD and body weight in males was negative and amounted to r = -0.71 in the 4 wk and r = -0.44 in the 6 wk. In the middle of the diaphyses, decrease in vBMD was observed in both sexes in the 4 wk and 8 wk. Our research showed that changes in vBMD and BMC are disorders in the process of mineralisation. In both sexes, they caused reluctance to walk and increased the likelihood of deformities and fractures.

Keywords: Bone mineral density, Bone mineral content, Tibiotarsal bone, Mineralisation, Quails

Bıldırcınların Yumurtadan Çıkışını Takip Eden Dönemde Tibiorsal Kemikte Yaş ve Cinsiyet İle İlişkili Mineral Yoğunluk ve Mineral İçerik Değişiklikleri

Özet

Bıldırcınların yumurtadan çıkışını takip eden süreçte, ilk kez bilgisayarlı tomografi kullanılarak tibiotarsal kemik mineral yoğunluğu (vBMD) ve mineral içeriği (BMC) analizi gerçekleştirilmiştir. Analiz, yaşları 2, 4, 6 ve 8 haftalık seksen erkek ve dişi bıldırcın üzerinde gerçekleştirilmiştir. İstatistiksel hesaplamalar iki yönlü ANOVA kullanılarak yerine getirilmiştir. vBMD'nin genellikle, metafiz ile karşılaştırıldığında, bıldırcınların tibiotarsal kemik içi diyafizinde 2 kat daha fazla olduğu saptanmıştır. Erkek bıldırcınların tibiotarsal kemik içi metafizinde vBMD ve BMC azalması, yumurtan çıkışını takip eden gelişme döneminin 4. ve 6. haftalarında meydana gelmektedir; erkeklerde en düşük vBMD (217 mg/ccm) ve BMC (3.68 mg/mm) değerleri 6. haftada gözlemlenmiştir. Buna karşın, dişilerde metafiz içinde vBMD azalması 4. ve 8. haftalarda oluşmuştur. Metafiz vBMD ve erkek vücut ağırlığı arasındaki Pearson'ın korelasyon katsayısı negatif olup 4. haftada r = -0.71 ve 6. haftada r = -0.44 değerine sahiptir. Diyafizde ise her iki cinsiyet için 4. ve 8. haftada vBMD'de azalma oluğu gözlemlenmiştir. Yaptığımız çalışma, vBMD ve BMC değişikliklerinin mineralizasyon sürecini bozduğunu göstermiştir. Her iki cinsiyete yürüme konusunda isteksizliğe neden olup şekil bozuklukları ve çatlama olasılıklarını yükseltmişlerdir.

Anahtar sözcükler: Kemik mineral yoğunluğu, Kemik mineral içeriği, Tibiotarsal kemik, Mineralizasyon, Bıldırcın

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INTRODUCTION

The Japanese quail (Coturnix coturnix japonica) is a subject of interest for poultry farmers for a very long time as it has been used for the production of both eggs and meat ^{1,2}. In both cases, the quail is very efficient by comparison with other species of poultry³. Due to its high yield efficiency, the quail, similarly to other poultry species in the research conducted by other authors 4-7, has problems with the skeleton. However, there are only few studies on skeleton formation, including the tibiotarsal bone during posthatching development of quails 8. In present literature, there is lack of data concerning mineral content BMC and bone density vBMD values in bone sections which are most exposed to deformities: proximal metaphysis and mid-diaphysis in situ of tibiotarsal bones in quails from the moment of hatching till slaughtering maturity in post hatching development. The bird is an experimental one used as an animal model in osteoporosis research and in mechanisms of bone turnover⁹. In some publications, posthatching development of the skeleton of the pelvic limb of Japanese quails, including bone formation processes, are described in details, however, it is done till 16 day of living ^{10,11}. That is why it is important to learn about processes describing bone mineralisation in further stages of quail development of a tibiotarsal bone, particularly exposed to deformities. Thus, the aim of the study was to analyse the changes in mineral content and density of the tibiotarsal bones during post-hatching development in quails as influenced by sex and age.

MATERIAL and METHODS

The study was conducted on 80 quails. The birds were divided into the following age groups: 2, 4, 6, and 8 weeks (wk). The quails were kept in cages. 3-wk-old birds were moved to cages for adult birds. The height of the cage was 22 cm, the length - 90 cm, the width - 53 cm. The floor of the cages was honeycomb too with eyeholes not bigger than 1 cm. The birds had three birdbaths and the access to feed ad libitum. In one cage there were 15 females and 5 males. The quails were fed with full-portion feed prepared especially for quails. The feed has a consistence of crumble (crumbled granulate).

