Ann. Anim. Sci., Vol. 22, No. 4 (2022) 1363–1372 *DOI: 10.2478/aoas-2022-0061*

sciendo

CHEMICAL BODY COMPOSITION AND BONE GROWTH OF YOUNG PIGS AS AFFECTED BY DEFICIENT, ADEQUATE AND EXCESS DIETARY PHOSPHORUS SUPPLY*

Monika Sobol[1], Grzegorz Skiba[1♦], Stanisława Raj[1], Paweł Kowalczyk[1], Karol Kramkowski[2], Małgorzata Świątkiewicz[3], Eugeniusz R. Grela[4♦]

[1]Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka 3, 05-110 Jabłonna, Poland
[2]Department of Physical Chemistry, Medical University of Bialystok, Kilińskiego 1, 15-089 Bialystok, Poland
[3]Department of Animal Nutrition and Feed Science, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[4]Institute of Animal Nutrition and Bromatology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
♦Corresponding author: eugeniusz.grela@up.lublin.pl, g.skiba@ifzz.pl

Abstract

The objective of the experiment was to study the effect of deficient, adequate and excess dietary phosphorus supply on growth performance, retention and utilisation of phosphorus, length, mass and geometry measurements of the femur shaft, content of protein, ash, phosphorus in viscera, edible (meat and fat) and inedible (bones and skin) parts of the body in pigs aged from 33 to 110 days. It was found that compared to animals fed according to phosphorus requirement the deficiency and excess of dietary phosphorus did not influence total feed intake (mean 120.6 kg) and feed conversion (mean 1.9 kg/kg gain). However phosphorus deficiency lowered total gain of the body mass (P=0.0072), diminished weight of the inedible part of the carcass (P=0.0229), decreased the content of body protein (P=0.0171), ash (P=0.0001), and phosphorus (P=0.0001). Whereas, over-supply of dietary phosphorus did not cause any change of these components. Utilisation of the total phosphorus was diminished (P=0.0001) in pigs fed diet with both excess (by 16.26%) and deficiency (by 12.28%) of the phosphorus, but excess had much lower negative impact than its deficiency. When available form of this element was considered over-supply still reduced (P=0.0001) its utilisation the most (by 26.58%) but deficiency made utilisation the best (7.77%). Both dietary deficiency and over-supply of the phosphorus diminished (P=0.0001) femur mass (by 25 and 11 g, respectively). Thus negative impact of phosphorus deficiency was much stronger. Moreover, phosphorus deficiency diminished (P=0.0015) bone length (by 0.5 cm), however, excess did not change this feature. The response of animals to a decrease in bone mass and length due to disturbances in phosphorus supply (both deficiency and excess) was the increase in the vertical external diameter of the femur shaft.

Key words: pigs, dietary phosphorus supply, body phosphorus retention, bone growth

Phosphorus is an essential element for all living beings. Skeleton is the main place of the phosphorus location in the body because 80% of this element is present in the bone as hydroxyapatite. Remaining 20% are key elements of the cells as nucleic acids, nucleotides, phospholipids and numerous phosphorylated compounds that function in metabolism (Suttle, 2010).

Dietary phosphorus is not fully absorbed by monogastric animals, and non-utilised part of this element is excreted to the environment being the main factor of water eutrophication (Cordell et al., 2011; Sørensen et al., 2018 a; Yang et al., 2008). Nevertheless, the commercial recommendation for the phosphorus content in pigs feed advises dietary excess of the phosphorus as a safety buffer to avoid the potential production losses and welfare concerns resulting from phosphorus underfeeding (Kyriazakis, 2008), such as reduced average daily gain (Harper et al., 1997; Gonzalo et al., 2018) and inadequate body mineral balance (Veum and Ellersieck, 2008; Gutierrez et al., 2015) and bone mineralisation and development (Sørensen et al., 2018 b). According to Becker et al. (2020) the reduction in growth performance, and in bone development, is progressively worsened as the ratio of calcium/available phosphorus widens and the response of all parameters measured has a linear manner. In turn, over-supply of dietary phosphorus reduces growth and bone strength in rats, even if calcium supply is at an adequate level (Huttunen et al., 2007). Moreover, high level of dietary phosphorus and calcium does not increase bone mineral accretion, but reduces bone resorption in growing pigs (Fernández, 1995 a, b). A recent study carried out by Gutierrez et al. (2015) showed that

*This work was supported by the statutory funds of The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences.

femur mineral content measured by dual energy X-ray absorptiometry method increased with available phosphorus intake but reached a plateau, when dietary ratio of Ca/available phosphorus was relatively constant, however, recommended by NRC (2012).

Thus, optimal minerals supply to pigs is not a matter of increasing the mineral intake, but should be estimated to maintaining animals' welfare and growth performance. This strategy should include increased digestive efficiency and metabolic utilisation, minimised excretion, maximized bone growth, mineralization and strength. Till now research works focus mainly on the dietary phosphorus supply as a single factor and its influence on the overall mineral balance and physiological response in the pig, thus ratio of dietary calcium/phosphorus differed between groups of pigs (Sørensen et al., 2018 a, b, 2019), only in the study by Pettey et al. (2015) dietary calcium/phosphorus ratio was similar between groups (NRC, 2012), just like in the study by Gutierrez et al. (2015). Although, only Pettey et al. (2015) determined deposition of chemical body component (including phosphorus) only in body parts, however only in viscera and whole carcass at several points of the pigs' growth, but at all growth stages pigs were fed a diet adequate in phosphorus content. According to our best knowledge the present study is the first to determine responses of young pigs to varied supply of dietary phosphorus in relation to: 1) growth performance; 2) phosphorus balance; 3) weight of the body components: viscera, edible parts (meat and fat), inedible parts (bones and skin); 4) accretion of the chemical components (including minerals) in the body parts; 5) bone growth (length, mass, geometry measurements) when dietary ratio of calcium/available phosphorus is constant and agreed with nutrient requirements of young growing pigs. The objective was to study the effect of deficient, adequate and excess dietary phosphorus supply on growth performance, retention and utilisation of phosphorus, length, mass and geometry measurements of the femur shaft, content of protein, ash, phosphorus in viscera, edible (meat and fat) and inedible (bones and skin) parts of the body in pigs aged from 33 to 110 days. It was hypothesised that various intake of dietary phosphorus (below or above nutritional requirements), while preserving the ratio of calcium/available phosphorus as constant, will not have a marked effect on feed intake, growth performance, bone morphometry and geometry parameters, and content of the main chemical body components (excluding phosphorus).

