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Original article

The influence of feed phosphates on the structural, mechanical and chemical properties of bone tissue in pigs

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Abstract

The aim of the study was to assess the influence of various feed phosphates on the structural and mechanical properties as well as on the chemical composition of femurs in adult pigs (weight approx. 110 kg). Three types of phosphates – monocalcium phosphate (MCP), dicalcium phosphate (n-DCP) and calcium-sodium phosphate (CSP) – were used alternatively in pigs fed with the standard feed mixture. The MCP and CSP phosphates were typical, imported products used traditionally in pig feeding. Dicalcium phosphate (n-DCP) was manufactured in Poland on the basis of phosphoric acid with the new pro-ecological method. The following parameters were determined: the mean physical density of the samples of the compact and spongy bone tissue, values of Young's modulus, strength and the energy of deformation, and Vickers microhardness (HV). Also the content of C, O, Na, Mg, Al, and Si, as well as Ca, P and Sr was determined. Significant differences in mean values of the mentioned parameters occurred between the studied groups. The best mechanical properties were shown by the bones from the n-DCP group, and the compact bone tissue (diaphysis) contained the most Ca, P, and Sr when compared to the MCP and CSP groups.

Key words: pig, feed, phosphate, bone tissue, mechanical properties

Introduction

The basic role in the development of the skeletal system of pigs is played by calcium and phosphorus, which have to be provided in the form of mineral additives (Mollard et al. 2004, Ruan et al. 2007, Feng and Jasiuk 2011). A very important role is played by phosphorus, in particular in rapidly growing pigs. Although it is present in the main components of feed mixtures, i.e. in grains and corn, it is poorly absorbed. The absorption is slightly improved after an application of a special enzyme, i.e. microbial phytase (Peter et al. 2001, Woyengo et al. 2008).

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A common source of phosphorus (and partly of calcium) are feed phosphates: monocalcium, dicalcium and tricalcium phosphates, calcium-sodium phosphates, calcium-magnesium-sodium phosphates, ammonium phosphates and others. Bioavailability of phosphorus from these phosphates is very variable and ranges from 50 to 91%, depending mainly on the quality of feedstock and the applied production technology (Eckhout et al. 1995, Jongbloed et al. 2000, Poulsen 2007).

As mentioned before, mineralization processes and physical and chemical properties of bones depend on the bioavailability of phosphorus (and also Ca). The structure of the bone tissue is connected with mechanical properties; its adaptation to changing load conditions implicates changes in the structure of the whole tissue. On one hand, due to such properties as fragility and stiffness, the bone tissue is counted among ceramic materials (Jackson et al. 1978), while on the other hand, collagen fibers give it a specific elasticity. Hence, bone tissue should be considered as a biphasic composite material, comprising a collagen matrix of a low modulus of elasticity in which hydroxyapatite crystals of a high modulus of elasticity are "suspended" (Uklejewski et al. 2006, Nikodem 2010).

Collagen fibers comprise mainly type I collagen, which determines most processes dependent on the organic substance of the bone such as the process of bone formation, its mineralization and achieving correct properties. Non-organic elements, hydroxyapatite crystals, carbonate ions and phosphate ions are deposited along the collagen fibers. Hydroxylapatite crystals (taking up 65% of the total bone mass) are constructed from the basic units ($Ca_{10}(PO_4)_6(OH)_2$) surrounded by a small layer of water thanks to which the exchange of ions takes place. The main mineral components of these crystals are calcium and phosphorus (Kuryszko and Zarzycki 2000).

The aim of the study was an assessment of the influence of the three different feed phosphates used in the standard nutrition of pigs on the structural and mechanical properties, and the chemical composition of the bone tissue (femur).

Materials and Methods

Animals and nutrition

The experimental fattening was carried out at the Experimental Station of Animal Nutrition in Gorzyń (Poznań University of Life Sciences). The studied material consisted of 24 porkers (3 groups with 8 animals in each one) with a mean initial body weight of 20 kg that were fed to the weight of 110 kg. During the whole experimental period the animals were kept in identical conditions. Three types of basic feed mixtures, adjusted to the fattening period (Starter, Grower and Finisher type) were used, but in each of them the appropriate quantity of feed phosphates for the complete cover of the requirement for this macroelement was introduced. The level of protein, amino acids, energy, mineral components and vitamins was adjusted in these feed mixtures to the level recommended in the Polish Swine Feeding Standards (1993). According to the type of the phosphate used the animals were divided into three groups: group I – individuals fed with the complete ration mix with participation of monocalcium phosphate (MCP); group II – with participation of dicalcium phosphate (n-DCP); and group III – with participation of calcium-sodium phosphate (CSP). MCP and CSP were typical, imported phosphates, used traditionally in pig feeding. In group II a new dicalcium phosphate (n-DCP) was used, manufactured on the basis of phosphoric acid with a new, proecological method (Table 1). Detailed feed recipes and chemical properties of n-DCP have been presented in separate papers (Hoffmann et al. 2009, Dobrzański et al. 2010). Results of fattening were presented in another paper (Korniewicz et al. 2012). It may be concluded from them that the basic indices such as an average daily gain ADG (992 – 1002 g), daily feed intake DFI (2.58 – 2.59 kg), feed conversion rate FCR (2.58 – 2.65 kg/kg) did not differ between the groups of pigs (MCP, n-DCP, CSP).

