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Variability of the proximal phalanx in warmblood and coldblood horses – morphological and structural analyses

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ABSTRACT. Anatomical shape as well as bone geometry are important factors for the mechanical properties of stiffness and strength in bones. In the light of this statement, the primary aim of the study was to evaluate the variable forms of the proximal phalanx in different types of horses. Multivariate analyses of data from 81 horses revealed that the proximal phalanx has diverse spatial forms. Differences were observed particularly in the length of the bone and the breadth of its diaphysis. In horses with lighter morphotype, the phalanx is significantly narrower in its middle section. In the second stage of the study, geometrical parameters of the phalanx were analysed with the use of peripheral quantitative computed tomography (pQCT). Tomographic analysis was conducted at three levels: at 15%, 50% and 85% of the bone length. Based on the analysis, we concluded that most of the geometric parameters have higher values in coldbloods but only at the mid-diaphysis (50%) and at 85% of the bone length. Moreover, in coldblood horses, higher strength of the phalanx at these levels, expressed by Strength Strain Index, was observed. We did not observe any significant differences between warmblood and coldblood horses in the metaphyseal proximal region which is located at 15 % of the bone length.

KEY WORDS: bone, computed tomography, horses, proximal phalanx

INTRODUCTION

Diseases of limb bones in horses are one of the pathologies most commonly encountered in this species. Statistical data from many countries show that proximal phalanx fractures in the thoracic limbs are one of the most common injuries in horses (Yovich & McIlwraith, 1986; Ellis et al., 1987; Parkin et al., 2004; Dzierzęcka et al., 2006). On the basis of our four-year-long study conducted on 850 thoroughbred race horses, we concluded that diseases of the musculoskeletal system usually applied to segments of autopodium in thoracic limbs and were caused by injuries. Among the injuries, fractures comprised over 30% of all the registered cases and applied mostly to proximal phalanges (Dzierzęcka et al., 2006, 2007, 2008).

Proximal phalanges are usually subject to longitudinal and sagittal fractures (Yovich & McIlwraith, 1986; Ellis et al., 1987; Parkin et al., 2004, 2004; Powell, 2012). Such fractures are probably facilitated by the characteristic base shape at the proximal bone end. On the articular surface, there is an articular pit for articulation with the sagittal crest of the third metacarpal bone (in thoracic limb), or the third metatarsal bone (in pelvic limb). The crest "squeezes into" the articular foveola of the proximal phalanx acting like a splitting wedge, which often leads to this type of fracture (Dubs & Nemeth, 1975; Markel & Richardson, 1985; Ellis et al., 1987).

The mechanic endurance of the bone tissue, which determines its supporting functions, depends not only on the mineral composition but also on its spatial architecture (Mow & Hades, 1991; Alho, 1993). In human medicine, densitometric methods are usually used for the evaluation of bone tissue quality. These methods are imperfect as they are unable to evaluate the spatial structure of the bone trabeculae. However, not only the mineralization level but also the trabecular architecture, which forms the bone tissue geometry, plays an important role in creating the tissue strength. In spatial constructions, durability is affected not only by the quality of the material but also by its proper architectural structure allowing for the distribution of the load on the bone (Mow & Hades, 1991; Czerwiński, 1994; Claes et al., 1995). The characteristics of macroarchitecture are most commonly shown by the use of osteometric techniques. However, it is the quantitative computed tomography (QCT) that delivers full information on the micro-architecture, i.e. geometric parameters of the investigated part of the skeleton (Charuta, et al. 2012; Dzierzęcka & Charuta, 2012).

Computer tomography is a non-invasive method of 3D imaging, which enables researchers to evaluate the densitometric and geometric parameters of the scanned bone fragment. Additionally, based on the assumptions made by Ferreti, the use of this method allows for determining the strain strength index (SSI). Ferreti assumed that bone durability depends on the properties of compact osseous tissue such as density and distribution on the circuit of the cross-section. The software calculates SSI based on tomographically-obtained volumetric bone mineral density (vBMD) and bone radius.

