

Effect of Various Feed Phosphates on Biochemical Indices of Blood and Mineral Composition of Bones in Finishing Pigs

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Abstract

The aim of this study was to evaluate the effect of three different chemical feed phosphates on the blood biochemical indicators and the content of main minerals of bones in finishing pigs.

Over a period of 85 days of fattening, monocalcium (MCP, Finnish product), dicalcium (DCP, Polish product) and calcium-sodium (CSP, Russian product) phosphates were used in fattener feeding. The feeding was based on standard mixtures of starter, grower and finisher type. Dicalcium phosphate was produced according to the new, pro-ecological technology based on phosphoric acid. The content of Ca, Na, P, solubility of P in citric acid, and the concentration of undesirable substances (As, Cd, F, Hg and Pb) were determined in feed phosphates.

At the end of the fattening period, blood was collected from 36 finishing pigs (12 from each group) and the following biochemical indicators were determined in the serum: enzymatic activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase (GGT), creatine kinase (CK), lactic dehydrogenase (LDH), lactic acid (LA); the concentration of total protein, albumins, glucose, urea, creatinine, content of triglycerides, cholesterol and its high density lipoproteins (HDL) and low density lipoproteins (LDL) fractions, and mineral components concentration (Ca, Cl, Cu, Fe, K, Mg, Na, P, Zn). Basic macroelement content (Ca, Mg, P) was determined in the thigh bones from 30 pigs (10 from each group). Significant differences ($p < 0.05$) between groups were observed only in some biochemical indicators, i.e. CK, LDH and LA. The highest content of Ca, Mg and P was found in the bones of pigs fed mixtures supplemented with DCP which indicates improved bioavailability of main macroelements from that phosphate.

Feed supplements, fatteners, blood serum, bone composition

Cereal grain, i.e. the main source of phosphorus, is the basic fodder in pig feeding. In cereal grain, phosphorus is present in the form of sparingly available phytates. The introduction of formulation containing an exogenous microbiological phytase to feeding mixtures is a common way of improving phosphorus availability from phytic compounds (Krasucki and Grela 1997; Radcliffe et al. 1999; Steiner et al. 2006). Such procedure may considerably reduce the amount of phosphorus expelled in faeces which is important in terms of environmental protection.

However, the amount of phosphorus available in feed of plant origin, even with the addition of microbiological phytase does not fulfill the demands of intensively growing pigs, and supplementation of the feeding dose with additional 30%-50% mineral phosphorus is necessary (Fandrejewski 1997).

Mono-, di-, and tricalcium, calcium-sodium, sodium-calcium-magnesium and ammonium phosphates are available on the fodder market. The production of dicalcium phosphate (DCP) using a new pro-ecological method has been recently introduced in Poland (Hoffmann et al. 2008).

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The apparent digestibility of phosphorus in these phosphates is often extremely variable, from 51 to 90%, and depends mainly on the technology of production used and the purity of the phosphoric acid employed (Eckhout and Paepe 1997; Poulsen 2007; Gołębiowska et al. 2008).

The aim of the present study was to evaluate the effect of three different feed phosphates including DCP produced using the new method, on the blood biochemical indicators and the content of the main minerals in bones of finishing pigs.

Materials and Methods

Animals and feeding

Experimental fattening was conducted at the Experimental Animal Feeding Plant in Gorzyń that belongs to the Poznan University of Life Sciences, Poland. The study was conducted on 60 piglets (originating from Large Polish White × Polish Landrace crossbred sows and Hampshire × Pietrain crossbred boars) of an average initial body mass of 20 kg. The animals were divided into 3 groups of 20 individuals each, according to the rule of analogues. The fattening lasted 85 days, i.e. until the body mass reached about 110 kg.

The division of piglets into 3 feeding groups followed from the contribution of phosphates analysed in mixtures used during the experimental fattening: group 1 (control) – mixtures with monocalcium phosphate (MCP); group 2 – mixtures with dicalcium phosphate (DCP); group 3 – mixtures with calcium-sodium phosphate (CSP).