Material and methods

Ethics

The design of the study, animal care, and all procedures applied during the experiment were performed in accordance with the relevant principles of the European Union and the Polish Animal Protection Act and were approved by the II Local Ethics Committee on Animal Experimentation of Warsaw University of Life Sciences, SGGW, Warsaw, Poland (Resolution 1/2013). The experiment was designed to minimise the number of animals whilst maintaining high statistical power according to the principles of the 3Rs (replacement, reduction and refinement).

Animals, diets, housing, and treatment

The experiment was carried out on 18 female piglets (crossbreds of ♀ Polish Large White × ♂ Danish Landrace) aged from 28 to 110 days. After transport to the experimental piggery animals were subjected to five days of acclimatization. Then at the age of 33 days the piglets were assigned to three experimental groups (I, II, III) and till day 110 of age were fed with diets differing in calcium and phosphorus content, but the proportion between these components (calcium/available phosphorus) was the same in all diets and consistent with the nutritional recommendation (NRC, 2012). Mono- and dicalcium phosphate were the source of inorganic calcium and phosphorus. Depending on group changes in calcium and phosphorus content were achieved by increased or decreased amount of mono-calcium phosphate at the expense of corn in the diet. Design of the experiment is presented in Table 1.

Table 1. Design of the experiment

Treatment	Group	Intake (% of recommended amount)				Ca/available P
		protein/amino acids/energy	vitamins	Ca	available P	
Ca and P (both –50%)	II	100	100	50	50	2.19
Control	I	100	100	100	100	2.19
Ca and P (both +50%)	III	100	100	150	150	2.19

Ca – calcium; P – available phosphorus.

The piglets used in the experiment were characterized by high growth potential. The study was conducted in the period of the rapid bone growth and mineralization, thus during this time animals are very sensitive to experimental factors. The animals were kept individually in pens (3.2 m^2) equipped with nipple drinkers, on a concrete floor without straw. Pigs received a dry pelleted feed offered *ad libitum*. Central heating and air conditioning systems (Fancom, model ISM0.12; Fancom BV) in the piggery made it possible to keep the animals under thermo-neutral conditions according to EC regulation (No 1924/2006). The diets offered to pigs were formulated based on cereals (barley, wheat), protein (soybean meal) and energetic (rapeseed oil) compounds and were supplemented with mineral–amino acid–vitamin mixes. These mixes provided the diets with the same amount of vitamins, amino acids, but different amount of calcium and phosphorus (Table 2). All diets were balanced in the content of energy, protein, and amino acids. Lysine content amounted to 11.8 g (standardized ileal digestible) per kg feed. Remaining essential amino acids (methionine:

threonine: tryptophan) were balanced in proportion to the lysine content (100: 33: 63: 18). Metabolisable energy, protein, amino acids and minerals content was calculated according to RFES (2003). Composition, nutritive and energetic value are presented in Table 2.

Slaughter of the animals

At 110 day of age the pigs were slaughtered in the experimental slaughterhouse of The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Science after a fasting period of 16 h. Before slaughter the pigs were electrically stunned (STZ 3 apparatus; P.P.H. MASTER Sp. J., Solec Kujawski, Poland) and then exsanguinated. The blood was collected and weighed. Next, the abdominal cavity was opened and the entire gastrointestinal tract was removed. The stomach, large and small intestines were separated, next emptied and weighed. The liver, kidneys, pancreas and spleen were separated and their weights recorded. Lung with tongue, heart, brain and spinal cord were also removed and weighed together with added blood.

Carcasses were chilled overnight at 4°C by 24 hours and weighed. After the chilling period, each right half carcass was weighed and next dissected to primal cuts (ham, loin, belly and shoulder) according to a standardised procedure described by Różycki (1996). Each primal cut was further weighed and dissected into soft tissues (muscle and fat) and inedible parts (skin, bones). Inedible parts and viscera/internal organs were separately autoclaved (at 110°C and a pressure of 1.25 atm.) for 12 h. Next, inedible parts, viscera and soft tissues were separately ground and mixed, and from each body part (soft tissue, inedible part of carcass and viscera/internal organs) a sample of 500 g was randomly taken and homogenized. Afterwards from each part a representative sample was taken for chemical determinations. The scheme of assigning individual body parts to the analyzed samples is presented in Figure 1.

Table 2. Ingredients, chemical composition and nutritive value of the diets

Item	Diet		
	(II)	(I)	(III)
Ingredients (g/kg)			
barley	205		
wheat	205		
maize	280		
rapeseed meal	50		
soybean meal	170		
rapeseed oil	50		
mineral–amino acid–vitamin mix*	40		
Analysed chemical composition (g/kg)			
dry matter	878	882	882
organic matter	853	853.6	851.8
nitrogen free extract	573.4	574	572.2
crude protein	165	165	165
fat (ether extract)	75	75	75
crude fiber	39.6	39.6	39.6
crude ash	25.0	28.4	30.2
Calculated nutritive value (g/kg)**			
total lysine	11.8	11.8	11.8
methionine	3.9	3.9	3.9
threonine	7.4	7.4	7.4
tryptophan	2.1	2.1	2.1
sodium	1.5	1.5	1.5
total phosphorus	4.21	5.81	9.29
available phosphorus	1.59	3.19	5.25
calcium	3.50	7.00	11.52
calcium/available phosphorus	2.20	2.19	2.19
metabolisable energy (MJ/kg)	14.1	14.1	14.1
lysine/metabolisable energy	0.84	0.84	0.84

Ca – calcium; P – available phosphorus; I – control diet; II – diet containing 50% less of Ca and available P; III – diet containing 50% more Ca and available P; *Mineral-vitamin mixtures provided per kg of complete diet the same amount of: Fe, 62.5 mg; Zn, 62.5 mg; Mn, 18 mg; Cu, 4 mg, J, 0.2 mg; Se, 0.2 mg; Vitamin A, 2000 IU; Vitamin D_3, 245 IU; Vitamin E, 40 mg; Vitamin K_3, 2 mg; Vitamin B_1, 2 mg; Vitamin B_2, 2.5 mg; Vitamin B_6, 3.1 mg; Vitamin B_{12}, 0.02 mg; lysine, 5.27 g; methionine, 1.55 g; threonine, 2.37 g; tryptophan, 0.53 g; NaCl, 1.31 g. Mineral-vitamin mixtures provided per kg of complete diet different amounts of calcium (diet I, 5.68 g; diet II, 2.18 g; diet III, 9.18 g) and phosphorus (diet I, 1.92 g; diet II, 0.32 g; diet III, 3.52 g); **RFES, 2003.