The study was conducted with a permission of 2nd Local Ethics Committee of Experiments on Animals in Wrocław (Decision No. 15/2007).

Preparation of samples

The studied material was collected in slaughterhouses, directly after the slaughter and dissection of pigs of the weight of approx. 110 kg. Six femurs from the right half-carcass from each of the 3 groups were analyzed. The upper part (trochanter with head – see Fig. 1) was cut from each bone using a special saw. Soft parts were removed mechanically using a knife and hot water, and then they were air-dried. Bones were chilled and brought in plastic containers to the laboratory of the Division of Biomedical Engineering and Experimental Mechanics of Wrocław University of Technology.

Due to the fact that the spongy and compact bone tissue are characterized by different mechanical parameters, two groups of samples were analyzed. The

Table 1. Chemical properties of feed phosphates (kg s.m.)*.

Specification	Monocalcium phosphate	Dicalcium phosphate	Calcium-sodium phosphate
Chemical formula	(MCP) $Ca(H_2PO_4)_2$	(DCP) $Ca\ HPO_4 \times 2H_2O$	(CSP) $Na_2Ca_5(PO_4)$
Content of total phosphorus – P (g)	227	185	180
Solubility of phosphorus in 2% citric acid (%)	99	98	98
Content of calcium – Ca (g)	177	250	310
Content of sodium – Na (g)	7.6	4.7	49
Origin of phosphorus	Finnish production	Polish production	Russian production

* according to Hoffmann and Hoffmann (2009) in own modification

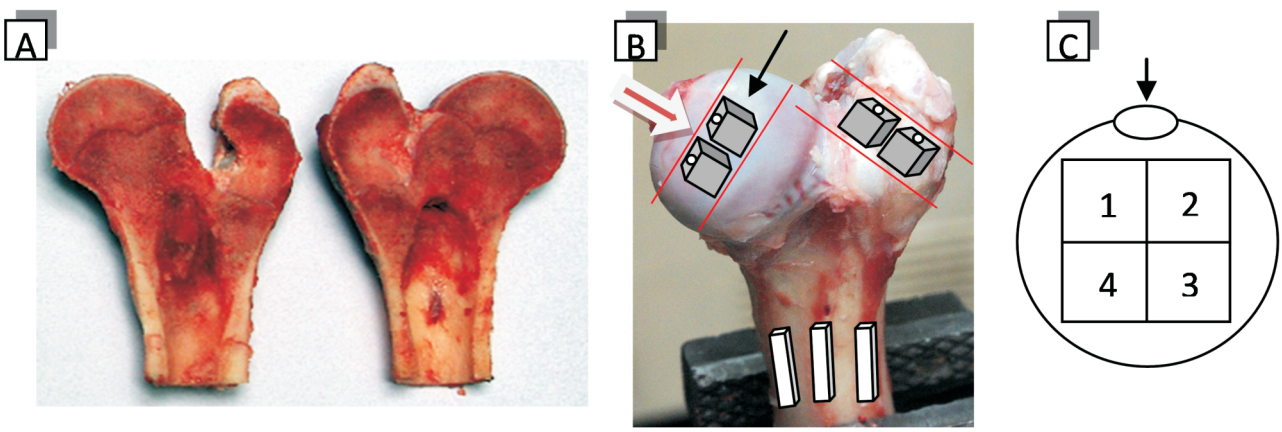

Fig. 1. A – Cross-section of the head of the femur, B-C – Position and way of preparing the samples of spongy bone tissue (trochanter and head) and compact bone tissue (diaphysis) from the head of the femur.