Table 1. Nutritional values of “starter”, “grower” and “finisher” mixtures

Feed materials	Units	Mixture		
		Starter	Grower	Finisher
In 1 kg of a mixture:				
Net energy	Kcal	2340	2280	2281
Metabolic energy	MJ	13.60	13.25	13.25
Dry matter	%	87.3	87.3	87.3
Total protein	%	17.4	15.7	14.5
Lysine	%	1.17	0.93	0.85
Methionine	%	0.39	0.28	0.26
Methionine+cystine	%	0.71	0.60	0.55
Threonine	%	0.75	0.59	0.54
Tryptophan	%	0.23	0.20	0.16
Isoleucine	%	0.66	0.59	0.51
Total calcium	%	0.73	0.68	0.60
Total phosphorus	%	0.55	0.50	0.43
Mineral phosphorus ^{*)}	%	0.16	0.15	0.13
Digestible phosphorus	%	0.34	0.30	0.25
Bacterial phytase	FTU	500	510	425
Total sodium	%	0.20	0.20	0.14
Iron	mg	198	183	172
Manganese	mg	91	92	73
Copper	mg	167	25	21
Zinc	mg	157	148	126
Iodine	mg	1.66	1.49	1.26
Cobalt	mg	0.88	0.81	0.68
Selenium	mg	0.49	0.49	0.41

^{*)} Mineral phosphorus in equal amount derived from monocalcium (Group I, MCP), dicalcium (Group II, DCP) or calcium-sodium phosphate (Group III, CSP)

F, Hg and Pb. The analyses were conducted in a specialist chemical laboratory at the Institute of Inorganic Technology and Mineral Fertilizers in Wrocław University of Technology using mandatory chemical methods (AOAC 1990).

The contribution of particular feed phosphates resulted from the optimisation of P content in the mixtures. It was assumed that mineral phosphorus makes up 30% of the demand for total phosphorus. The contribution of phosphorus in the three mixtures used was as follows: MCP 0.57 – 0.73%, DCP 0.70 – 0.89%, and CSP 0.72 – 0.92%. The nutritional value of starter, grower and finisher mixtures, with mineral components, is presented in Table 1.

Feed phosphates

Three kinds of phosphates were used in the experiment: monocalcium (MCP, Finnish product), calcium-sodium (CSP, Russian product) and dicalcium (DCP, Polish product). Dicalcium phosphorus was produced from calcium oxide and calcium carbonate, using concentrated phosphoric acid as the phosphorus source. The product obtained in a direct chemical reaction between phosphoric acid and calcium was a phosphoric acid salt with the following chemical formula: $\text{CaHPO}_4 \times 2\text{H}_2\text{O}$. The technology applied was non-scrap and non-sewage autothermal technology using the heat of the reaction to dry the product. Because of the lack of direct drying with gases, the final product thus obtained did not contain any harmful organic substances (Hoffmann and Hoffmann 2009).

Within the chemical assessment of phosphates, the following were determined: the content of Ca, total P, P soluble in 2% citric acid, and the content of undesirable substances such as As, Cd,

Blood analyses

Blood for laboratory analysis was collected once from 36 pigs (12 from each group) at the end of the fattening period, i.e. day 85 of life, with a body mass of about 110 kg. The blood was collected in the morning, before feeding, from the zygomatic vein, into test tubes containing agents activating the coagulation process (Sigma, Poland). The blood samples were delivered within 2 h from collection to the biochemical laboratory of the Department of Environment Hygiene and Animal Welfare of Wrocław University of Environmental and Life Sciences, where serum was separated and the following biochemical indicators were determined: enzymatic activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase (GGT), creatine kinase (CK), concentration of total protein, albumin, glucose, urea, creatinine, triglycerides, cholesterol and its HDL and LDL fractions, and the concentration of mineral components – Ca, Cl, Cu, Fe, K, Mg, Na, P, Zn.