Empty body		
Viscera/internal organs	Chilled carcass	
stomach, intestines (small and large), liver, bladder, pancreas, spleen, heart, lung, tongue, brain and spinal cord, blood	Edible part	Inedible part
	meat, fat	skin, bones including skull and feet

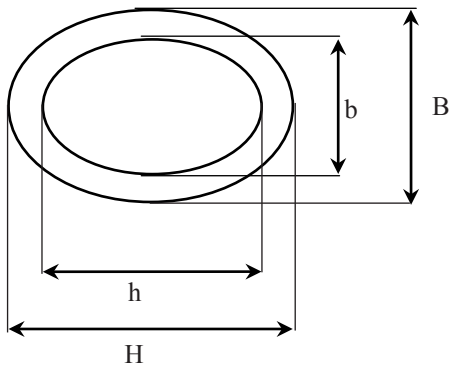
Figure 1. Scheme of assigning individual body parts to the analysed samples

Chemical analysis of the samples

In each sample of feed the dry matter, nitrogen, crude fibre, extract ether, ash and phosphorus contents were analysed using the official methods (AOAC, 2011) 934.01; 984.13, 978.10; 920.39; 942.05, 965.17, respectively. In each sample of the body the content of nitrogen, ash and phosphorus were determined using the official methods (AOAC, 2011) 928.08; 920.153; 991.27, respectively. Protein content in the samples of the body and feeds was estimated as N × 6.25. Based on the content of chemical components and weight of half-carcasses the chemical composition of the carcass (whole) was determined.

Bones collection

After exsanguination, the left femur was dissected from each carcass. After excision, the bones were cleaned of any remaining flesh, weighed and length measured. Each bone was cut to measure its horizontal and vertical diameter (both external and internal), Figure 2.



where: B – vertical external diameter, H – horizontal external diameter, b – vertical internal diameter, h – horizontal internal diameter.

Figure 2. Measurements of external and internal diameters of bone cross-section

Statistical analyses

Statistical analyses were performed using Statistica (version 13.1, StatSoft, Tulsa, OK, USA). The examined bone characteristics in different groups are presented as mean values. The results were analysed with one-way ANOVA. When the F ratio was significant, the Tukey test was used to determine differences between the groups. Statistical significance was set at $P<0.05$. A borderline significant trend was set at $P<0.09$.

Results

Performance

Pigs of the groups III and I reached a higher final body weight (Table 3) compared to pigs of the group II (75.3 and 73.8 vs. 71.7 kg, respectively for groups III, I and II; $P = 0.0033$). The total weight gain took a similar course (66.1 and 65.8 vs. 62.3 kg, respectively for groups III, I and II; $P = 0.0072$). Values of the remaining performance indicators did not differ between groups of pigs.

Body partition according to the analysed body parts

Pig's body partition according to the analysed body parts is presented in Table 4. The final empty body weight of pigs of the groups III and I was similar, however higher than in pigs of the group II (72119 and 71057 vs. 68372 g, respectively, $P = 0.0050$). Pigs of the group III tended ($P = 0.0671$) to have heavier mass of viscera/internal organs compared to pigs of the groups I and II (15375 vs. 13821 and 13664 g, respectively). Mass of the inedible parts (bones and skin) of the body was the heaviest in pigs of the group I, lower in group III and the lowest in group II (19144, 18168, 17333 g, respectively), however statistical difference was found only between pigs of the groups I and II ($P = 0.0229$). In turn, mass of the edible parts (meat and fat) did not differ between the groups of pigs.

Chemical composition of the body parts

Chemical composition of the body parts is presented in Table 5. Compared to pigs of the group II, pigs of the groups I and III had similar but greater content of the protein (12591 v.s 13275 and 13236 g, respectively, $P = 0.0171$), ash (1322 vs. 2200 and 2326 g, respectively; $P = 0.0001$) as well as phosphorus (239 vs. 291 and 417 g, respectively; $P = 0.0001$) in empty body.

Concerning composition of the viscera/internal organs the pigs of the group III had greater ($P = 0.0335$) protein content than pigs of the groups II and I in which mass of this body component was similar (2657 vs. 2299 and 2329 g, respectively). Compared to pigs of the group II pigs of the groups I and III had similar but greater content of ash (94 vs. 145 and 162 g, respectively; $P = 0.0001$) and phosphorus (17 vs. 27 and 30 g, respectively; $P = 0.0001$).

Protein content in the inedible part of the carcass was similar in animals of the groups II and III, however lower

than in pigs of the group I (3587 and 3738 vs. 3958 g, respectively; P = 0.0031). Compared to pigs of the group II pigs of the groups I and III had similar but greater content of ash (980 vs. 1701 and 1804 g, respectively; P = 0.0001) and phosphorus (173 vs. 295 and 314 g, respectively; P = 0.0001).

Table 3. Performance of the pigs during growth from 33 to 110 days of age

Item	Group/diet			SE pooled	P-value
	Ca and P (both –50%) (II)	control (I)	Ca and P (both +50%) (III)		
Initial body weight (kg)	9.4	8.0	9.2	0.31	0.1389
Final body weight (kg)	71.7 A	73.8 B	75.3 B	0.48	0.0033
Total weight gain (kg)	62.3 A	65.8 B	66.1 B	0.60	0.0072
Total feed intake (kg)	121.3	119.2	121.4	1.38	0.7932
Feed conversion ratio (kg/kg body gain)	1.95	1.81	1.83	0.03	0.1437

Ca – calcium; P – available phosphorus; Means with different letters are significantly different (A, B for P<0.01); SE pooled – standard error for total animals.