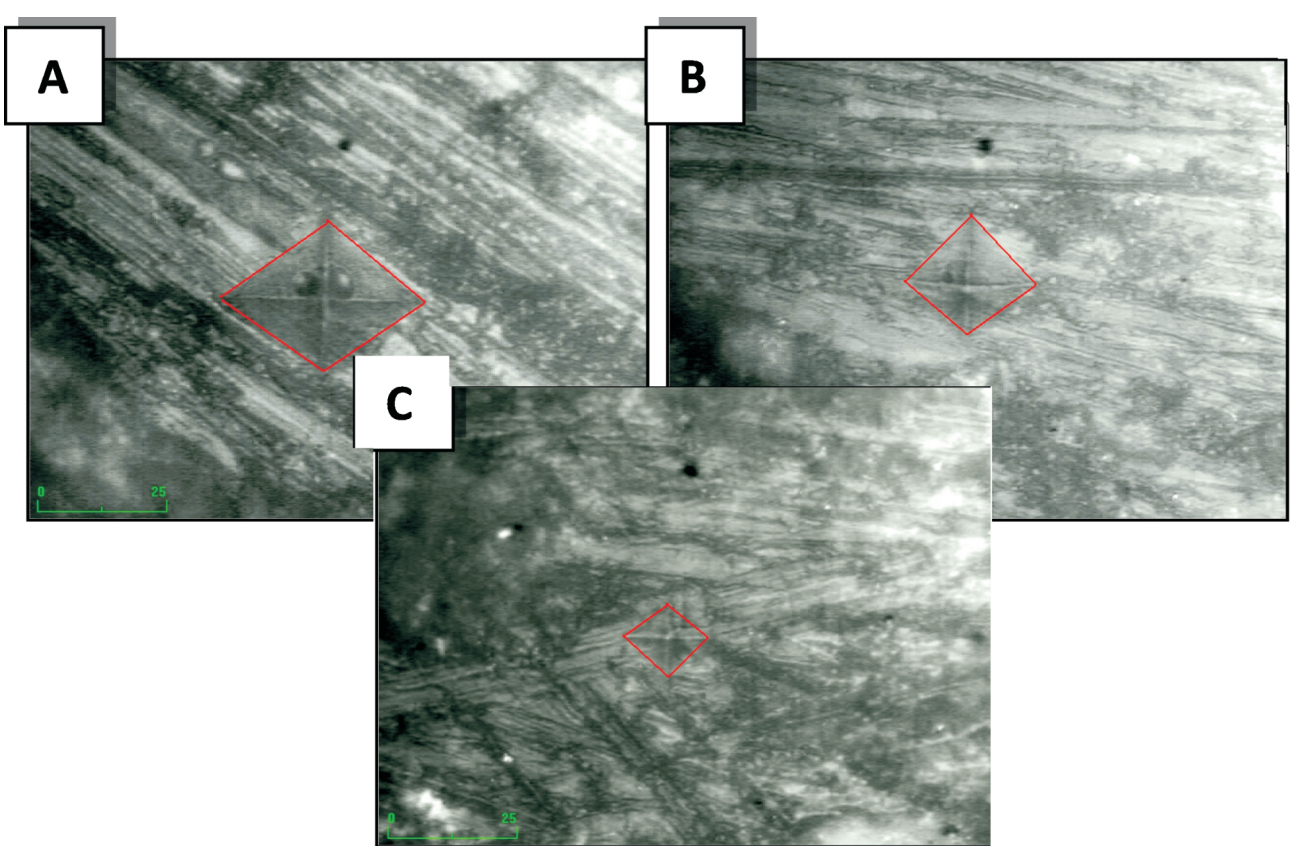

Fig. 2. Image of imprints (obtained using the hardness tester) of exemplary samples of the compact bone tissue from A – group I; B – group II; C – group III (scale in μm).

first group comprised samples of compact tissue from the core of the femur, whereas the second group included samples of spongy tissue from the region of the head of the greater trochanter of the femur. The studied samples (n = 18), prepared with the help of the precise cut-off machine Accutom-5® by STRUERS, had the shape of 10x10x10 mm cubes (Fig. 1). The image of imprints from compact bone tissues samples obtained using hardness tester is presented in Fig. 2. Their weight was determined using the RADWAG digital scale model WPS 110/C/2 with an accuracy ± 10 mg.

Range and methods of measurement

Mechanical properties. Studies on mechanical properties of bones were carried out during the uniaxial compression test in the MTS 858 MiniBionix and MTS Synergie 100 universal testing machines. The use of two machines was dictated by a different range of load moved by the analyzed groups of tissues. In order to eliminate the influence of the marginal effects connected with the destruction of the material on the probe ends, and in the case of the spongy tissue bending of individual bone trabeculae on the preparation margins, 80 grit sandpaper was used additionally. The loading velocity used in the tests was 0.01 s^{-1}, which corresponds to the loading velocity under physiological conditions (Ashman et al. 1988, An and Draughn 2000).

Ninety compact tissue and spongy tissue samples from the region of the head of the femur and greater trochanter were studied, for which in total 270 measurements were made. In these measurements tension-deformation characteristics were determined on which basis characteristic parameters describing mechanical properties – Young's modulus, yield strength, shearing strength, energy of deformation as well as ductility and density were determined (Yamada and Evans 1973).