The feeding of nestlings (1-7 day), during this period 1 kg of the feed had 12.56MJ of the metabolic energy, 28% protein, 1.0% calcium. The second stage of feeding was 8-28 day (12.14 MJ metabolic energy, 24% protein, 0.80% calcium). The third stage was from 29 to 42 day and longer (11.72 MJ of metabolic energy, 20% of protein, 0.80% calcium.

Tibiotarsal bones to be analysed were obtained from 10 males and 10 females from each of the age groups. The bones were thoroughly cleaned from soft tissues (muscles were removed, the periosteum, epiphyseal and metaphyseal cartilages were left). Each bone was weighed. A laboratory

weight was used to measure body weight and bone weight (AXIS, type AG500C, max loading 500 g with the read graduation of 0.001 g).

Then the bones were frozen in the temperatures from -25°C to -30°C. Next, XCT Research SA Plus Peripheral Quantitative Computed Tomography (pQCT) Scanner (Stratec Medizintechnik GmbH. Pforzheim Germany) was used to analyse the *in situ* structure of the proximal metaphyses and in the middle of the diaphyses of the tibiotarsal bones. Before the pQCT analysis, the bones were defrosted.

The following densitometry parameters were determined:

vBMD - Total volumetric bone mineral density (mg/ccm). The mean density of the total bone.

BMC - Total bone content per 1 mm slice (mg/mm). The mineral content of the total bone within a 1 mm slice.

The tomographic analysis of the proximal metaphysis was conducted at 18% of the bone length, whereas the analysis of the in the middle of the diaphyses of the tibiotarsal bone was performed at 50% of the bone length with the voxel size of 0.07 mm and scanning speed of 4 mm/min. The area of analysis was determined following preliminary scanning (20 mm/s) and the bone length was measured. Threshold coefficient, differentiating compact bone from trabecular bone, was determined at the level of 0.900 cm⁻¹.

The obtained results underwent statistical analysis. All calculations were performed using the Statistica 9.0 software (StatSoft, Inc. Tulsa, USA), at P \leq 0.05. The two-way ANOVA analysis was conducted in accordance with the model:

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

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

T-Tukey's- Kramer 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 use of Pearson's correlation coefficient.

The analyses were conducted after the Local Ethics Committee had accepted and approved the planned procedures on the experimental birds (33/2009). There was no conflict of interest during the course of the study.

RESULTS

Mean values of BMC and vBMD for tibiotarsal sections and body weight depending on age and sex, are presented in the following tables and figures:

Differences in Bone Density vBMD and Mineral Content BMC in Males as Influenced by Age

Analysing mineral density vBMD in proximal metaphyses of tibiotarsal bones in post-hatching development of males, it was stated that the first decrease of vBMD occurred in proximal metaphyses in 4 wk, then between 4 and 6 wk vBMD in proximal metaphyses in males of tibiotarsal bones significantly attenuated by 102.68 mg/ccm, at P \leq 0.05 (*Table 1*).

Another analysed parameter in the proximal metaphysis of males was mineral content BMC, which amounted to 4.18 mg/mm in 2 wk. During the whole post-hatching development, the lowest mineral content in the proximal metaphysis of males was observed in 6 wk, it was only 3.68 mg/mm. BMC attenuated by 1.52 mg/mm, at P \leq 0.01 in 6 wk (in comparison to 4 wk) (*Table 1*). It is woth noting that BMC values attenuated in proximal metaphyses in 6 wk, whereas vBMD values in 4 and 6 wk. At that age, unwillingness to move and more bone fractures were observed, whereas at 6 wk – macroscopically deformed tibiotarsal bones.

Analysing densitometric parameters in the middle of the diaphyses of the tibiotarsal bone in males, it was stated that vBMD in the diaphyses was twice bigger than in the metaphyses (*Table 1*). BMC in the diaphyses was lower than in the proximal metaphyses (*Table 1*). The highest vBMD and BMC in the middle of the diaphyses was observed in 6 wk males and amounted to 669.83 mg/ccm and 3.95 mg/mm, respectively. The lowest vBMD in mid-diaphysis in males was observed in 4 wk, in relation to values achieved in 6 wk it was lower by 95 mg/ccm, at P \leq 0.05 (*Table 1*).

Differences in Bone Density vBMD and Mineral Content BMC in Females as Influenced by Age

Bone density vBMD of the tibiotarsal bones in proximal

metaphyses in 2 wk females amounted to 313.48 mg/cm³. In 4 wk in proximal metaphyses, there was a slight decrease in vBMD values, whereas in 6 wk the highest values of vBMD in females in the whole post-hatching development in proximal metaphyses were observed in 6 wk (450 mg/ ccm). Significant statistical differences were found between vBMD values between 4 and 6 wk, at P≤0.05.