Table 4. Weight of the empty body, viscera/internal organs, edible and inedible parts at the age of 110 days

Item	Group/diet			SE pooled	P-value
	Ca and P (both –50%) (II)	control (I)	Ca and P (both +50%) (III)		
Empty body weight (g)	68372 A	71057 B	72119 B	480.01	0.0050
Viscera/internal organs (g)	13664	13821	15375	340.75	0.0671
Edible parts (meat, fat) (g)	37375	38092	38576	425.36	0.5389
Inedible parts (bones, skin) (g)	17333 a	19144 b	18168 ab	282.25	0.0229

Ca – calcium; P – available phosphorus; Means with different letters are significantly different (A, B for P<0.01; a, b for P<0.05); SE pooled – standard error for total animals.

Table 5. Chemical composition of the body parts of pigs at the age of 110 days

Item	Group/diet			SE Pooled	P-value
	Ca and P (both –50%) (II)	control (I)	Ca and P (both +50%) (III)		
Empty body (g)					
protein	12591 A	13275 B	13236 B	118.71	0.0171
ash	1322 A	2200 B	2326 B	111.38	0.0001
phosphorus	239 A	391 B	417 B	19.72	0.0001
Viscera/internal organs (g)					
protein	2299 a	2329 a	2657 b	65.14	0.0335
ash	94 A	145 B	162 B	8.02	0.0001
phosphorus	17 A	27 B	30 B	1.52	0.0001
Inedible part of the carcass (bones, skin)					
protein	3587 A	3985 B	3738 A	54.33	0.0031
ash	980 A	1701 B	1804 B	92.56	0.0001
phosphorus	173 A	295 B	314 B	15.95	0.0001
Edible part of the carcass (meat, fat)					
protein	6705	6961	6841	87.85	0.5209
ash	248 A	353 B	359 B	13.75	0.0001
phosphorus	48 A	69 B	72 B	2.69	0.0001

Ca – calcium; P – available phosphorus; Means with different letters are significantly different (A, B for P<0.01); SE pooled – standard error for total animals.

Compared to pigs of the group II, animals of the groups I and III were characterised by greater content of ash (248 vs. 353 and 359 g, respectively; P = 0.0001) and phosphorus (48 vs. 69 and 72 g, respectively; P = 0.0001) in edible part of the carcass.

Phosphorus balance

The phosphorus balance is presented in Table 6. According to the assumption the total intake of the crude and available phosphorus was the highest in the pigs of the group III, lower in the group I and the lowest in the pigs of the group II (1079, 692, 510 g; P = 0.0001 and 586, 382, 194 g; P = 0.0001, respectively). Pigs of the groups II and III contained and deposited in the body more amount of this element compared to pigs of the group I (417 and 391 vs. 239g and 367 and 341 vs. 189 g, respectively; P = 0.0001). Pigs of the group I were characterised by better utilisation of total phosphorus compared to pigs of the groups II and III (49.25 vs. 36.97, 32.99%, P = 0.0001). However, utilisation of the available phosphorus by pigs took the following order (P = 0.0001): group II (97.04%) > group I (89.27%) > group III (62.69%).

Mass, length and diameters of the femur

Mass, length and diameters of the femur are presented in Table 7. Mass of the femur differed (P = 0.0001) between groups of animals and was the lowest in pigs of the group II (233 g), higher in the group III (247 g) and the highest in pigs of the group I (258 g). Length of the femur was similar in pigs of the groups I and III, however greater than in pigs of the group II (18.0 and 18.3 vs. 17.5 cm, respectively; P = 0.0015). Vertical external diameter of the femur was similar in pigs of the groups II and III, and greater compared to animals of the group I (23.3 and 23.4 vs. 22.5 mm, respectively; P = 0.0003). However, values of vertical internal diameter tended to have similar relations (13.9 and 12.5 vs. 11.7 mm, respectively, P = 0.1019). Values of horizontal diameter (both external and internal) did not differ between animals.

Discussion

Performance

In the current study pigs had *ad libitum* access to feeds, which differed only in total and available phosphorus content (deficient, accurate, excess), however had similar ratio of calcium/available phosphorus, and were balanced with respect to all other nutrients. Results of the present study indicated that both groups of pigs fed a diet with high phosphorus deficiency and phosphorus excess did not modify their feed consumption. However, according to some authors phosphorus may be involved in appetite control (Ruan et al., 2007), and its deficiency can reduce feed intake in piglets and in growing–finishing pigs (Sørensen et al., 2018 a; Vier et al., 2019). Other authors found no changes in feed intake by growing pigs

Table 6. Phosphorus balance in the pigs during growth from 33 to 110 days of age

Item	Group/diet			SE pooled	P-value
	Ca and P (both –50%) (II)	control (I)	Ca and P (both +50%) (III)		
Intake of total phosphorus (g)	510 A	692 B	1079 C	57.91	0.0001
Intake of available phosphorus (g)	194 A	382 B	586 C	39.19	0.0001
Content in the empty body (g)	239 A	391 B	417 B	19.72	0.0001
Deposition in the empty body (g)	189 A	341 B	367 B	20.18	0.0001
Utilisation of total phosphorus (%)	36.97 B	49.25 C	32.99 A	1.68	0.0001
Utilisation of available phosphorus (%)	97.04 C	89.27 B	62.69 A	3.71	0.0001

Ca – calcium; P – available phosphorus; Means with different letters are significantly different (A, B for P<0.01); SE pooled – standard error for total animals. Initial phosphorus content in the body of pigs amounted to 50 g (Kotarbińska, 1969).

Table 7. Mass, length and diameters of the femur in pigs at 110 days of age

Item	Group			S.E. pooled	P-value
	Ca and P (both –50%) (II)	control (I)	Ca and P (both +50%) (III)		
Mass (g)	233 A	258 C	247 B	3.580	0.0001
Length (cm)	17.5 A	18.0 B	18.3 B	0.128	0.0015
Horizontal external diameter (mm)	22.6	22.2	22.6	0.402	0.7243
Horizontal internal diameter (mm)	13.5	12.6	12.6	0.513	0.3432
Vertical external diameter (mm)	23.3 B	22.5 A	23.4 B	0.137	0.0003
Vertical internal diameter (mm)	13.9	11.7	12.5	0.696	0.1019

Ca – calcium; P – available phosphorus; Means with different letters are significantly different (A, B for P<0.01); SE pooled – standard error for total animals.

