ASSESSMENT OF THE MINERAL DENSITY AND MINERAL CONTENT OF THE EQUINE PROXIMAL PHALANX DEPENDING ON DIFFERENT MORPHOLOGICAL TYPES AND SEX

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Abstract. So far, the analysis of the bone mineral density (BMD) and the bone mineral content (BMD) of proximal phalanges, which would simultaneously include the differences resulting from the different morphological types of horses has not been investigated. In humans, these parameters are inextricably linked with age, sex and breed. It is not known if this is the case in horses as well. The research material consisted of isolated proximal phalanges derived from 37 horses. The individuals represented different morphological types: respiratory-dolichomorphic type, respiratory-muscular-mesodolichomorphic type, muscular-lymphatic-mesometric type and lymphatic-brachymorphic type. The research was conducted with the use of densitometer Norland model Excell Plus (Fort Atkinson WI, USA). Numerical material reflecting BMD and BMC of the pastern bones of horses was characterized on the basis of descriptive statistics. In order to check the significance of differences between the studied morphological types one-factor analysis of variance was applied, whereas differences between individuals of various sexes were examined with the use of t-student test. No significant differences (P) between average values of BMD and BMC for the studied morphological types and sexes of horses were found.
Keywords: horses, densitometry, proximal phalanges, thoracic limbs.

PRIEKINĖS KOJOS VIRŠUTINIŲ PIRŠTAKAULIŲ MINERALŲ TANKIO IR KIEKIO PRIKLAUSOMYBĖS NUO MORFOLOGINIO ARKLIŲ TIPO IR LYTIES VERTINIMAS

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Santrauka. Skirtingų morfologinių tipų arklių priekinių kojų viršutinių pirštakaulių mineralų kiekio (BMD) ir tankio (BMD) tyrimų kol kas nėra atlikta. Žmonių kauluose šie parametrai neatskiriamai susiję su amžiumi, lytimi ir rase. Nebuvo žinoma, ar tas būdinga ir arkliams. Tyrimo metu ištirti 37 arklių priekinių kojų viršutiniai pirštakauliai. Tirti arkliai priklausė skirtingiems morfologiniams tipams: respiratoriniam-dolichomorfiniam, respiratoriniam-raumeniniam-mezodolichomorfiniam, raumeniniam-limfatiniam-mezometriniam ir limfatiniam-brachimorfiniam. Tyrimas atliktas tankio matavimo prietaisu „Norland model Excel Plus“ (Fort Atkinson WI, JAV). Skaitmeniniai duomenys apie čiurnos kaulų BMD ir BMC apibūdinti remiantis aprašomąja statistika. Skirtumams tarp morfologinių tipų reikšmingumo nustatyti pritaikyta vieno faktoriaus variantiškumo analizė. Tuo tarpu skirtumai tarp įvairių lyčių individų buvo nustatomi pagal Stjudento t-testą. Reikšmingų BMD ir BMC vidutinių verčių skirtumų (p) tarp morfologinių arklių tipų ir lyčių nenustatyta.
Raktažodžiai: arkliai, tankio matavimai, viršutiniai pirštakauliai, priekinės galūnės.

Introduction. One of the difficulties in the interpretation of the results of densitometry of horse bones is the lack of a justified way of presenting measurements of bone mineral density (BMD) and bone mineral content (BMC). In order to make the results of measurements clinically useful for individual patients, they must be referred to similar values achieved from a healthy referential population. A referential population is usually described in terms of an average BMD and standard deviation values (SD) in a population appropriate in terms of age, sex and breed. As far as humans are concerned, the values are strictly defined both for age, sex and race (Hui et al., 2003). It is known that individuals of the black race have a bigger bone mineral density in comparison with those of the white race (Abrams et al. 1995; Bryant et al. 2003). It is found that especially at the age of puberty, significant differences in the amount of calcium stored in the skeleton were observed. This higher calcium retention by females of the black race may be responsible for the increased density of the bone tissue in adult black females in reference to females of the white race (Braun et al. 2007).