Mineral content of BMC in proximal metaphyses in females was the lowest during the post-hatching development in 2 wk (3.65 mg/mm), and the highest in 6 wk (5.88 mg/mm). The values of densitometric parameters in the middle of the diaphyses were as follows: vBMD was twice bigger in relation to metaphyses. During the post-hatching development in females, the highest vBMD in the middle of the diaphyses was observed in 6 wk (748.00 mg/ccm, at P≤0.05 (*Table 1*). The highest BMC values in mid-diaphysis were observed in 8 wk (5.04 mg/mm).

Differences in Bone Density vBMD and Mineral Content BMC between Males and Females as Influenced by Age

No significant differences in vBMD were observed between males and females in the proximal metaphyses in 2 wk vBMD increased both in males and females during the post-hatching development till 4 wk. There were statistically significant differences in vBMD in proximal metaphyses between 6 wk males and females at P<0.01. vBMD amounted to 217.29 mg/mm for 6 wk males and 450 mg/mm for females at the same age.

During the analysis, it was stated that BMC was higher in males in the proximal metaphyses 2 and 4 wk (*Table* 1). However, no statistically significant differences were observed. Statistically significant differences in BMC values were found between males and females in 6 wk, at $P \le 0.01$.

Item	Section Within Bones								
BMC mg/mm		Proximal Meta	physis	Middle of the Shaft					
Age	Males ± SD	Females ± SD	Pooled Sexes ± SD	$Males \pm SD$	Females ± SD	Pooled Sexes ± SD			
2wk	4.18±0.83 ªA	3.65±0.66 ^{aA}	3.81±0.70 ªA*	1.74±1.13ªA	1.93±0.31 ^{aA}	1.83±0.26ªA*			
4wk	5.20±0.78 ªA	4.23±0.85 ^{aA}	4.62±0.94 ªA*	3.11±0.47 ^{ьв}	3.16±0.51 bB	3.14±0.49 ^{bB*}			
6wk	3.68±0.81 bB**	5.88±0.0 ^{bB**}	4.69±0.99 ^{aAbB*}	3.95±0.41 ыв	4.15±0.00 ^{bB}	3.97±0.38 cC*			
8wk	4.47±0.56 ªA	4.48±0.49 ªA	4.51±0.55 ^{bB*}	3.78±0.33 ^{bB**}	5.04±0.75 ^{cC**}	4.41±0.66 ^{cC}			
BMD mg/ccm		Proximal Metaphysi	s	Middle of the Shaft					
Age	Males ± SD	Females ± SD	Pooled Sexes ± SD	$Males \pm SD$	Females ± SD	Pooled Sexes ± SD			
2wk	327.30±43.16ª	313.48±15.18ª	336.04±66.87 ^{c*}	628.34±39.23ª	650.61±37.31ª	638.82±38.85 c*			
4wk	329.97ª±33.09	285.15±36.57ª	346.04±58.90 ^{ab*}	574.60±55.13ª	609.78±100.93ª	597.47±87.69 ^{ab*}			
6 wk	217.29 ^{b±} 60.36	450.10±0.00 ^b	371.10±64.49 ^{b*}	669.83±79.58ª	748.00±0.00 ^b	680.02±78.70 ^{b*}			
8 wk	337.65ª±62.36	340.92±30.90 ^a	338.31±57.13ª*	593.61±40.38ª	686.57±28.29ª	612.21±53.56ª*			

^{*a,b*}Means within a column with different superscripts are significantly different ($P \le 0.05$), ^{*A,B*}Means within a column with different superscripts are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$), ^{***}Means within a row are significantly different ($P \le 0.01$).

Age	BMC											
	Proximal Metaphysis				Middle of the Shaft							
	м	ales	Females		Males		Females					
	BW	Bone Mass	BW	Bone Mass	BW	Bone Mass	BW	Bone Mass				
2 wk	1.00*	1.00*	0.81*	0.77*	0.74*	0.74*	0.48	0.83*				
4 wk	0.15	0.49	0.62*	0.80*	0.75*	0.40	0.20	0.63*				
6 wk	-0.90*	-0.07	0.24	0.17	0.10	0.45*	0.44	0.21				
8 wk	0.55*	0.24	-0.79*	-0.12	0.30	0.77*	0.78*	0.83*				
Age	BMD											
2 wk	1.00*	1.00*	0.06	0.04	-0.06	-0.09	0.28	0.15				
4 wk	-0.71*	-0.58*	0.45	0.46	0.31	-0.11	0.08	0.52				
6 wk	-0.44*	-0.15	-0.95*	-0.34	-0.10	-0.02	0.23	0.38				
8 wk	0.29	-0.12	-0.89*	-0.48	0.14	-0.21	0.66*	0.31				