It is worth mentioning that the third metacarpal bone (MC3) has been a subject of numerous studies. Nevertheless, we focused our studies on the proximal phalanx because of the high frequency of fractures in this bone (Clegg, 2011; Ramzan & Palmer, 2011; Jacklin & Wright, 2012). Our analyses partially fill the gap in research in the field of osteological problems regarding this species. In horses, the mechanical properties of stiffness and strength in a bone also depend on the physical properties of the bone material, and the overall anatomical shape or bone geometry (Davies, 2002; Meulen et al., 2001; Radzki et al., 2006, 2007). As a result, we hypothesize that some horses may be subject to higher risk of fractures of the proximal phalanx related to its less favorable anatomical shape. For that reason, it may be informative to compare the spatial form of this bone in horses of different breeds. We can assume that the spatial form will be different in warmblood horses and in coldblood horses due to different morphotypes they represent (Komosa et al., 2006; Brooks et al., 2010). The proximal phalanx may have a variable shape in cross-bred horses as their phenotype is not uniform. Some may be more osteologically-related to warmbloods or to coldbloods.

As a result of the above hypothesis, the primary aim of this study was to determine the shape of the proximal phalanx in warmblood, coldblood and cross-bred horses as an intermediate type. This part of our study included osteometry involving the use of multivariate statistical methods.

The secondary goal of this study was to show the diversity of geometrical parameters of bones with the use of peripheral quantitative computer tomography (pQCT) and compare the SSI values in warmblood and coldblood horses as extremely diverse types of horses.

MATERIAL AND METHODS

The study was conducted on 81 horses destined for slaughter for reasons unrelated to this research. Among them were 33 Polish Halfbred Horses. This is a warmblood X thoroughbred used for sports purposes. Their height at the withers ranges between 165 - 174 cm, and their body weight between 540 - 620 kg. The second group comprised 30 coldblood horses. These were Polish Coldblood Horses, which are mainly used as draft animals. They are shorter, standing 148-160 cm . Their mass, however, is higher; between 600 - 800 kg. Individuals weighing over 900 kg can also occur. The remaining 18 horses were cross-breds with intermediate characteristics compared to the other two types. The animals were aged between 3 and 15 years.

In the first stage of the study, the proximal phalanx of the left thoracic limb was dissected and measured in all the horses. Using the standard method by Driesch (1976), five measurements were performed on every preparation. The following parameters of the phalanx were analysed:

1. Greatest length (GL)
2. Smallest breadth of the diaphysis (SD)
3. Breadth of the proximal end (Bp)
4. Breadth of the distal end (Bd)
5. Depth of the proximal end (Dp)

These metric features comprised benchmark data for further multivariate statistical analyses. Firstly, an exploratory method - Principal Components Analysis - was used to determine similarities in the phalanx between the three groups of horses. The second method applied was the Discriminant Analysis, which indicated those features that were most efficient in differentiating the phalanx in the studied horses. Next, based on the obtained parameters, a size index (expressed as a percentage) was developed with regard to the proximal phalanx. Statistical analyses were performed using the Statistica v 9.0 software.

The second stage of the study involved the comparison of geometrical parameters at three levels (15%, 50% and 85%) of the pastern bone in warmbloods and coldbloods. The length of every analysed bone was measured with a digital slide caliper at three locations. The obtained values were then input into the CT software. After initial scanning, it was possible to establish the referential line that was tangent to the articular surfaces. Since the bone length was already input, adding the measuring lines on any given bone area had to be performed next. In the case of our bones, the lines were drawn at 15%, 50% and 85% of the bone length (Fig. 1). The thickness of the analyzed slices was 0.07 mm.

However, at this stage of the study, the number of horses was limited to 20. Ten phalanges of warmblood horses and ten of coldblood horses were randomly selected. Cross-breds were not included. Peripheral Quantitative Computed Tomography (pQCT) with XCT Research SA Plus (Stratec Medizintechnik GmbH, Pforzheim Germany) was used to analyse the structure of the bones according to Ferretti's method (Ferretti et al., 1995, 1996).