So far most of the research concerning the parametric evaluation of horses' bones has been conducted mainly to determine the influence of physical activity and food types on BMD and BMC values of the long bones (Porr et al. 1998; Hoffman et al. 1999; Firth et al. 2005; Tóth et al. 2010). Due to the fact that the proximal phalanges in the thoracic limbs of the horses are one of the most exposed to injuries (Hodson et al. 2001; Dzierzęcka et al. 2007), it became the subject of the present research. The aim of the study was to evaluate the dependence of BMD and BMC on sex and morphological type of this species.

Table 1. **Morphological types*, usage, sex, age in years and cause of death of 37 horses in which studies of the first phalanges were conducted focusing on their bone mineral density and bone mineral content**

Bone marking**	Morpholo-gical types	Usage	Gender	Age (year)	Cause of death
1P, 1L	O	jumper	male	7	Bone fracture (euthanasia)
2P, 2L	OM	recreational	male	7	Tendon injury (euthanasia)
3P, 3L	ML	farm	male	4	Slaughtering house
4P	O	racehorse	male	7	Colic
5L	L	farm	male	3	Slaughtering house
6L, 6P	ML	farm	male	15	Slaughtering house
7L	ML	farm	female	5	Slaughtering house
8P	O	jumper	male	5	Colic
9L, 9P	L	farm	female	6	Heart disease (euthanasia)
10P	ML	farm	female	4	Slaughtering house
11P, 11L	O	jumper	female	4	Tendon injury (euthanasia)
12L, 12P	OM	recreational	male	7	Colic (euthanasia)
13P	L	farm	male	3	Slaughtering house
14 L	ML	farm	female	4	Slaughtering house
15L	ML	farm	female	4	Slaughtering house
16P	ML	farm	male	10	Slaughtering house
17L	O	racehorse	female	4	Bone fracture (euthanasia)
18L, 18P	ML	farm	female	15	Metritis (euthanasia)
19L	ML	farm	male	3	Electrocution
20L, 20P	ML	farm	male	4	Colic
21L, 21P	OM	recreational	female	7	Colic
22L, 22P	OM	recreational	female	4	Bone fracture (euthanasia)
23L, 23P	OM	recreational	female	4	Bone fracture (euthanasia)
24L	OM	recreational	male	12	Colic
25P	O	racehorse	male	5	Bone fracture (euthanasia)
26P, 26L	ML	farm	male	12	Slaughtering house
27L	L	farm	female	8	Slaughtering house
28P, 28L	OM	recreational	male	3	Colic (euthanasia)
29L, 29P	L	farm	female	7	Slaughtering house
30L, 30P	ML	farm	male	8	Slaughtering house
31P	ML	farm	female	15	Slaughtering house
32L, 32P	OM	recreational	female	8	Colic
33L,33P	ML	farm	female	5	Colic
34L, 34P	ML	farm	female	7	Slaughtering house
35L, 35P	ML	farm	male	4	Slaughtering house
36L, 36P	ML	farm	male	5	Slaughtering house
37L, 37P	ML	farm	male	5	Slaughtering house

*Morphological types:
O – respiratory, OM – respiratory-muscular, ML-muscular – lymphatic, L-lymphatic; **P – right bone, L – left bone

Material and Methods. The research material consisted of isolated proximal phalanges of the thoracic limbs derived from 37 individuals (range age 3–15 years). In 22 cases, bilateral proximal phalanges of the thoracic limb were examined, whereas, unfortunately, only one of the proximal phalanges from each 15 other cases were available (in 7 cases the right one and in 8 cases the left one). There were no animals in which any anomalies concerning the proximal phalanges had been stated intravitally, and their slaughtering or death were not the result of any diseases. Before slaughtering or on the basis of the corpse observation, their morphological characteristic was conducted as depending on a morphological type. Each individual, according to features of its built, represented an example of one of the morphological types (somatotype constituted): respiratory-dolichomorphic type (about 400–500 kg) – 6 individuals, respiratory-muscular-mesodolichomorphic type (about 450–550 kg) – 8 individuals, muscular-lymphatic-mesometric type (about 500–600kg) – 18 individuals and lymphatic-brachymorphic type (about 600–800 kg) – 5 individuals (Table 1). The morphological types were determined in accordance with the criteria suggested by Komosa et al. (2006).