Additionally, measurements of microhardness of the compact tissue were carried out, using the HMV Micro Hardness Tester Shimadzu®. The conditions of the examination were determined by the PN-EN ISO 6507-1, requirements concerning hardness testers by PN-EN ISO 6507-2, hardness calibration by PN-EN ISO 6507-3, and the hardness value tables by PN-EN ISO 6507-4:2006 standards.

Microelemental analysis. Nine samples of both compact tissue and spongy tissue taken in each group as in the previous stages from the trochanter and the head of the femur (27 samples in total) were subjected to microanalysis of the elemental composition, using X-ray techniques (scanning electron microscope LEO 435 VP by LEO, equipped with the X-ray elemental analyzer Roentec M1). According to the requirements of the method, the samples were additionally dehydrated in alcohol solution.

The content of 9 elements comprising the components of the bone tissue was analyzed in the study. The percentage (in the composition of the external layer of the bone tissue) of Al, C, Ca, Mg, Na, O, P, Si and Sr was determined.

The techniques of the elements determination in biological material using the SEM method are presented elsewhere (Tsezos et al. 1996, Michalak et al. 2011).

Statistical analysis

The measurement results were subjected to statistical analysis using OriginPro 8.0 software. The results in particular tables present the arithmetic means, standard deviations and the range of obtained values of the given set. The Shapiro-Wilk normality test was used for an assessment of distribution of the obtained results. Additionally, in order to carry out the analysis of significant differences between the means for different groups, the analysis of variance (ANOVA) was applied, with the level of significance of $p<0.05$.

Results

Strength parameters of the bone tissue. An important feature, i.e. physical density of the spongy tissue, was within the range from 1.166 to 1.219 g/cm^3, whereas that of the compact tissue was in the range from 1.746 to 1.842 g/cm^3 depending on the group (Table 2). For the latter, statistically significant differences were noted in group II (n-DCP) when compared to group I (MCP) and group III (CSP).

Other mechanical parameters of bones are presented in Table 3 and in Fig. 3. The mean value of Young's modulus in the samples of the compact tissue was the highest in group II (8.68 GPa), and was significantly different when compared to group I and III ($p<0.05$). However, maximum deformation (2.32%) and energy of deformation (353.2 J) were significantly ($p<0.05$) lower in group n-DCP when compared to the groups receiving MCP or CSP in their diet. Compression strength was also the highest in group II (115.8 MPa), but did not statistically differ when compared to the other groups. Ductility was the highest in group III (0.77%), but when compared to the other groups (I and II) the differences were not significant statistically. The highest values of micohardness HV of the

compact tissue were obtained for the samples in group III ($\bar{x}$ = 28.76 HV), and the differences were statistically significant ($p<0.05$) when compared to group I and II.

Different values, usually lower (Table 4 and Fig. 4), were obtained in the samples of the spongy tissue of the femur. In group II the mean value of Young's modulus was the highest (147.87 MPa), simi-

Table 2. Physical density of the samples of the compact and spongy tissue in g/cm^3 ($\bar{x}$ ± SD).

Tissue	Group (n = 90)		
	I – MCP	II – n-DCP	III – CSP
Spongy – greater trochanter	1.17 ± 0.08	1.19 ± 0.09	1.18 ± 0.07
Compact – diaphysis	1.77^{b} ± 0.10	1.84^{a} ± 0.05	1.75^{b} ± 0.10
Spongy – head of the femur	1.22 ± 0.08	1.19 ± 0.06	1.17 ± 0.04

a-b – $p<0.05$

Table 3. Values of mechanical parameters for the samples of the compact tissue of the femur ($\bar{x}$ ± SD).

Specification	Group (n = 32)		
	I – MCP	II – n-DCP	III – CSP
Young's modulus (GPa)	7.79^{b} ± 1.78	8.68^{a} ± 1.52	6.78^{b} ± 2.06
Maximum deformation (–)	2.59^{b} ± 0.46	2.32^{a} ± 0.23	2.67^{b} ± 0.69
Energy of deformation (J)	419.72^{b} ± 147.10	353.21^{a} ± 8.63	499.89^{b} ± 183.24
Compression strength (MPa)	109.41 ± 16.3	115.78 ± 20.05	106.49 ± 24.47
Ductility (%)	0.66^{b} ± 0.45	0.44^{a} ± 0.11	0.77^{b} ± 0.51
Vickers microhardness HV	19.45^{b} ± 4.58	18.65^{b} ± 4.79	28.76^{a} ± 6.25