In the analysed phalanges, the following geometrical parameters were determined: total bone area (TOT_A) mm^2, trabecular area (TRAB_A) mm^2, cortical thickness (CRT_THK_C) mm, periosteal circumference (PERI C) mm, endocortical circumference (ENDO_C) mm and Strength Strain Index (SSI = RP_CM_W) mm^3. The analysis was performed with the voxel size of 0.07 mm and scanning speed of 4 mm/min. The area for the analysis was determined after preliminary scanning (20 mm/s). Threshold coefficient, differentiating compact bone from

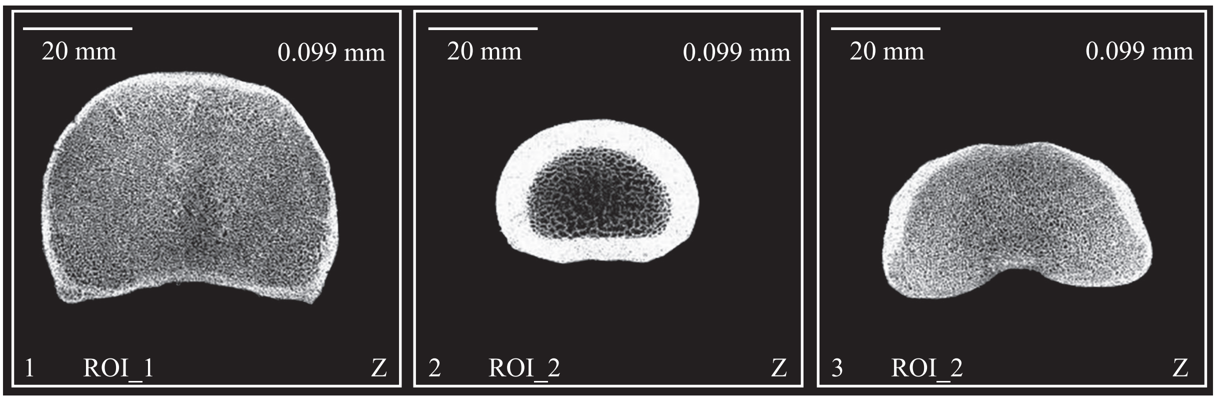

Fig. 1. – The view of three measurements of proximal phalanx (successively 15, 50 and 85% of the bone length) from the computed tomography image.

TABLE 1

Basic statistics of the proximal phalanx parameters in the analysed groups of horses.

Variable	N	Mean value (cm)	Minimum (cm)	Maximum (cm)	Standard deviation
Warmblood horses					
Greatest length	33	10.3	8.9	11.7	0.7
Smallest breadth of the diaphysis	33	4.0	3.1	5.0	0.4
Breadth of the proximal end	33	6.4	5.1	7.6	0.5
Breadth of the distal end	33	5.5	4.5	6.5	0.4
Depth of the proximal end	33	4.3	3.5	5.1	0.4
Coldblood horses					
Greatest length	30	9.7	8.6	10.4	0.4
Smallest breadth of the diaphysis	30	4.5	3.9	4.9	0.2
Breadth of the proximal end	30	6.8	5.6	7.8	0.5
Breadth of the distal end	30	5.7	4.7	6.6	0.4
Depth of the proximal end	30	4.3	3.7	4.9	0.3
Cross-bred horses					
Greatest length	18	9.7	9.1	10.2	0.3
Smallest breadth of the diaphysis	18	4.2	3.6	4.8	0.3
Breadth of the proximal end	18	6.6	6.0	7.1	0.3
Breadth of the distal end	18	5.5	5.1	6.3	0.3
Depth of the proximal end	18	4.4	4.0	4.9	0.2

trabecular bone, was determined at the level of 0.900 cm^{-1}.

RESULTS

Multivariate analyses of the proximal phalanx

I.1. Basic statistics and Principal Components Analysis.