After isolating the proximal phalanges from the soft tissues, the bones were marked, placed in air-tight plastic bags and then kept 4 months in the temperature of -20°C. Having collected the whole research material, proximal phalanges were transported and examined in Densitometer and Computed Tomography Laboratory at the Faculty of Veterinary Medicine of University of Life Sciences in Lublin. The research was conducted with the use of a densitometer Norland, Excell Plus (Fort Atkinson WI, USA), using the affinited beam X-ray technology and the programme for animal research (Research Scan, 3.9.6. version) at the following parameters: scanning resolution of 1.5 x 1.5 mm, scanning speed of 60 mm/s. The densitometer was calibrated with the use of a phantom provided by the producer before each measuring series. Then, the mineral content of the bones was analysed and given in g/cm^2 for BMD and in g for BMC.

In the second part of the study, the differences between male and female were examined. Due to a small number of researches conducted on some morphological types of horses and recognised lack of significant differences between BMD and BMC values between the right and left limb (Dzierzęcka and Czerwinski, 2010, 2012), the values for comparative analysis were taken both from the right and left limb. So we had a total of 32 proximal phalanges of males and 27 pastern bones from females (Table 1).

Descriptive statistics (the averages, standard deviation and coefficient of variability) were used to analyse the numeral material concerning density and mineral content in the pastern bones. To check whether the BMD and BMC values of the pastern bones taken from horses of various morphological types differ significantly, one-factor analysis of variance (ANOVA) was applied randomly. BMD and BMC parameters of the proximal phalanges were compared between individuals of different sexes with the use of t-Student test. Measurements were conducted using statistical software Statgraphics.

Results. Descriptive statistics of the studied morphological types for BMD and BMC of the proximal phalanges of horses' thoracic limbs is shown in Table 2, whereas averages and standard deviations of the examined features are presented in Fig. 1 – BMD and Fig. 2 – BMC.

Table 2. **Average (** $\bar{x}$ **), standard deviation** (SD)**, variability coefficient** (V%)**, bone mineral density** (g/cm) **and bone mineral content** (g) **values of the equine proximal phalanges in thoracic limbs for the investigated morphological types***

Type	Sample number	BMD			BMC		
		Average $\bar{x}$	SD	V%	Average $\bar{x}$	SD	V%
O	8	1.83	0.25	13.48	96.39	15.20	15.77
OM	15	1.91	0.16	8.31	95.92	18.11	18.88
ML	29	1.84	0.21	11.51	88.67	14.56	16.43
L	15	1.75	0.26	14.85	86.85	14.83	17.07

The achieved variability coefficients presented in Table 2 show a smaller diversity of BMD values compared with BMC values for all morphological types. Among all the types, the respiratory-muscular type (OM) was characterised by the smallest diversity of BMD and the highest of BMC.

The average values of both features within the examined types were similar. To check whether the BMD and BMC values of the proximal phalanges taken from horses of various morphological types differ significantly, one-factor analysis of variance (ANOVA) was applied. The achieved results are presented in Table 3. At fulfilled assumptions of the variance analysis, checked by the Bartlett's test, no significant differences between the average values of BMD for the studied morphological types were found. It is possible to assume that BMD values are almost the same for the studied types. A similar conclusion may be drawn on the basis of results of the variance analysis achieved for BMC. The averages of the feature for the studied types of horses also do not differ significantly (Table 3).