All analyses were done using a Pentra 400 biochemical analyser manufactured by Horiba ABX (Japan), with reagents from the same company. An exception was the determination of γ -glutamyltransferase concentration which was carried out using reagents produced by Alpha Diagnostics (Poland), and zinc and copper concentration analysis were carried out with Randox (UK) reagents. The concentrations of sodium, chlorides and potassium were conducted via the ionoselective method using the ionoselective attachment integrated with a Horiba ABX Pentra 400 analyser.

Activity of AST, ALT, CK and γ -glutamyltransferase was determined at a temperature of 37 °C, using the kinetic method according to IFCC (International Federation of Clinical Chemistry) recommendations.

The concentration of total protein, albumin, glucose, creatinine, triglycerides, cholesterol and its fractions was determined by colorimetric tests. In the case of total protein it was a burette reaction, while in the case of albumin concentration, the method using bromocresol green was used. Glucose concentration was determined using an enzymatic method with oxidase reaction. Urea concentration was assessed using a UV enzymatic test, with the contribution of urease and glutamate dehydrogenase, while creatinine was analysed using a modified kinetic method.

Lactic acid (LA) concentration was determined using the enzymatic colorimetric method applying the Trinder's end-point method, where the lactate is an intermediate product of glucose combustion. Lactic oxidase causes the release of hydrogen peroxide that reacts with the two compounds creating a coloured complex in the presence of peroxidase. The colour intensity is proportional to lactate concentration in the sample.

Lactic dehydrogenase (LDH) content was determined using the enzymatic kinetic method optimized in accordance with recommendations of the German Society of Clinical Chemistry (DGKC). LDH activity was determined in a reaction of pyruvate with hydrogen ions and NADH where lactate and NAD⁺ dinucleotide were formed with the contribution of LDH.

Globulin concentration was determined according to the following formula: globulins [g/l] = total protein [g/l] – albumins [g/l]. Cholesterol and triglyceride concentration was determined using the enzymatic colorimetric method. HDL fraction was assessed using cholesterol oxidase, and LDL fraction by enzymatic reaction in the presence of a coupling agent.

The content of inorganic phosphorus was determined using the UV-VIS method with the application of phosphomolybdate. Calcium was determined using the photometric method with the application of ortho-cresolphthalein complexone. Magnesium was determined using the photometric method with xylytol blue. The iron content was determined using the photometric test with pherene; zinc and copper by colorimetric tests.

The concentration of sodium, chlorides and potassium was determined using biochemical analyser Pentra 400 of Horiba ABX Company with ISE module, where the three ionoselective electrodes were used (for Cl, K and Na, respectively) and one reference electrode as well.

Chemical composition of bones

The determination of basic macroelement (Ca, P, Mg) content in the thigh bones of pigs was conducted at the Food and Environment Analysis Laboratory of the Meat and Fat Research Institute in Warsaw (Poland). Thirty thigh bones from the right half-carcass (10 from each group) were subjected to analysis. Ring shaped samples of a thickness of 3 mm were collected from each bone. They were cut with a saw from the narrowest part of the thigh bone. Soft parts were removed mechanically using a knife and hot water, and then air-dried. Double weighted amounts of a mass of 0.5–0.6 g each were prepared. In order to determine the content of Mg, P and Ca, the following dilutions were made: $\times 50$; $\times 500$; $\times 1000$. Samples subjected to analysis were mineralized using concentrated nitric acid in a microwave furnace (Milestone of 1200 Mega type). Metals were determined by inductively coupled plasma atomic emission spectroscopy (JY- 138-ULTRACE, Jobin Yvon, France).

Statistical analysis

The results of the study were statistically processed. Mean values and standard deviations were calculated using one-factor analysis of variance, and significance of differences between groups was determined by Duncan's test using Statgraphics v. 5.0 software.