a-b – $p<0.05$

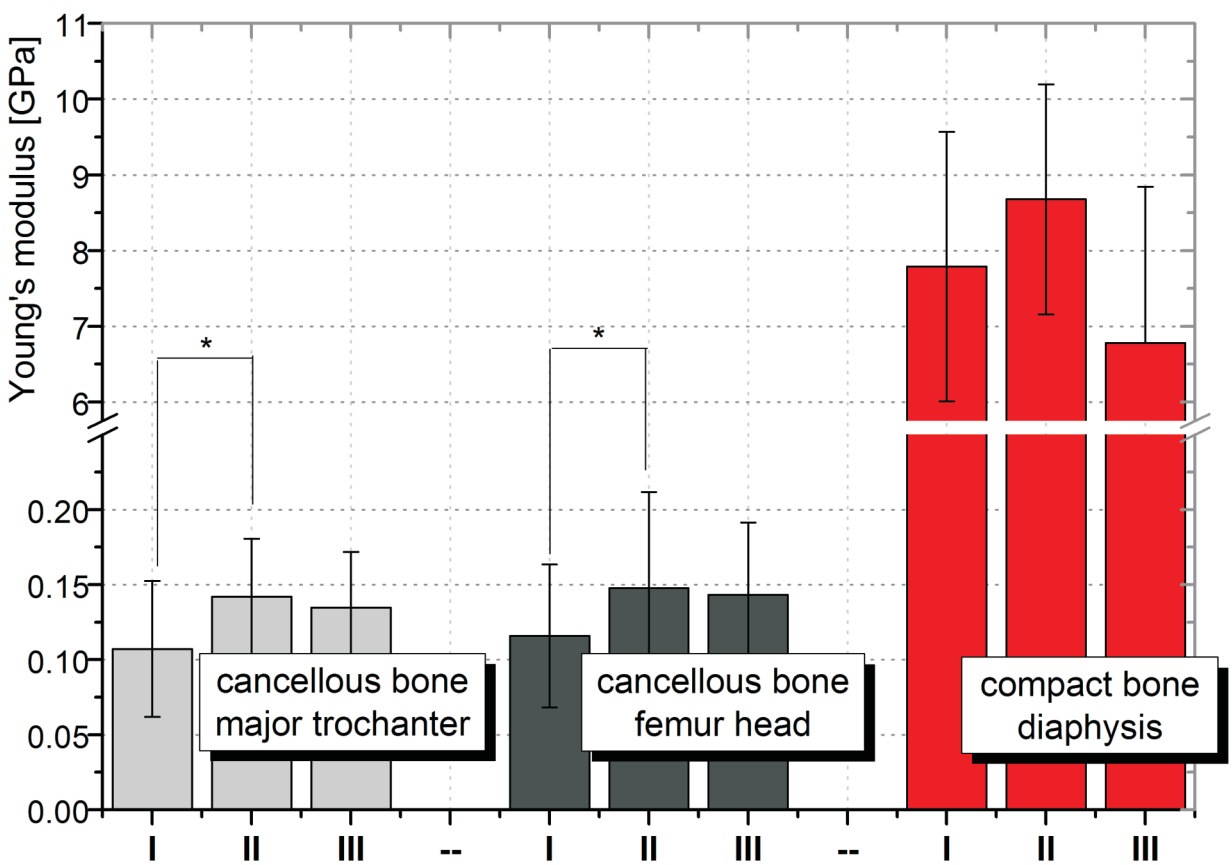

Fig. 3. Comparison of the values of compression strength obtained for the samples of the spongy tissue (greater trochanter and head of the femur) and the compact tissue in groups I – III ($p<0.05$).

lar to the compression strength (9.74 MPa), but statistical differences occurred only with respect to the group I ($p<0.05$). The parameter of maximum deformation was the highest in group III (14.38%) and was statistically different when compared to group II. Other indices, such as energy of deformation and ductility, showed certain differences between groups I-III, but they were not significant statistically.

Elemental composition. C and O made the highest contribution to composition (over 95%) on the surface of the bone samples in the spongy tissue (Table 5). Next was phosphorus (max. 1.02%), strontium (max. 1.06%) and calcium (max. 0.59%). Further positions were occupied by Al, Si and Mg. Fe or Zn was discovered in none of the groups. It is interesting that no Na, Al, Mg or Si was discovered in the greater trochanter of group II (n-DCP). Statistically significantly higher amount of Sr was found in group II when compared to group I or III. In the head of the femur statistically significant differences occurred between group I and II (for Ca), and I and others for phosphorus.

The highest content of C and O (approx. 90%) was also noted in the compact tissue samples. The next was phosphorus (max. 4.25%), then calcium (max. 3.25%) and strontium (max. 1.31%). Further positions were occupied by Al, Mg and Si. The latter

Table 4. Values of mechanical parameters for the samples of the spongy tissue from the head of the femur ($\bar{x} \pm SD$).