(Arouca et al., 2012; Nieto et al., 2016), broilers (Valable et al., 2018) and laying hens (Yan et al., 2005). However, opposite results concerning feed intake were observed in laboratory animals (juvenile rats) as some authors (Czarnogorski et al., 2004; Sweeny et al., 1998) observed that phosphorus deficient feeds led to an increase in the overall feed intake. The difference between our and cited results (presenting reduced or increased feed intake) was probably due to the fact that the main factor regulating feed intake is the requirement for protein and energy (Symeou et al., 2014), which in the present study was fully covered in all groups of pigs. Thus, in this case feed intake was independent from dietary phosphorus.

In the present study, despite no difference between groups in feed consumption, the pigs given feed with deficient phosphorus content grew slower and utilized feed slightly less efficiently than the control animals and those fed a diet with an excess of phosphorus. Albeit, dietary phosphorus deficiency decreased body weight gain and thus final mass. Similarly, Reinhart and Mahan (1986) and Vier et al. (2019) showed that low dietary P concentrations can negatively affect growth rate (daily gain). Opposite response of pigs was presented by Nieto et al. (2016) as they found that daily gain, final body weight, and feed conversion were not affected by increasing available phosphorus in the diet. Similarly, O'Quinn et al. (1997 a, b) and Hastad et al. (2004), who evaluated the effects of increasing available phosphorus levels on performance of growing pigs, observed no effect on growth rate. Lack of difference in weight gain between pigs offered high and medium phosphorus content, regardless of calcium content, was found by Pond et al. (1978). Other authors reported no difference in body weight gain in growing pigs fed diets solely deficient in calcium (Eklou-Kalonji et al., 1999) or deficient in both calcium and phosphorus (Nicodemo et al., 1998). However, Fammatre et al. (1977) found increasing effects of higher calcium and phosphorus supply to diets on growth in young pigs.

Weight of the analysed body parts

Average empty body weight of pigs in the present study amounted to 95.8% of the live weight and was slightly greater (approximately by 2%) than in the study by Pettey et al. (2015) and Mahan and Shields (1998) and over 3% greater than in the study by Wagner et al. (1999). However, results of the present work indicated that phosphorus deficiency decreased the empty body weight of pigs, whereas it was unchanged when phosphorus supply was in excess. However, the largest changes were visible in the mass of inedible part of the carcass, which was lighter in the animals fed a diet with phosphorus deficiency and similar/unchanged in pigs with adequate and excessive supply of phosphorus. Since a great part of the phosphorus (ca. 80%) is located in the skeleton (Suttle, 2010), it is no surprise that this group of pigs had the lightest mass of the inedible parts of the carcass (bones and skin). In turn, Létourneau-Montminy et al. (2015) found that a decrease in dietary calcium and phosphorus led to a decrease in mass of soft tissue in the body.

Chemical composition of body parts

In recent literature there is a lack of data concerning influence of dietary phosphorus level on weight and chemical composition of body parts of pigs, which makes it significantly difficult to conduct an extensive discussion. Results of the present study indicated that dietary phosphorus deficiency decreased content of measured chemical components of the empty body such as protein, ash and phosphorus in all investigated parts of the pigs' body. Interestingly, over-supply of dietary phosphorus did not cause an increase of these components in the empty body. Moreover, the under- and over-supply of the phosphorus did not influence the ratio of ash/phosphorus, which was similar in all measured parts of the body. Pettey et al. (2015) studied deposition of chemical components in the body parts (viscera and whole carcass) in several "points" of pigs', however, they fed in accordance with nutritional requirements a diet with ratio of calcium/available phosphorus similar to our control diet. Results presented by these authors indicated that pigs at the weight ca. 70 kg had similar mass of the carcass to pigs of the control group in our study mass but lighter viscera. Comparing the ratios of ash/phosphorus in body parts presented by Pettey et al. (2015) it was higher by 3–6% than in the present study. It could be probably due to the difference in the phosphorus absorption between pigs of the cited and present study.

Results of the present study indicate that dietary phosphorus deficiency lowered its content in all investigated parts of the body (viscera, edible end inedible). Depending on the body parts, content of the phosphorus in feed/group deficient in this element was lower by 37% in the viscera, by 41% in the inedible parts (bones, skin) and by 30% in the edible tissue (meat, fat). Thus, it seems that strong deficiency of the dietary phosphorus forces animals to reduce their deposition to a greater extent in viscera and inedible parts than in the soft tissue of the carcass. Analysis of the data presented by Lüdke et al. (1999) carried out on weaned piglets allows us to draw a similar conclusion. Similarly, Misiura et al. (2020) in a recent meta-analysis of literature data on this issue using statistical/mathematical models stated that there is evidence for prioritization of phosphorus retention in soft tissue when the supply of this nutrient is deficient. Narcy et al. (2015) found that during phosphorus deficiency this element is fixed at 40% in the soft tissue; moreover, Létourneau-Montminy et al. (2015) concluded that in such a situation the phosphorus is fixed in the soft tissue and has a priority over phosphorus deposition in the bones. Our results and available literature data indicate that the response of animals to a reduction in phosphorus supply is to reduce its deposition in the bones. This may be because bone tissue, which is the primary site of phosphorus accumulation, has a greater capacity to cover phosphorus deficiencies than the soft tissues. Albeit, over-supply of

the phosphorus did not change content of this element in any part of the body. Although, due to a small number of papers on this issue, a broad discussion is difficult.

Concerning influence of phosphorus supply on deposition of nitrogen/protein in the body Sørensen et al. (2018 a) found that the dietary phosphorus deficiency resulted in worse absorption of nitrogen, regardless of the age of pigs, which consequently led to a lower content of protein in the pigs' body. However, in this study response of animals to over-supply of the phosphorus depended on age of animals. In early grower phase absorption of nitrogen decreased, whereas in older age it was similar to control animals. Vipperman et al. (1974) and Sands et al. (2001) also found that the nitrogen retention was reduced in pigs fed a phosphorus deficient diet, and that the nitrogen retention was more affected by phosphorus than calcium supply. A similar conclusion was drawn by Fernández (1995 a) who found the most favourable nitrogen retention with the highest supply of phosphorus in the diet. Results of the present study indicate that lower nitrogen/protein deposition during phosphorus deficiency is due mainly to lower accretion of nitrogen/protein in the viscera and inedible (bones, skin) parts of the body.