Results and Discussion

The basic nutrient contents found in 1 kg of starter, grower and finisher mixtures used in pigs fattening are presented in Table 1. The content of metabolic energy of amino acid

Table 2. The results of the chemical analysis of feed phosphates (in 1 kg of DM)

Specification	Monocalcium phosphate (MCP) Ca (H ₂ PO ₄)	Dicalcium phosphate (DCP) Ca HPO ₄ x2H ₂ O	Calcium-sodium phosphate (CSP) Na ₂ Ca 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)	-	-	49
Undesirable substances:			
Fluorine (F) mg	450	350	800
Lead (Pb) mg	5.2	1.4	4.8
Cadmium (Cd) mg	5.0	2.3	0.5
Arsenic (As) mg	8.1	0.7	2.4
Mercury (Hg) mg	0.02	0.008	0.02

protein and mineral components of these mixtures correspond to recommendations included in the standards for pig feeding (Standards of Pigs Feeding 1993).

The results of the chemical analysis of the phosphates used are presented in Table 2 and prove that the phosphorus content was the highest in MCP (227 g/kg), and calcium content was the highest in CSP (310 g/kg). The solubility of phosphorus in 2% citric acid was very high (98-99%). Phosphates also contained fluorine and toxic metals (As, Cd, Hg, Pb) but in amounts acceptable for feed within the EU. The values obtained are comparable or lower from the values given by another authors (Wzorek 2006; Poulsen 2007).

Table 3 presents the results concerning the analysis of biochemical indicators of pigs' blood serum. The concentrations of total protein (64.0–65.6 g/l), albumin (39.4–41.8 g/l) and globulin (23.8–24.9 g/l) were similar in all groups and corresponded to reference values (Winnicka 2008). No significant differences were noted in glucose concentration which ranged from 5.10 mmol/l in group 3 to 5.23 mmol/l in group 2. Mean concentration values of lactic acid which is the final product of oxygen-free glucose combustion differed significantly between 4.49 µmol/l in group 1 and 4.97 µmol/l in group 3 ($p < 0.05$).

The activity of ALT in blood serum was different, ranging from 0.553 to 0.638 µkat/l, however, the differences were not significant. The observed activity of AST was similar in groups 2 and 3; a slight increase of its activity was observed in group 1, i.e. 1.053 µkat/l, a value close to the upper limit of reference values for this indicator in pigs (Winnicka 2008).

The activity of γ -glutamyltransferase in the serum of pigs from all groups was similar (0.548- 0.578 µkat/l), and exceeded reference values. Analysing the activity of lactic dehydrogenase, significant differences ($p < 0.05$) were found between groups 1 and 3. In the group with MCP addition, the activity of LDH was the highest – 15.08 µkat/l on average.

Differences were observed between groups 2 and 3 in the activity of creatine kinase, a key enzyme in the bioenergetic processes of cells with an important role in adenosine triphosphate (ATP) homeostasis. The CK value in the latest group was the highest, i.e. 28.83 µkat/l on average.

Average concentrations of urea and ceratinine, i.e. indicators reflecting kidney function, were similar in all the groups. A slightly higher urea concentration compared to other groups was observed in group 1 (5.71 mmol/l); similar concentrations of creatinine were found in groups 1 and 3, and the lowest one in group 2 (114.1 µmol/l). Values for GGT, LDH,

Table 3. Biochemical indices of blood serum of fatteners

Specification	Groups - phosphate		
	I Monocalcium	II Dicalcium	III Calcium-sodium
Total protein (g/l)	65.6 ± 6.5	64.0 ± 4.7	65.5 ± 6.7
Albumins (g/l)	41.8 ± 3.8	39.4 ± 4.9	41.7 ± 1.7
Globulins (g/l)	23.8 ± 6.1	24.6 ± 4.2	23.9 ± 7.6
Glucose (mmol/l)	5.13 ± 0.86	5.23 ± 0.71	5.10 ± 0.61
LA (µmol/l)	4.49 ^a ± 2.00	2.40 ± 0.49	4.97 ^b ± 2.23
ALT (µkat/l)	0.553 ± 0.199	0.638 ± 0.181	0.608 ± 0.209
AST (µkat/l)	1.053 ± 0.177	0.984 ± 0.192	0.982 ± 0.268
GGT (µkat/l)	0.578 ± 0.147	0.572 ± 0.112	0.548 ± 0.131
LDH (µkat/l)	15.08 ^a ± 2.57	14.78 ± 2.86	13.90 ^b ± 2.64
CK (µkat/l)	24.75 ± 20.86	28.83 ± 31.87	20.46 ± 15.40
Urea (mmol/l)	5.71 ± 0.75	5.22 ± 0.79	5.28 ± 0.68
Creatinine (µmol/l)	119.6 ± 13.0	114.1 ± 13.1	119.9 ± 8.6
Total cholesterol (mmol/l)	2.40 ± 0.29	2.40 ± 0.49	2.30 ± 0.16
HDL (mmol/l)	1.12 ± 0.18	1.08 ± 0.26	1.06 ± 0.11
LDL (mmol/l)	1.05 ± 0.14	1.08 ± 0.24	1.03 ± 0.14
Triglycerides TG (mmol/l)	0.22 ± 0.06	0.23 ± 0.08	0.25 ± 0.06