The first stage of the study involved basic statistical analysis of all the metrical features of the proximal phalanx. The analysis was performed separately for warmblood, coldblood and cross-bred horses (Table 1). These parameters were compared between groups of horses with the use of one-way ANOVA. Average values of GL and SD are highly significantly different ($p \leq 0.001$), while Bp is significantly different ($p \leq 0.05$). No statistical differences were observed between the average values of Bd and Dp.

The next stage was to present the morphometric differences of the proximal phalanx between the horse types mentioned. Principal Components Analysis was used for that purpose. The idea of the method is to display and describe the variability of the phalanx based on all its

TABLE 2

Correlations between metric features and principal components after varimax rotation. Load values in bold show strong correlation with a given component.

Variable	Principal Component 1	Principal Component 2
Greatest length	0.14	**0.96**
Smallest breadth of the diaphysis	**0.94**	0.03
Breadth of the proximal end	**0.90**	0.31
Breadth of the distal end	**0.89**	0.34
Depth of the proximal end	0.65	0.58

measurements altogether. Moreover, the analysis revealed new, so-called hidden factors, also referred to as components. From among the principal components selected, the highest two jointly explain 87.8% of the total variance determined by all the primary variables, i.e. measurements of the phalanx. The result is very high. Therefore, the two-dimensional graph based on the two components is highly representative of the proximal phalanx variability in the studied horses (Fig. 2).

Following a varimax rotation, each measurement was ascribed a load that expressed its correlation with a given principal component (Table 2). Principal component 1 is highly correlated with all the parameters of the proximal phalanx that describe its breadth. Thus, it can be called a "breadth component". Principal component 2, on the other hand, is a "length component" because the feature it correlates with to the highest extent is GL. The two-dimensional diagram clearly showed the variability of the proximal phalanx between the warmblood and coldblood horses. In most cases, however, cross-

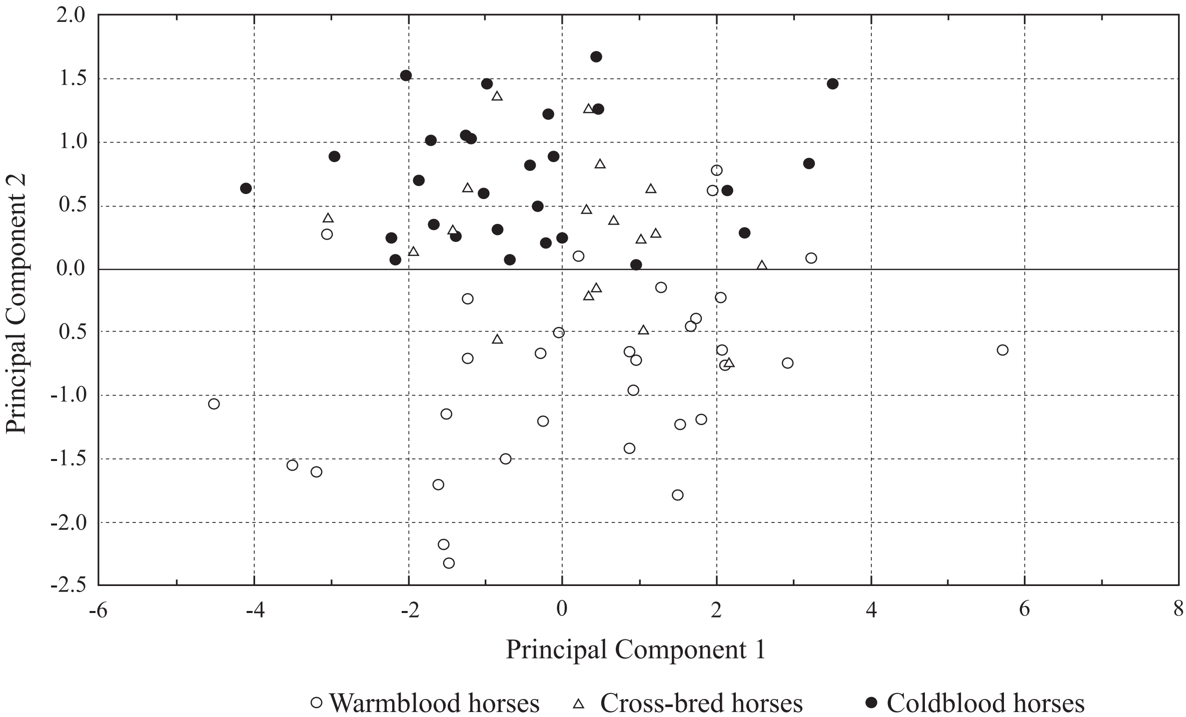

Fig. 2. – Two-dimensional graph showing two strongest principal components.