Phosphorus balance

Results of the present study indicate that utilisation of the total phosphorus strongly depended on its content in the feeds and was much less when over-supply or deficiency of the phosphorus occurred. Moreover, over-supply had a much more negative effect than its deficiency. However, when available form of this element was considered over-supply still reduced its utilisation the most, but deficiency made its utilisation the best. There are some studies on this subject, however results are inconclusive, and concern usually total phosphorus. Symeou et al. (2014) showed that efficiency of utilisation of dietary phosphorus is independent of the calcium level in the feed. Similarly, Oster et al. (2018) reported that reduction in phosphorus content had no effect on its utilisation in piglets. By contrast, Varley et al. (2011) found that phosphorus utilisation increased as its content in feed was reduced. Similar results, as in the present study, were reported by Sørensen et al. (2018 a), Poulsen (1994) and Fernández (1995 a) who stated that both excessive phosphorus supply and deficiency may result in low utilisation. Data presented by Sørensen et al. (2018 a) indicated that in the case of excessive supply worse utitlisation was mainly due to elevated phosphorus excretions. Analysis of the data shows that the lower phosphorus utilisation in the phosphorus deficient group was mainly due to lower digestibility of this element. The "savings" made by reducing to the necessary minimum the amount of phosphorus allocated to processes other than deposition in tissues (e.g. energy, hormonal, enzymatic conversion etc.) resulting in less excretion in the urine may turn out to be insufficient to increase its use.

Generally, results of the present study indicated that available phosphorus utilisation was strongly reduced along with increasing its intake and reached up values from about 97 to 63%. Similar data was presented by Sørensen et al. (2018 a) who reported phosphorus retention efficiency of about 99 to 69% in the group with the smallest and the greatest phosphorus intake.

Fandrejewski et al. (1999) reported that the utilisation of available phosphorus was significantly better (85–87%) in pigs fed the diet with low phosphorus content than in the pigs fed higher phosphorus diet (67–73%). Thus, authors concluded that increasing phosphorus retention by supplementing the diet with inorganic form of this element decreased its utilisation. Fernández (1995 c) also found that net retention of phosphorus increased as the amount of absorbed phosphorus increased.

Mass, length and diameters of the femur

Despite the fact that in the present study the ratio of calcium to available phosphorus in the feeds was constant, both deficiency and excess of the phosphorus influenced bone mass negatively. However, the negative impact of phosphorus deficiency was much stronger. Regarding the growth of the femur the phosphorus deficiency diminished its length. Albeit, unlike bone mass, the excess of the dietary phosphorus did not change its length. This may suggest that both the deficiency and the excess of phosphorus in the feed are not conducive to the growth of bone mass and its length. Most likely, this is due to the fact that osteoblasts, whose role is to synthesize and mineralize the bone matrix, are derived from mesenchymal stem cells. Fat cells are also formed from these cells (Pierre, 2008). Thus, it seems that disturbances in calcium and phosphorus supply (deficiency and possibly excess), can promote the formation of fat cells and not osteoblasts from mesenchymal stem cells.

Literature data concerning the influence of phosphorus supply on bone growth are inconclusive. Swelum et al. (2017) in the study with lambs showed that similar to our results lower phosphorus and calcium intake diminished femur length. Liesegang et al. (2002) have shown that a low phosphorus diet, irrespective of calcium supply, fed to young pigs did not affect the length of the femur compared to a sufficient supply. While another study with growing rats (Huttunen et al., 2007) showed that a high phosphorus content in the feed, however with unchanged calcium level, decreased the femur length. In the study by Sørensen et al. (2018 a), the dietary phosphorus supply did not influence the length and weight of the bones (femur and humerus) although, similarly to the present study, the body weight growth of the pigs fed low phosphorus diet was reduced. However, if weight of the defatted bone was considered Sørensen et al. (2018 a) found that phosphorus deficiency significantly lowered bone mass. This finding is in agreement with Nicodemo et al. (1998), who found no difference in the wet weight of femur and tibia-fibula of pigs fed calcium and phosphorus deficient feeds, whereas authors also reported a reduction in dry weight of bone.

An interesting and rarely shown result of the present study is the increase in the vertical external diameter of the bone as a response to dietary phosphorus deficiency or excess. This may be related to the animals' response to a decrease in bone mass and length due to these factors. Probably, increasing the vertical external diameter and consequently the thickness of the bone cortical layer may have a positive effect on its biomechanical properties (e.g. strength).

Conclusions

The applied phosphorus deficiency worsened growth rate, decreased content of ash and phosphorus in all investigated parts of the pigs' body. Deficiency of this element forces animals to reduce their deposition to a greater extent in viscera and inedible than in edible parts of the body. The applied total dietary phosphorus excess influenced its utilisation much more negatively than its deficiency. When available form of this element is considered deficiency makes its utilisation the best. Both applied dietary phosphorus deficiency and excess influence bone mass negatively. However, the impact of deficiency is much stronger. Results indicate that increasing the phosphorus content in the feed for growing pigs does not improve their growth rate, body phosphorus content and bone growth. However, it may have a negative impact on the environment.

Author contributions:

Conceptualization, M.S., G.S., E.R.G. and S.R.; methodology, M.S., G.S. and S.R.; software, S.R., P.K. and K.K.; validation, M.S. and M.Ś.; formal analysis, M.S., M.Ś. and P.K.; investigation, M.S., G.S., M.Ś. and S.R.; resources, M.S., G.S. and S.R.; data curation, G.S. and S.R.; writing – original draft preparation, M.S., G.S. and S.R.; writing – review and editing, G.S., S.R. and E.R.G.; visualization, M.S.; K.K. and P.K.; supervision, G.S., E.R.G. and S.R.; project administration, G.S and S.R.; All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Reference

AOAC (2011). Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Arouca C.L.C., de Oliveira Silva F.C., de Oliveira Fontes D., Donzele J.L, de Oliveira R.F.M., Haese D., Kill J.L., de Paula E. (2012). Available phosphorus levels for 95 to 120 kg barrows genetically selected for lean gain. Rev. Bras. Zoo., 41: 1433–1441.