Specification	Group (n = 58)		
	I – MCP	II – n-DCP	III – CSP
Compression modulus (MPa)	$115.89^{b} \pm 47.71$	$147.87^{a} \pm 63.86$	143.34 ± 48.15
Maximum deformation (–)	14.69 ± 3.02	$13.75^{a} \pm 2.45$	$14.83^{b} \pm 3.98$
Energy of deformation (J)	424.61 ± 211.87	455.67 ± 211.61	459.94 ± 209.48
Compression strength (MPa)	$8.24^{b,c} \pm 3.20$	$9.74^{a} \pm 3.01$	$9.31^{d} \pm 2.82$
Ductility (%)	3.37 ± 1.72	3.40 ± 1.87	3.59 ± 1.61

a-b, c-d – $p<0.05$

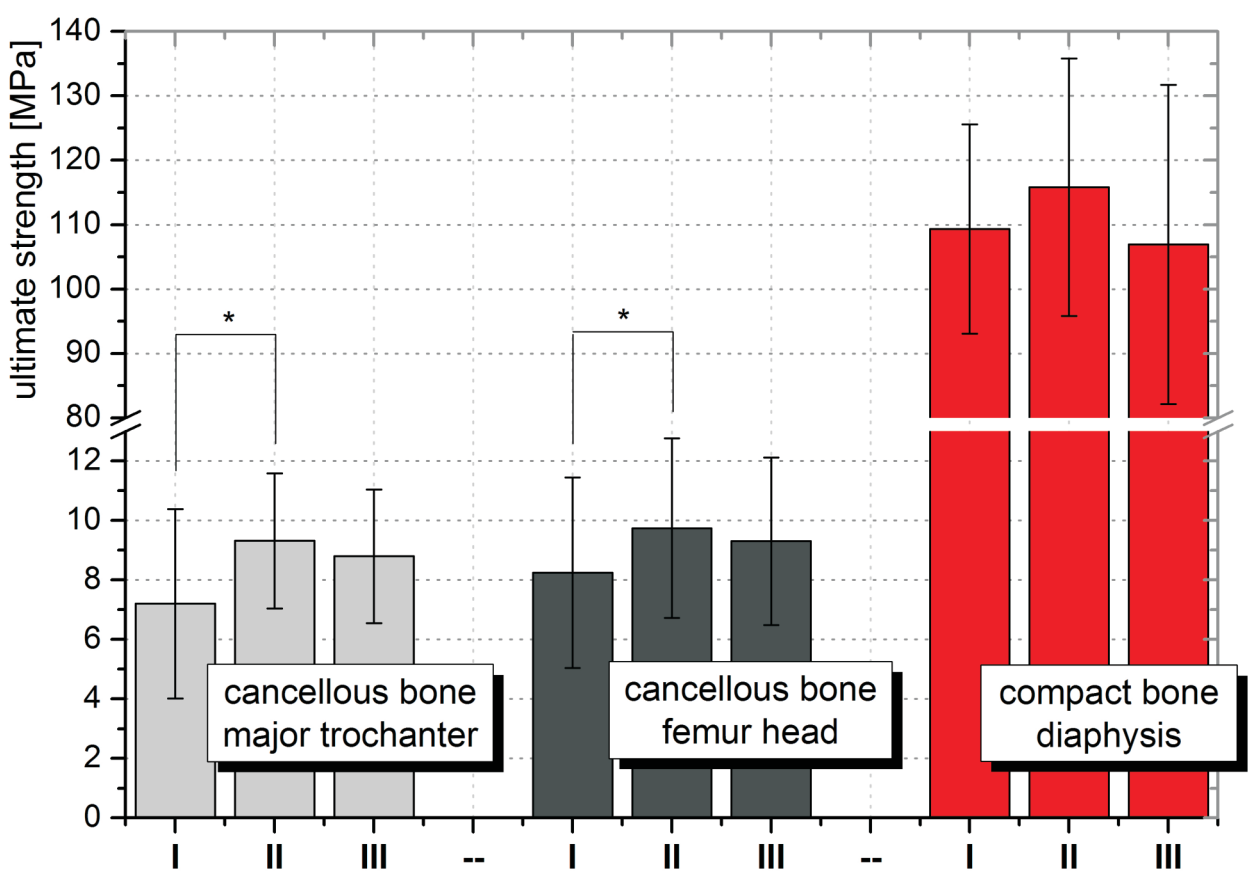

Fig. 4. Comparison of the values of Young's modulus from the compression test achieved for the samples of the spongy tissue (greater trochanter and head of the femur) and the compact tissue in groups I – III ($p<0.05$).

element did not occur in groups II and III. Statistically significant differences were noted for Ca and Sr between group II and other groups (Table 6).