TABLE 3

Results of the Canonical Discriminant Analysis.

Variable N=81	Wilks' Λ	Partial Wilks' Λ	F value (2.76)	Level of significance
Smallest breadth of the diaphysis	0.692	0.502	37.674	**<0.001**
Greatest length	0.542	0.641	21.240	**<0.001**
Depth of the proximal end	0.374	0.930	2,844	0.064
Outside the model:				
Breadth of the proximal end	0.339	0.976	0.924	0.401
Breadth of the distal end	0.343	0.988	0.458	0.634

breds resemble coldblood horses rather than warmblood ones as regards the shape of the bone.

I.2. Canonical Discriminant Analysis

The aim of the Canonical Discriminant Analysis was to identify those metrical features that had the highest influence on the classification of a given bone to one of the three groups of horses. The stepwise progressive analysis model was chosen. Apart from the F test, the discriminant analysis uses a parameter called Wilk's Lambdas (Λ). This parameter describes the statistical significance of the model's discriminant power following the introduction of a given metric feature. Partial Wilk's Lambdas, on the other hand, shows the input of a metric feature into the discrimination between the groups. Decrease of this value indicates that the input of the metric feature increases.

The discriminant analysis demonstrated that three out of five features determine the classification of the phalanx. However, only two of them were statistically significant - smallest

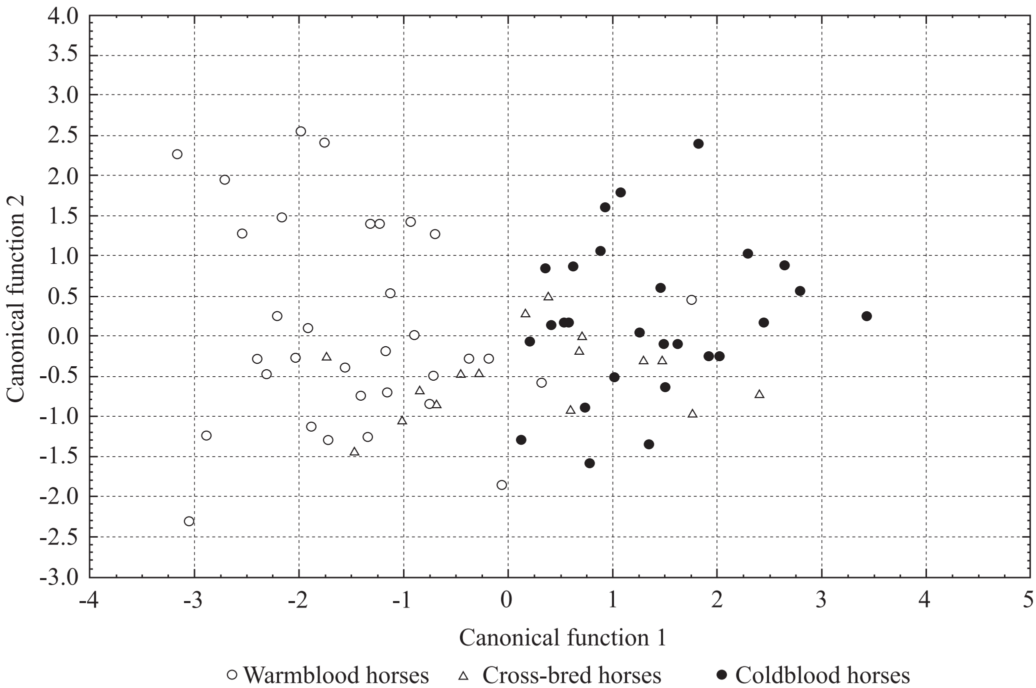

Fig. 3. – Two-dimensional graph showing canonical discriminant functions.