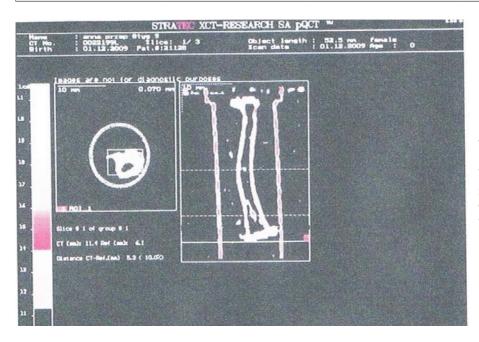


Fig 1. Tomographic analysis of a deformed tibiotarsal bone in quail male in 6 wk with marked analysis areas: proximal metaphysis and mid-diaphysis

Şekil 1. Bıldırcında işaretli analiz bölgesinde deforme tibiotarsal kemiğin tomografik analizi: proksimal metafiz ve orta diafiz

BMC in 6 wk was significantly higher in females (5.88 mg/ mm) than in males (3.68 mg/mm). It is worth emphasising that statistically significant differences in BMC in the middle of the diaphyses between the two sexes were observed in 8 wk, at P≤0.01. BMC amounted to 3.78 mg/mm for males and 5.04 mg/mm for females (Table 1). Statistically significant differences were also observed in vBMD between males and females in 6 and 8 wk ($P \le 0.05$) (*Table 1*).

Pearson's Correlation Coefficient

Pearson's correlation coefficient defined the relation between vBMD and BMC values and body and bone weight in particular bone sections. The BMC in the proximal metaphyses in 2 wk males depended on body weight r = 1.00and bone weight r = 1.00. In 2 wk females, BMC was also related to body weight r = 0.81 and bone weight r = 0.77. Therefore, BMC increased in tibiotarsal bones when body weight and bone weight grew. A similar relation was observed in the middle of the diaphyses in 2 wk individuals of both sexes (Table 2). The BMC in proximal metaphyses in 4 wk females depended on body weight (r = 0.62) and bone weight (r = 0.80). Thus, between 2 and 4 wk vBMD and vBMD values rose when body and bone weight increased.

In a group of males in proximal metaphyses, when BW increased in 6 wk, BMC decreased r = -0.90.

While analysing Pearson's correlation coefficient in 2 wk males, a strong positive correlation was noticed between vBMD, and BW (r = 1.0) and bone weight (r = 1.00) in proximal metaphyses. A strong negative s correlation between vBMD and body weight (r = -0.71) and bone weight (r = -0.58) was observed in 4 wk males. Thus when body weight and bone

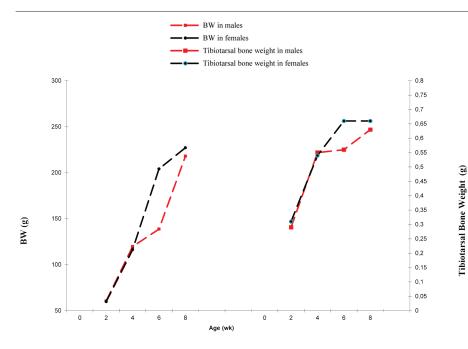


Fig 2. Mean values of BW (Body Weight g) and tibiotarsal bone weight in quails, depending on age and sex

Şekil 2. Yaş ve cinsiyete bağlı olarak bıldırcınlarda BW (vücut ağırlığı g) ve tibiotarsal kemik yoğunluğu ortalama değeri

weight increased in 4 wk males, the decrease of vBMD was observed. A negative correlation between vBMD and BW was also observed in a group of 6 wk males (r = -0.44).

In females, it was observed that when BW rose, BMC (r = -0.79) decreased in 8 wk and vBMD attenuated in 8 wk (r = -0.89).Whereas, BMC and vBMD grew in the middle of diaphyses in 8 wk females when body weight increased, Pearson's correlation coefficient amounted to (r = 0.78) and (r = 0.66), respectively (*Table 2*).

DISCUSSION

The tibiotarsal bone is the most frequently studied one in poultry, including quails and on influences of nutrition ¹² and biology ¹³. A lot of factors have an influence on shaping the structure of the skeleton and BMC and vBMD values in bones, such as food ¹⁴ or breeding method ¹⁵. The authors suggest ¹⁶ that birds kept in aviary system had stronger bones than those kept in cages. According to Jedral ¹⁷, birds kept in cages had lower vBMD.