Table 5. Content of the main elements in the samples of the spongy tissue in % ($\bar{x}$ ± SD).

Element	Group (n = 9)		
	I – MCP	II – n-DCP	III – CSP
Greater trochanter			
C	73.89 ± 1.86	71.20 ± 3.49	74.45 ± 2.81
O	22.56 ± 1.43	24.65 ± 2.26	21.90 ± 2.71
Sr	0.67b ± 0.31	1.06a ± 0.72	0.56b ± 0.30
P	0.57 ± 0.27	0.33 ± 0.14	0.68 ± 0.37
Al	0.36 ± 0.20	0.34 ± 0.19	0.46 ± 0.22
Si	0.23 ± 0.17	0.21 ± 0.14	0.35 ± 0.10
Ca	0.14 ± 0.13	0.13 ± 0.05	0.24 ± 0.23
Na	0.13 ± 0.15	0.08 ± 0.03	0.06 ± 0.02
Mg	0.09 ± 0.02	nd	nd
Head of the femur			
C	71.46 ± 2.56	71.62 ± 2.28	73.60 ± 2.54
O	23.90 ± 2.24	24.61 ± 2.02	22.56 ± 2.63
P	1.02^{a} ± 0.30	0.53^{b} ± 0.31	0.69^{b} ± 0.33
Ca	0.59^{a} ± 0.42	0.23^{b} ± 0.12	0.36 ± 0.23
Sr	0.57^{b} ± 0.18	1.00^{a} ± 0.49	0.66^{b} ± 0.34
Al	0.40 ± 0.22	0.36 ± 0.12	0.28 ± 0.18
Si	0.39 ± 0.21	nd	nd
Na	0.11 ± 0.08	nd	nd
Mg	0.06 ± 0.02	nd	nd

nd – not detected a-b – $p<0.05$

Table 6. Content of the main elements in the samples of the compact tissue (diaphysis) in % ($\bar{x}$ ± SD).

Element	Group (n = 9)		
	I – MCP	II – n-DCP	III – CSP
C	55.58^{b} ± 9.97	38.37^{a} ± 4.14	55.38^{b} ± 6.71
O	36.52^{b} ± 9.19	51.15^{a} ± 2.03	34.88^{b} ± 4.28
P	2.92^{b} ± 0.90	4.24^{a} ± 0.69	2.95^{b} ± 0.70
Ca	1.95^{b} ± 0.86	3.25^{a} ± 1.40	1.73^{b} ± 1.51
Sr	1.01^{b} ± 0.27	1.31^{a} ± 0.22	0.91^{b} ± 0.21
Al	0.55^{a} ± 0.19	0.48 ± 0.29	0.41^{b} ± 0.27
Si	0.34 ± 0.16	0.35 ± 0.12	0.24 ± 0.13
Mg	0.15 ± 0.08	0.12 ± 0.05	0.10 ± 0.07
Na	0.06 ± 0.02	0.10 ± 0.04	0.05 ± 0.03

a-b – $p<0.05$

Discussion

The results are difficult to discuss, especially because in the available literature similar papers were not found. Moreover, in the case of the biological material its mechanical properties depend on numerous other factors, such as preparation method and time of the samples storage (Turner and Burr 1993, Nikodem 2010).

In the present paper the authors present the results of the experimental study on the influence of various feed phosphates on physical and chemical properties of porcine bones. The results presented show that the studied fragments of the femur of these animals differed with respect to the chemical composition as well as structural and mechanical properties of the bone tissue, which are after all interconnected (Nikodem et al. 2004, Kuropka et al. 2006).

The highest values of the mechanical parameters (Young's modulus, compression strength) for the samples of the compact tissue of the femur were obtained for group II, i.e. derived from animals receiving the new dicalcium phosphate (n-DCP). As indicated by the analysis of the chemical composition, this is probably caused by the deposition of greater reserves of calcium in the cortical compact tissue (Pointillart et al. 1995). This shows that the compact bone tissue, forming the external bone cover and giving it specific strength, becomes a reservoir first of all for calcium salts and also phosphates salts. Other chemical studies of the same bones (whole ones) revealed that they contained significantly more calcium (262.1 g/kg) and phosphorus (124.2 g/kg) when compared to the groups receiving MCP and CSP in the feed mixtures (Dobrzański et al. 2010). It is worth adding that digestibility studies showed in young porkers slightly higher availability of Ca from the mixture with n-DCP (66.4%) when compared to MCP (63.9 %) and CSP (64.3%). These values for phosphorus were 76.8, 73.5 and 74.4%, respectively (Korniewicz et al. 2010). This answers why there was significantly more building material (Ca and P) in the bone tissue.