TABLE 4

Results of one way variance analysis for the proximal phalanx index in different types of horses.

Source	DF	SS	MS	F	P
Between groups	2	844.9	422.4	58.06	0.000
Within groups	78	567.5	7.3		
Total	80	1412.4			

breadth of the diaphysis (SD) and greatest length (GL) (Table 3).

The model did not include the parameters representing the breadth of the proximal and distal ends. Canonical discriminant functions allowed us to present once again the variability of the distal phalanx on the two-dimensional graph (Fig. 3).

I.3. Proximal phalanx index

Two most significant metric features of the phalanx were used to create a percentage index of its shape. The following formula was used: SD/GL x 100%. The mean value of the index was 38.91 %, 43.15 % and 46.21 % for the warmblood, cross-bred and coldblood horses respectively. One-way ANOVA conducted for the index showed that mean values for the groups mentioned above differ very significantly (Table 4).

Next, a *post hoc* procedure, Fisher's Least Significant Difference test, was conducted. It showed that the means for each group were significantly different from one another. The critical p-value was $p \leq 0.001$. Fig. 4 presents the examples of varied shapes of the distal phalanx in different types of horses.

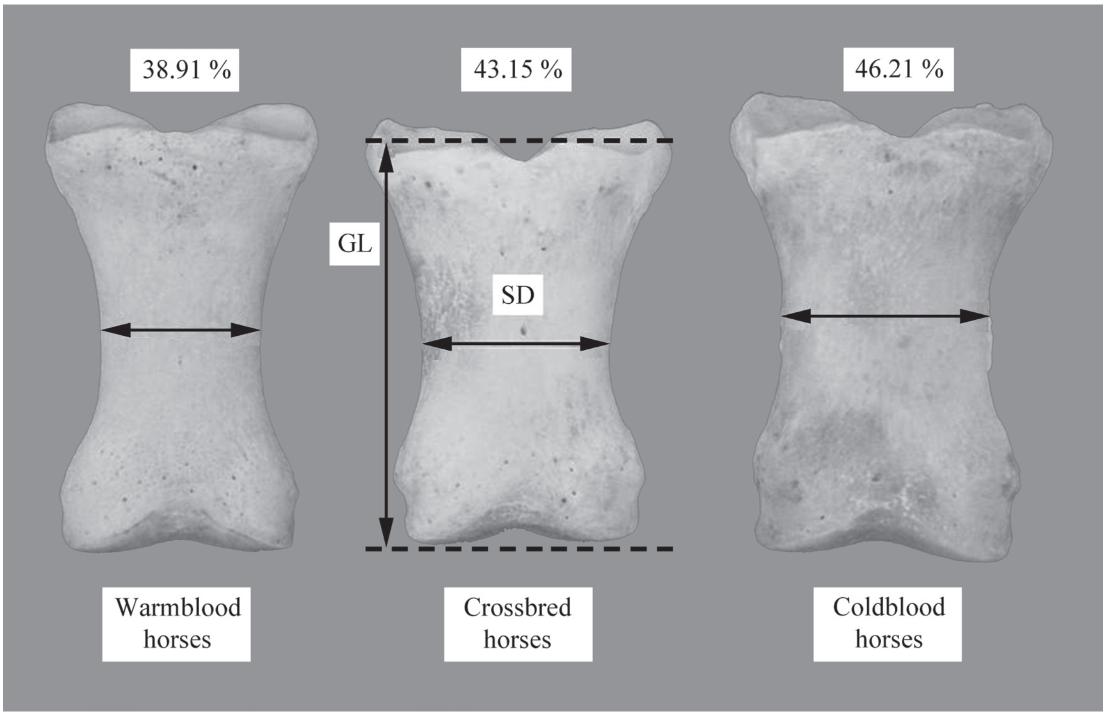

Fig. 4. – Shapes of the proximal phalanx and values of its index for Warmblood, Cross-bred and Coldblood horses.