The conducted research analysed the influence of age and sex on vBMD and BMC in tibiotarsal bones in quails which were kept in cages.

The research confirmed that BMC was higher in proximal metaphyses and vBMD was higher in diaphyses (twice). It is a normal situation resulting from a bone structure. In proximal metaphyses, there is more mineral, as rebuilding processes take place faster there and metabolism is 8 times higher in the cancellous bone of proximal metaphyses.

As far as differences in densitometric parameters in tibiotarsal bone as influenced by sex were concerned, it is essential to emphasize that quails experience a significant (P \leq 0.01) sexual dimorphism, which is noticeable from 4 wk of age. The birds studied showed clear differences between sexes. It mainly concerned the body weight, which was higher in 6 wk females (200 g) than in 6 wk males (140 g).

The achieved results showed that from the 2 wk BMC increased in proximal metaphyses when body weight grew in both sexes, which was confirmed by a positive correlation r = 1.00 for males and r = 0.81 for females. In 2 wk, a positive correlation between BMC, BW and bone weight was observed in the middle of the diaphysis for both sexes. Also in males in 2 wk, a strong positive correlation was noticed between vBMD, and BW (r = 1.00) and bone weight (r = 1.00). In the further post-hatching development vBMD values of proximal metaphyses of tibiotarsal bones in 4 and 6 wk males attenuated. It is worth noting that strong correlation between vBMD and body weight (r = -0.71) and bone weight (r = -0.58) was observed in 4 wk males. Another analysed parameter, BMC, achieved the lowest values in proximal metaphyses in 6 wk (r = -90). Thus, decreasing BMC values in 6 wk males attenuated bone resistance to fractures. The values of both densitometric parameters increased in 8 wk males. Whereas in females, the decrease of vBMD values was observed in proximal metaphyses in 4 and 8 wk. The attenuation of BMC values in metaphyses occurred in 8 wk. It is worth adding that in females between 4 and 6 wk, body weight increased by approximately 88g and the values of densitometric parameters increased in 6 wk. In males, body weight increased by 21 g between 4 and 6 wk, whereas BMC and vBMD values attenuated. It was also observed that the decreasing values of both densitometric parameters along with the increase of body weight and bone weight in 6 wk in males were the cause of deformities of tibiotarsal bones. Three males out of 10 (30%) had deformed tibiotarsal bones in 6 wk. In a group of females, limb problems were observed in 8 wk. Two out of 10 (20%) females had deformed tibiotarsal bones.

It is worth noting that a similar research was conducted in turkeys ¹⁸. During the post-hatching development of turkeys, it was stated that vBMD attenuated in proximal metaphyses in 9 wk males (261.05 mg/ccm) and females (295.15 mg/ccm). In 9 wk turkeys, numerous fractures and bone deformities of tibiotarsal bones were observed, whereas in quails bone deformities appeared in 6 wk.

Using computed tomography, vBMD was also analysed in tibiotarsal bones of ducks ¹⁹. It was stated that vBMD attenuated in proximal metaphyses between 4 and 6 wk in ducks of both sexes, which caused deformities in 6 wk, thus visible deformities of tibiotarsal bones of ducks and quails occurred at the same age. It should be emphasised that the decrease of vBMD in proximal metaphyses was observed earlier than in ducks, in 4 wk.

Trabecula[®] programme was used to analyse the structure of trabeculae in proximal metaphyses of tibiotarsal bones in ducks in post-hatching development. The number, volume and density of radiological trabeculae were determined. It was found that the density was the lowest in 6 wk ducks and amounted to 44.62%. Such a low density was the cause of numerous fractures of tibiotarsal bones ⁶. Trabecula[®] programme was also used to study the structure of tibiotarsal bones in the development of geese as influenced by age and sex. It was found that the lowest number of trabeculae in proximal metaphyses was observed in 6 wk males (6.34 mm²). Density (33.73%) and volume of trabeculae (1.50% mm) was also the lowest at that age group ⁷.

The achieved results concerning birds' development are a valuable source of information for poultry farmers because there is a critical moment in the development of the above mentioned species in which bone density BMD decreases causing bone deformities and making poultry breeding less profitable.

In conclusion, the presented reasearch determined the value of vBMD and BMC in quails during post-hatching development using a computed tomography. The gradual densitometric values in the proximal metaphyses were the cause of fractures and deformities bones in quails. Reduction of the densitometric values in the proximal metaphyses the ever increasing body weight caused deformation of the tibiotarsal bone. The results can provide valuable information for poultry farmers were the cause of fractures.

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