Superscripts a, b: $p < 0.05$

fraction (1.06–1.12 mmol/l). The LDL fraction of cholesterol was also similar in all groups (1.03–1.08 mmol/l). Triglyceride content in the blood serum of examined pigs was very similar, 0.22–0.25 mmol/l, i.e. half the reference range, on average (Winnicka 2008).

The results of a biochemical study are difficult to interpret unequivocally, since metabolic processes are extraordinarily complex and affected by numerous factors. Dietary protein and fats may have some influence on the results (Usydus 2005; Zraly et al. 2006), and also various inorganic and organic feed components may change the values of blood indicators (Korniewicz et al. 2007; Wang et al. 2009).

Average concentrations of mineral components in the blood serum of pigs are presented in Table 4. The results obtained prove that phosphates administered in the pigs' feed did not significantly influence the concentration of analysed macro- and microelements.

The concentration of calcium was within the range of 2.5–2.6 mmol/l, and phosphorus 3.0–3.1 mmol/l. These values were close to the upper limit of reference values given by Winnicka (2008). Magnesium concentration in the blood serum of pigs was the same in

Table 4. The content of mineral components in blood serum of fatteners

Specification	Group - phosphate		
	I Monocalcium	II Dicalcium	III Calcium-sodium
Calcium (Ca) mmol/l	2.6 ± 0.1	2.5 ± 0.2	2.6 ± 0.1
Phosphorus (P) mmol/l	3.0 ± 0.1	3.1 ± 0.4	3.1 ± 0.2
Sodium (Na) mmol/l	141.7 ± 1.3	141.4 ± 1.5	141.6 ± 1.4
Chlorine (Cl) mmol/l	102.0 ± 2.6	101.6 ± 2.3	101.1 ± 1.9
Potassium (K) mmol/l	5.1 ± 0.6	5.1 ± 0.5	5.1 ± 0.4
Magnesium (Mg) mmol/l	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Iron (Fe) µmol/l	25.8 ± 5.5	24.4 ± 5.1	24.3 ± 5.9
Zinc (Zn) µmol/l	10.3 ± 2.6	9.4 ± 1.2	9.8 ± 1.5
Copper (Cu) µmol/l	38.5 ± 4.7	38.5 ± 4.3	38.2 ± 4.4

CK, urea and creatinine concentration were within or slightly exceeded the range of reference values (Winnicka 2008).

Fat balance in fatteners was assessed, determining the content of triglycerides, total cholesterol, and its LDL and HDL fractions. No significant differences between groups were observed for all of these indicators. The average value of total cholesterol was 2.3–2.4 mmol/l, which exceeded the reference value (Winnicka 2008). Small differences were observed in the HDL