TABLE 5

The value of T– test, testing the difference between geometric parameters of the pastern bones of the Warmblood horses and Coldblood horses at 15%, 50% and 85% of its length.

Variable	**Meanvalue Warmbloodhorses**	**Meanvalue Coldbloodhorses**	**T value**	**Level of significance**
at 15% of its length				
Total bone area	2145.13	2001.36	1.079	0.295
Trabecular area	965.30	900.30	1.088	0.291
Cortical thickness	4.54	5.41	-1.792	0.090
Periosteal circumference	163.72	158.33	1.060	0.303
Endocortical circumference	135.22	124.33	1.973	0.064
Strength strain index	14121.05	15632.07	-0.818	0.424
at 50% of its length				
Total bone area	969.68	1244.20	-2.412	**0.027**
Trabecular area	436.15	559.63	-2.416	**0.026**
Cortical thickness	6.64	6.23	0.985	0.337
Periosteal circumference	109.88	124.31	-2.523	**0.021**
Endocortical circumference	68.16	85.16	-2.653	**0.016**
Strength strain index	6572.25	9484.25	-2.316	**0.032**
at 85% of its length				
Total bone area	1117.02	1370.91	-2.837	**0.011**
Trabecular area	502.09	616.56	-2.848	**0.011**
Cortical thickness	5.90	5.48	1.026	0.318
Periosteal circumference	117.99	131.00	-2.888	**0.010**
Endocortical circumference	80.88	96.54	-2.905	**0.009**
Strength strain index	8301.89	10434.70	-2.196	**0.041**

Comparison of geometrical parameters of the proximal phalanx between the warmblood and coldblood horses

The number of horses to undergo computed tomography was limited to 20 compared with the initial group. Ten warmblood and ten coldblood horses were randomly selected for the analysis. T-test was used to compare all the geometrical parameters between the two groups (Table 5).

The test showed that at 15% of the bone length, phalanges in both groups of horses did not present any significant differences regarding the geometrical parameters analysed. However, at the mid-diaphysis and at 85% of its length, the bones in warmblood and coldblood horses differ significantly with respect to all the parameters but one - cortical thickness (CRT_THK_C). Parameters that differed, i.e. TOT_A, TRAB_A, PERI_C, ENDO_C and SSI, were higher in coldblood horses. As for CRT_THK_C, it reached different values compared with other parameters. It was the highest at the mid-diaphysis and the lowest at 15 % of the bone length. It is an opposite relation or a mirror image, so to say, compared with the values of the other parameters (Table 5).

DISCUSSION

The present study indicates that the proximal phalanx has a significantly different shape in different groups of horses. The index we created compares the breadth of the diaphysis (SD) with the greatest length of the phalanx (GL). Since the index is expressed as a percentage, the meaning of a low value is that the shape of the bone resembles an hourglass. The diaphysis is the narrowest in warmblood horses, medium in cross-breds and the broadest in coldblood horses. Slim phalanx in warmbloods stems both from the increase of its total length as well as narrower diaphysis. Multivariate analyses, especially the discriminant analysis, indicated that the breadth of the phalangeal diaphysis and the length of the bone play the most important role in classifying the animal as a given morphological type. It is interesting in terms of the phalanx shape that cross-bred horses are more similar to coldbloods than to warmbloods.