Due to the fact that earlier statistical analyses showed that BMD and BMC values for various morphological types of horses do not differ significantly, in the second part of the research they were treated homogeneously. BMD and BMC values of the proximal phalanges were compared in stallions and mares for the whole population of the studied horses (all subgroups were analysed collectively). Descriptive statistics, average ($\bar{x}$), standard deviation (SD) BMD and BMC for sex are summarized in Table 4.

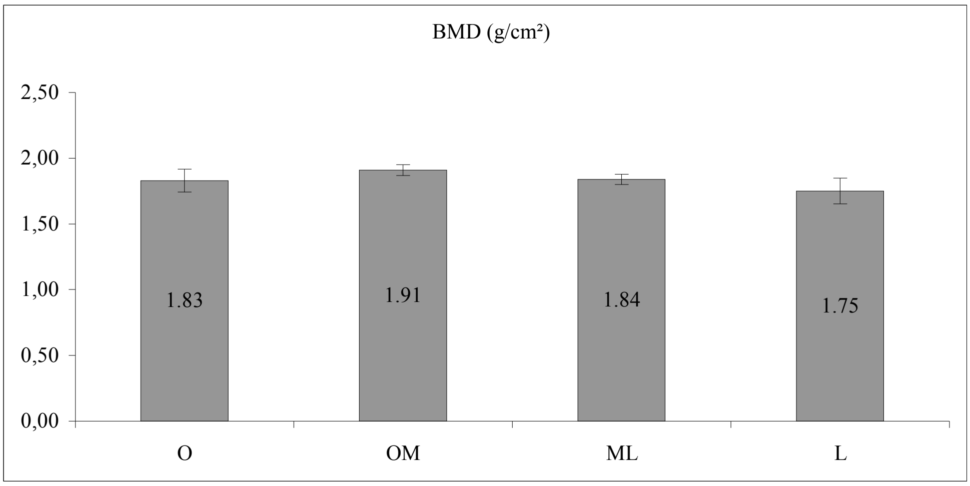

Fig. 1. **Average** ($\bar{x}$) **bone mineral density values and Standard deviation** (SD) **for the investigated morphological types***

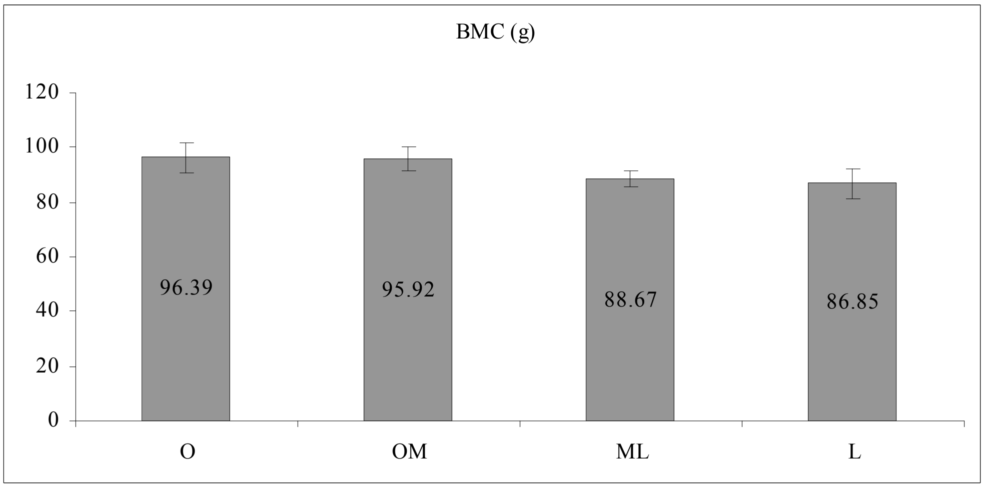

Fig. 2. **Average** ($\bar{x}$) **bone mineral content values and Standard deviation** (SD) **for the investigated morphological types***