fraction (1.06–1.12 mmol/l). The LDL fraction of cholesterol was also similar in all groups (1.03–1.08 mmol/l). Triglyceride content in the blood serum of examined pigs was very similar, 0.22–0.25 mmol/l, i.e. half the reference range, on average (Winnicka 2008). The results of a biochemical study are difficult to interpret unequivocally, since metabolic processes are extraordinarily complex and affected by numerous factors. Dietary protein and fats may have some influence on the results (Usydus 2005; Zraly et al. 2006), and also various inorganic and organic feed components may change the values of blood indicators (Korniewicz et al. 2007; Wang et al. 2009). Average concentrations of mineral components in the blood serum of pigs are presented in Table 4. The results obtained prove that phosphates administered in the pigs' feed did not significantly influence the concentration of analysed macro- and microelements. The concentration of calcium was within the range of 2.5–2.6 mmol/l, and phosphorus 3.0–3.1 mmol/l. These values were close to the upper limit of reference values given by Winnicka (2008). Magnesium concentration in the blood serum of pigs was the same in all groups (0.9 mmol/l), and the sodium content was 141.4–141.7 mmol/l. The content of chlorine and potassium was similar in all groups. Also the concentration of iron was similar in all groups (24.3–25.8 µmol/l), similarly as zinc (9.4–10.3 µmol/l) and copper (38.2–38.5 µmol/l). The lack of differences between groups may

Table 5. The content of mineral components in thigh bones (g/kg)

Component	Group - phosphate		
	I Monocalcium	II Dicalcium	III Calcium-sodium
Calcium (Ca)	250.6 ^b ± 7.5	262.1 ^a ± 9.1	253.0 ^b ± 5.4
Phosphorus (P)	118.6 ^b ± 3.5	142.2 ^a ± 2.4	122.2 ± 1.9
Magnesium (Mg)	3.85 ^b ± 0.17	4.24 ^a ± 0.24	4.16 ± 0.14

Superscripts a, b: $p < 0.05$

dicalcium phosphate retained significantly higher amounts of Ca, Cu, Mg, Mn and P.

It is known that organic forms of microelements are more easily available. Novotný et al. (2005) applied Fe, Cu, Mn, Zn and Se in a traditional or organic form to feed for piglets. They observed a significant effect of the organic form on the increase in concentrations of these microelements: Fe from 14.7 to 18.3, Cu from 17.8 to 22.7, Zn from 8.5 to 9.5, Mn from 0.4 to 0.6 $\mu\text{mol/l}$. Czech and Grela (2006) after administering mineral chelates in feed for fatteners observed a significant increase in the content of Cu, Mn and Zn in blood serum compared to the control group. Also, other authors noted the effect of various forms of iron or zinc on the concentration of some blood indicators in pigs (Svoboda 2004; Rekiel and Więcek 2005).

The results of the chemical analysis of thigh bones concerning Ca, P and Mg content are presented in Table 5. Calcium concentration in group 2 (DCP) was significantly higher (262.1 g/kg) compared to groups 1 and 3. DCP used in feed mixtures influenced the increase in the phosphorus content (124.2 g/kg), and the difference with respect to group 1 was significant. Magnesium content in the thigh bones of pigs from group 2 was also significantly higher (4.24 g/kg) than in pigs from group 1 (3.85 g/kg).

Similar concentrations of mineral components in thigh bones of sows were observed by Gajewczyk (1983). According to that author, calcium constituted 23%, phosphorus 15% and magnesium 0.55% of the dry matter of bone. When analysing the chemical composition of metacarpal, metatarsal and thigh bones of sows, Fuchs et al. (1993) demonstrated that Ca, P and Mg concentrations in the dry matter were the same in spite of administration of various feed phosphates. Slightly different data for the range of Ca and P accretion and resorption in pigs of a body mass of 65 kg are given by Fernandez (1995). The author observed that an accretion relative to bone ash declined with live weight, and conversely, daily accretion increased with live weight due to larger bone mass. Bone resorption of Ca and P was strongly and negatively related to mineral intake and accounted nearly exclusively for the increases in dietary intake and intestinal absorption.

Generally, the three different phosphates used in the experiment did not significantly influence the differentiation of biochemical indicators of the blood of pigs (except for CK, LDH and LA). The highest concentrations of Ca, P and Mg in the thigh bones of pigs in the group given DCP may prove better bioavailability of those building elements in pigs, which is of major importance in fast-growing animals.

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be explained by similar chemical composition of the phosphates administered; however, in the study conducted by Korniewicz et al. (2010) it was demonstrated that pigs fed mixtures with the addition of a new

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