Our results coincide with those obtained by Hildebrand (1987), Biewener (1991) and Pieszka et al. (2011), who demonstrated similar architecture of the third metacarpal bone, which is a part of the manus of the thoracic limb. Manus bones undergo temporary deformations when the horse gallops. Therefore, resistance to bending, which also takes place when a horse lands after a jump, is particularly important. In coldblood horses, on the other hand, diaphyses in limbs can be compared to columns, adapted to resist compressive forces, whose width is equal along the entire length (Bartosik, 1957). Compressive forces are related to high body mass of these horses. Physical activity exerts lower influence. In other words, the limbs of coldbloods are not exposed to such overloads as in saddle horses (galloping, jumping). Nevertheless they have to permanently withstand the vertical pressure of higher body mass. The body mass of a coldblood horse with height at the withers of approximately 160 cm is approximately 800 kg or more while a warmblood horse with the same height weighs only 500 kg. A similar problem was exposed by Nauwelaerts et al. 2011 when studying the inertial properties of separate sections of limbs. These researchers showed that the ratio between the total body mass and the foreleg pastern mass (body part composed of proximal and middle phalanx) is similar in horses of different morphotypes. We think that this conclusion confirms our belief because it suggests that higher pastern mass is obtained by increased breadth and circuit of the proximal phalanx. The deep digital flexor tendon and other accompanying anatomical structures are also broader. As far as the length of the pastern as a body part is concerned, it can be significantly different even within horses of the same breed or between related breeds (Komosa & Purzyc, 2009; Sobczuk & Komosa, 2012). Such diversity of shape among pasterns and bones that compose them confirms the need of conducting such anatomical analyses.

Moreover, in the context of bone strength, densitometric parameters should be considered as well as structural features, which are also referred to as architectural features (Ferretti et al., 1995, 1996; Rauch & Schoenau, 2001). In our study, structural features of the phalanx were determined by the evaluation of the following geometrical parameters: total bone area, trabecular area, cortical thickness, periosteal circumference and endocortical circumference. Our study is particularly valuable because the pQCT technique used allowed us to conduct an integrated evaluation of both material and structural features of the bone tissue through the determination of the Strength Strain Index.

The first important observation resulting from our study was that bone strength at 50% of the diaphysis was significantly higher in coldblood horses, whose total bone area and trabecular area measured in this region were significantly higher. Similarly, the values of periosteal circumference and endocortical circumference measured at the mid-diaphysis of the phalanx were also higher in coldblood horses. As in the case of the mid-diaphysis, bone strength and thus, total bone area, trabecular area, periosteal circumference and endocortical circumference, were significantly higher in coldblood horses.

Similar observations regarding long bones were made by other researchers, who compared geometrical parameters of the radius and tibia in trained and untrained horses. When the geometrical parameters of bones increased, as occurred in the trained horses, bone strength expressed as the Strength Strain Index grew as well (Nicholson & Firth, 2010). Larger diameter of the bones, i.e. more peripheral location of the cortical substance, increases bone resistance to bending and twisting forces. Therefore, the bone whose cortical substance constitutes the farthest periphery, i.e. whose section is close to a circle, is stronger than other bones with the same cross-sectional area (Khan et al., 2001). In our study, the location of the compact bone was determined using periosteal circumference and endocortical circumference. The results indicate that the phalanges in coldblood horses are more resistant to forces both at the mid-diaphysis and at 85% of the bone length. In the areas discussed, the diameter of the pastern bone is close to a circle.

At the same time, our study did not show any significant differences in cortical thickness between warmblood and coldblood horses in any of the measurement areas, nor did the studies on the radius and tibia in trained and untrained horses (Nicholson & Firth, 2010).

Our study, together with the results of research conducted on radius and tibia by Nicholson & Firth (2010) and on adult rats by Jee et al. (1991), confirms that varying impact of different types of genetic strain induces the increase of periosteal circumference, endocortical circumference, total bone area and trebecular area in the bones. However, it does not lead to the increase of cortical thickness (Firth et al., 2005; Verheyen et al., 2006; Nicholson & Firth, 2010). The differences in cortical thickness in the three measurement areas are particularly noticeable in the warmblood horses. In these horses, the parameter in question is, on average, even 30% higher at the mid-diaphysis than at 15% of the phalanx length. From the anatomical perspective, cortical thickness corresponds to the substantia compacta, which builds the bone at its circumference. The increase in thickness of this layer inside the phalangeal diaphysis is accompanied by a simultaneous decrease of diaphyseal breadth (SD), which occurs mostly in warmblood hoses. The correlation between the shape of the bone and its geometry visible on computed tomography can be related to the adaptation of the proximal phalanx to deformations that take place when warmblood horses gallop.

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