Becker S.L., Gould S.A., Petry A.L., Kellesving L.M., Patience J.F. (2020). Adverse effects on growth performance and bone development in nursery pigs fed diets marginally deficient in phosphorus with increasing calcium to available phosphorus ratios. J. Anim. Sci., 98: 1–8.

Beyer M., Chudy A., Hoffmann L., Jentsch W., Laube W., Nehring K., Schiemann R. (2003). Rostock Feed Evaluation System. Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany.

Cordell D., Rosemarin A., Schroder J.J., Smit A.L. (2011). Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options. Chemosphere, 84: 747–758.

Czarnogorski M., Woda C.B., Schulkin J., Mulroney S.E. (2004). Induction of a phosphate appetite in adult male and female rats. Exp. Biol. Med., 229: 914–919.

Eklou-Kalonji E., Zerath E., Colin C., Lacroix C., Holy X., Denis I., Pointillart A. (1999). Calcium-regulating hormones, bone mineral content, breaking load and trabecular remodeling are altered in growing pigs fed calcium-deficient diets. J. Nutri., 129: 188–193.

Fammatre C.A., Mahan D.C., Fetter A.W., Grifo A.P., Judy J.K. (1977). Effects of dietary protein, calcium and phosphorus levels for growing and finishing swine. J. Anim. Sci., 44: 65–71.

Fandrejewski H., Weremko D., Raj S., Skiba G, Han I.K. (1999). Performance, body and carcass composition and bone characteristics of pigs fed rapeseed and soybean meal-cereal diets supplemented with microbial phytase. J. Anim. Feed Sci., 8: 533–547.

Fernández J.A. (1995 a). Calcium and phosphorus metabolism in growing pigs. I. Absorption and balance studies. Livest. Prod. Sci., 41: 233–241

Fernández J.A. (1995 b). Calcium and phosphorus metabolism in growing pigs. II. Simultaneous radio-calcium and radio-phosphorus kinetics. Livest. Prod. Sci., 41: 243–254.

Fernández J.A. (1995 c). Calcium and phosphorus metabolism in growing pigs. III. A model resolution. Livest. Prod. Sci., 41: 255–261.

Gonzalo E., Létourneau-Montminy M.P., Narcy A., Bernier J.F., Pomar C. (2018). Consequences of dietary calcium and phosphorus depletion and repletion feeding sequences on growth performance and body composition of growing pigs. Animal, 12: 1165–1173.

Gutierrez N.A., Serão N.V.L., Elsbernd A.J., Hansen S.L., Walk C.L., Bedford M.R., Patience J.F. (2015). Quantitative relationships between standardized total tract digestible phosphorus and total calcium intakes and their retention and excretion in growing pigs fed corn–soybean meal diets. J. Anim. Sci., 93: 2174–2182.

Harper A.F., Kornegay E.T., Schell T.C. (1997). Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci., 75: 3174–3186.

Hastad C.W., Dritz S.S., Tokach M.D., Goodband R.D., Nelssen J.L., DeRouchey J.M., Boyd R.D., Johnston M.E. (2004). Phosphorus requirements of growing-finishing pigs reared in a commercial environment. J. Anim. Sci., 82: 2945–2952.

Huttunen M.M., Tillman I., Viljakainen H.T., Tuukkanen J., Peng Z., Pekkinen M., Lamberg-Allardt C.J.E. (2007). High dietary phosphate intake reduces bone strength in the growing rat skeleton. J. Bone Mineral Res., 22: 83–92.

Kotarbińska M. (1969). Badania nad przemianą energii u rosnących świń (in Polish). Instytut Zootechniki, Wrocław, pp. 1–67.

Kyriazakis I. (2008). A review of our current understanding of phosphorus requirement of pigs. Kenilworth: BPEX. https://pork.ahdb.org.uk/media/2135/RIA-1-Phosphorus-req.pdf.

Létourneau-Montminy M.P., Narcy A., Dourmad J.Y., Crenshaw T.D., Pomar C. (2015). Modelling the metabolic fate of dietary phosphorus and calcium and the dynamics of body ash content in growing pigs. J. Anim. Sci., 93: 1200–1217.

Liesegang A., Ursprung R., Gasser J., Sassi M.L., Rristeli J., Riond J.L., Wanner M.J. (2002). Influence of dietary phosphorus deficiency with or without addition of fumaric acid to a diet in pigs on bone parameters. Anim. Physiol. Anim. Nutr., 86: 1–16.

Lüdke H., Schöne F., Geinitz D., Brys J. (1999). Untersuchungen zur Phosphorversorgung von Schweinen im Lebendmasseabschnitt 11–38 kg. Archiv. Anim. Nutr., 11/12: 1085–1095.

Mahan D.C., Shields Jr R.G. (1998). Macro- and micromineral composition of pigs from birth to 145 kilograms of body weight. J. Anim. Sci., 76: 506–512.

Misiura M.M., Filipe J.A.N., Walk C.L., Kyriazakis I. (2020). How do pigs deal with dietary phosphorus deficiency? Brit. J. Nutr., 124: 256–272.

Narcy A., Rousseau X., Même N., Magnin M., Nys Y. (2015). Impact of dietary phosphorus and calcium levels on their deposition in soft and bone tissues in chickens. Proc. 20th European Symposium of Poultry Nutrition, 24–27.08.2015, Prague, Czech Rep., pp. 217.

Nicodemo M.L., Scott D., Buchan W., Duncan A., Robins S.P. (1998). Effects of variations in dietary calcium and phosphorus supply on plasma and bone osteocalcin concentrations and bone mineralization in growing pigs. Physiol. Soc., https://doi.org/10.1113/expphysiol.1998.sp004147.

Nieto S., Kiefer C., de Souza K.M.R., Gonçalves L.M.P., Bonin M.N., Santos T.M.B., Carvalho K.C.N., Santos A.P. (2016). Digestible phosphorus levels for barrows from 50 to 80 kg. R. Bras. Zootec., 45: 242–249.