Table 3. **Results of the variance analysis** (ANOVA) **and Bartlett's test of homogeneity of variance for bone mineral density and bone mineral content of the proximal phalanges for the investigated morphological types*of horses**

Feature of bone	ANOVA	Test Bartlett'a
BMD	F_{emp}=0.95, p-value=0.421	B_{emp}=1.05, p-value=0.432
BMC	F_{emp}=1.18, p-value=0.326	B_{emp}=1.02, p-value=0.816

Table 4. **Average** ($\bar{x}$)**, atandard deviation** (SD) **and variability coefficient** (V%)**, bone mineral density** (g/cm^2)**, and bone mineral content** (g) **of proximal phalanges by gender**

Gender	Sample number	BMD			BMC		
		Average $\bar{x}$	SD	V%	Average $\bar{x}$	SD	V%
Male	32	1.81	0.23	12. 3	90.81	19.66	21.65
Female	27	1.89	0.18	9.28	91.97	9.46	10.28

An average BMD value for male in the studied attempt was slightly lower than for female. On the basis of t-Student test it was observed that (t=1.48, p-value=0.143) the difference between the averages is not statistically significant. Similar results were achieved for BMC (t=0.28, p-value=0.780), which allows to confirm lack of significant differences in values of the feature between male and female. The BMD changeability coefficient for both sexes was similar, whereas its BMC value was twice as high in male as in female. It proves that the mineral content in the bone tissue of male was more diversified than in female.

Discussion. The lack of significant differences between the values of bone mineral density and the bone mineral content in the proximal phalanges of horses' thoracic limbs for various morphological types of horses proves that there is smaller diversity as far as bone mineral density is concerned in comparison with the human population (Abrams et al. 1995; Bryant et al. 2003; Hui et al. 2003; Braun et al. 2007). Lack of significant differences concerning the studied BMD and BMC parameters in the pastern bones of both sexes is a clear difference in relation to the results achieved from the human population. Human BMD and BMC values are significantly higher in males than in females, which is connected with significant differences in the level of such sex hormones as: estrone sulfate (E1), estradiol (E2), testosterone (T), androstenedione (4-A), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) - Finkelstein et al. (2002). Additionally, menopausal women experience a sudden decrease of the female sex hormones, mainly of estradiol (E2) which has a significant influence on bone metabolism. It also causes the increased activity of the osteoclasts and the decreased activity of the osteoblasts. The latter have receptors which are stimulated by estrogens. Men, whose sexual activity lasts till their death, do not experience menopause and their hormonal spectrum do not change significantly, as opposed to women. When the number of the estrogens falls, it is more difficult to rebuild bones, as the osteoblasts work slower and weaker at a low level of estrogens. The phenomenon is present in women (Abrams et al. 1995; Finkelstein et al. 2002; Hui et al. 2003) whereas it is not observed in female horses.

Nowadays a wide range of observations aiming at the evaluation of the influence of early trainings of horses and other animals on the structure of their long bones are conducted all over the world. It appeared that appropriately dosed exercises may prevent bone injuries in the future. Horses, starting trainings at the age of two, have significantly better shaped bones, as a properly chosen dose of movement is advantageous for modelling their bones, even at a very young age (Iwamoto et al. 2002; Bell et al. 2001; Firth et al. 2005). Stimulating the bone system through exercises of a particular intensity causes the growth of thickness of the cortical bone of the studied bone mainly from the medial and lateral side of the bone shaft, and to a smaller extent from the palmar and the dorsal side (Hiney et al. 2004).

Conclusions. The conducted research as well as the analysis of world writings makes it possible to formulate a conclusion that physical stimulation in a form of physical activity and food are the most significant factors stimulating the bone growth and influencing its physio-chemical parameters, including BMD and BMC. The influence of sex and a morphological type on the bone mineral density and the bone mineral content in the bone tissue of the proximal phalanx of horses' thoracic limbs was not observed.

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