Our study showed that the bone samples (compact tissue) of this group (n-DCP) were characterized by the smallest ductility and the smallest value of the energy of deformation, which proves significantly higher fragility of this material. These tissues also showed the smallest microhardness HV, but the physical density was the highest. On the other hand, the compact tissue from group III (CSP) was characterized by the smallest values of physical density, and at the same time the greatest microhardness HV. The results may suggest that the increase in density of the sample caused by the increase of the mineral stage does not concurrently cause an increase in hardness of this sample but on the contrary the fragility of this material increases. This is because the number of calcium crystals increases (and not their size), which probably determines the hardness of the material (Todoh et al. 2004, Nikodem and Ścigała 2012).

Mechanical parameters of the samples of spongy tissue (the head of the femur) were characterized by, apart from the similar energy of deformation, completely different values, and also by a greater range of results than that of the compact tissue. This results from the fact that relatively young animals were

studied, with the developed bone growth plate, which proves the occurrence of processes of reconstruction of the bone tissue. A similar tendency as before could be noted; better strength indices were noted in group II, and in particular when compared to group I.

The values of both Young's modulus and the compression strength for the spongy bone tissue from the head of the femur are higher only by approx. 7% when compared to the samples cut from the greater trochanter. This result indicates that the load burdened on by the pelvis-femur system in pigs seems to be quite even. The pelvis pressure on the femur seems to be balanced by the strength of muscles in the region of the greater trochanter of the femur.

Feng and Jasiuk (2011) gave the values of mechanical parameters for the femur (cortex) of pigs of different age (6, 12 and 42 months). Young's modulus was 15.1, 19.9 and 22.5 GPa, respectively, and the ultimate tensile strength was within 99-119 MPa. The older the animal was, the harder and more durable was the bone; this strength increased from 0.23 up to 0.86 GPa in the oldest animals.

The chemical composition of bones influenced the mechanical parameters, although these relations are complex. As the presented data indicate, the content of C and O was similar in the spongy tissue (external layer) in all the groups. However, smaller participation of carbon, and greater of oxygen, in the compact tissue when compared to the spongy tissue is noteworthy. Other elements, i.e. Al, Na, Mg and Si, occurred at low concentrations (in total below 1%). The presence of Fe and Zn was not detected with the SEM technique.

The elements which are directly connected with metabolism of the bone tissue are Ca, P and Sr. The samples of the compact tissue of group II (n-DCP) were characterized by the highest contents of these elements, and the differences were statistically significant when compared to groups I and II. In the case of the spongy bone tissue only the strontium concentration showed this relation. Ca and P levels were the highest in group III (CSP). Although in animals of group III sodium was present in the phosphate feed, no increased content of this important metal was found during the analysis of the elemental composition of the bone tissue. Sodium is regulated in feed mixes; in all groups its content was similar (0.2%).

The fact of the increase of the strontium content in the bone tissue in pigs receiving n-DCP is interesting. It is difficult to explain this mechanism because this element was not compared in pig feeding. Its biological role is known; it is used in the form of ranelate in prophylaxis and therapy of a dangerous disease of the skeletal system like osteoporosis (Ammann 2006, Marie 2006). The preliminary clinical studies showed that giving 2000 mg of strontium ranelate/day and standard calcium and vitamin D_3 preparations for 3 years causes an increase of bone mineral density (BMD) in the lumbar spine to 14% and reduction of the relative risk of vertebral fractures (by 41%), as well as new non-vertebral fractures (neck of femur) in the 1st as well as in the 3rd year of treatment (Leszczyński et al. 2007). In this context, the results of study by Pagano et al. (2007) are interesting. In feeding piglets, they applied 50 mg Sr/kg of feed with a dose of 500 U/kg of microbiological phytase. A beneficial increase of the bone breaking strength (from 250 in the control group to 326 kg in the experimental group) and the BMD (respectively from 0.62 to 0.73 g/cm^2) occurred in the femurs. Also certain changes in the content of Fe, Zn Cr and Mn in bones were observed. The obtained results additionally show that strontium can actively participate in the process of mineralization and reconstruction of bone tissue.

Conclusion

The study showed the influence of various feed phosphates in the pig mixtures on the mechanical and chemical properties of bone tissue, and in particular its external part (cortical). Generally, the best effects, visible in particular in the values of the compression strength and the hardness of the bone tissue, were achieved through the use of the new phosphate (n-DCP).

The result of the present study is the information crucial from the point of view of understanding the phenomena occurring during metabolic processes of mineral compounds and reconstruction of the bone tissue (e.g. after implantation). This may contribute to better knowledge of the causes of developing, as well as prevention of the consequences of numerous skeletal system diseases, both in animals and humans.

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