NRC (2012). Nutrient requirements of swine. 11th ed. National Academy Press, Washington, DC.

O'Quinn P.R., Knabe D.A., Gregg E.J. (1997 a). Digestible phosphorus needs of terminal-cross growing-finishing pigs. J. Anim. Sci., 75: 1308–1318.

O'Quinn P.R., Knabe D.A., Gregg E.J., Lusas E.W. (1997 b). Nutritional value for swine of soybean meal produced by isopropyl alcohol extraction. J. Anim. Sci., 75: 714–719.

Oster M., Gerlinger C., Heide K., Just F., Borgelt L., Wolf P., Polley C., Vollmar B., Muráni E., Ponsuksili S., Wimmers K. (2018). Lower dietary phosphorus supply in pigs match both animal welfare aspects and resource efficiency. Ambio, 47(suppl. 1): S20–S29.

Pettey L.A., Cromwell G.L., Jang Y.D., Lindemann M.D. (2015). Estimation of calcium and phosphorus content in growing and finishing pigs: Whole empty body components and relative accretion rates. J. Anim. Sci., 93: 158–167.

Pierre J.M. (2008). Transcription factors controlling osteoblastogenesis. Arch. Biochem. Biophys., 473: 98–105.

Pond W.G., Walker E.F., Kirtland Jr D. (1978). Effect of dietary Ca and P levels from 40 to 100 kg body weight on weight gain and bone and soft tissue mineral concentrations. J. Anim. Sci., 46: 686–691.

Poulsen H.D. (1994). Reduced dietary phosphorus for growing and finishing pigs: effects on performance, retention and excretion. Forskningsrapport. Statens Husdyrbrugsforsoeg (Denmark), pp. 29.

Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims.

Reinhart G.A., Mahan D.C. (1986). Effect of various calcium : phosphorus ratios at low and high dietary phosphorus for starter, grower and finishing swine. J. Anim. Sci., 63: 457–466.

Różycki M. (1996). Rules at evaluating the pigs in Pig Slaughter Testing Station. State of pig breeding and pig evaluation results (in Polish). National Research Institute of Animal Production, Kraków, pp. 69–82.

Ruan Z., Zhang Y.G., Yin Y.L., Li T.J., Huang R.L., Kim S.W., Wu G.Y., Deng Z.Y. (2007). Dietary requirement of true digestible phosphorus and total calcium for growing pigs. Asian-Austr. J. Anim. Sci., 20: 1236–1242.

Sands J.S., Ragland D., Baxter C., Joern B.C., Sauber T.E., Adeola O. (2001). Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J. Anim. Sci., 79: 2134–2142.

Sørensen K.U., Tauson A.H., Poulsen H.D. (2018 a). Long term differentiated phosphorus supply from below to above requirement affects nutrient balance and retention, body weight gain and bone growth in growing-finishing pigs. Livest. Sci., 211: 14–20.

Sørensen K.U., Marlena C., Kruger M.C., Hansen-Møller J., Poulsen H.D. (2018 b). Bone biochemical markers for assessment of bone responses to differentiated phosphorus supply in growing-finishing pigs. J. Anim. Sci., 96: 4693–4703.

Sørensen K.U., Shiguetomi-Medina J.M., Poulsen H.D. (2019). Mineralisation of tubular bones are affected differently by low phosphorus supply in growing-finishing pigs. J. Sci. Food Agric., 99: 3628–3634.

Suttle N.F. (2010). Mineral nutrition of livestock, 4th edition. Suttle N.F. (ed.) Wallingford, CABI, http://www.cabi.org/cabebooks/ebook/20103291114.

Sweeny J.M., Seibert H.E., Woda C., Schulkin J., Haramati A., Mulroney S.E. (1998). Evidence for induction of a phosphate appetite in juvenile rats. Am. J. Physiol. Regul. Integr. Comp. Physiol., 275: R1358–R1365.

Swelum A.A., Saadeldin I.M., Alowaimer A.N., Mahmoud A., Abouheif M.A. (2017). Impacts of restricted feeding and realimentation on bone development and plasma concentrations of bone-specific biomarkers in lambs. J. Anim. Feed Sci., 26: 116–122.

Symeou V., Leinonen I., Kyriazakis I. (2014). Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pigs: model description. Animal, 8: 1612–1621.

Valable A.S., Narcy A., Duclos M.J., Pomar C., Page G., Nasir Z., Magnin M., Létourneau-Montminy M.P. (2018). Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. Animal, 12: 1555–1563.

Varley P.F., Sweeney T., Ryan M.T., O'Doherty J.V. (2011). The effect of phosphorus restriction during the weaner-grower phase on compensatory growth, serum osteocalcin and bone mineralization in gilts. Livest. Sci., 135: 282–288.

Veum T.L., Ellersieck M.R. (2008). Effect of low doses of *Aspergillus niger* phytase on growth performance, bone strength, and nutrient absorption and excretion by growing and finishing swine fed corn-soybean meal diets deficient in available phosphorus and calcium. J. Anim. Sci., 86: 858–870.

Vier C.M., Dritz S.S., Wu F., Tokach M.D., de Rouchey J.M., Goodband R.D., Gonclaves M.A.D., Orland U.A.D., Chitakasempornkul K., Woodworth J.C. (2019). Standardized total tract digestible phosphorus requirement of 24 to 130 kg pigs. J. Anim. Sci., 97: 4023–4031.

Vipperman Jr P.E., Peo Jr E.R., Cunningham P.J. (1974). Effect of dietary calcium and phosphorus level upon calcium, phosphorus and nitrogen balance in swine. J. Anim. Sci., 38: 758–765.

Wagner J.R., Schinckel A.P., Chen W., Forrest J.C., Coe B.L. (1999). Analysis of body composition changes of swine during growth and development. J. Anim. Sci., 77: 1442–1466.

Yan F., Angel R., Ashwell C., Mitchell A., Christman M. (2005). Evaluation of the broiler's ability to adapt to an early moderate deficiency of phosphorus and calcium. Poultry Sci., 84: 1232–1241.

Yang X.E., Wu X., Hao H.L., He Z.L. (2008). Mechanisms and assessment of water eutrophication. J. Zhejiang Univ., 9: 197–209.

Received: 27 V 2022
Accepted: 21 VIII 2022