



# The quality of different mono- and dicalcium phosphates estimated on the basis of their crystalline phases, chemical composition, solubility, and biological parameters of broiler chickens

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**ABSTRACT.** The quality of five commercial monocalcium phosphates (MCP) and dicalcium phosphates with natural admixtures of hydrated and dehydrated forms was assessed on the basis of their crystalline phases determined by roentgenographic irradiation, Ca and P contents, and solubility in water, 2% citric acid, 0.4% HCl, and ammonium citrate solutions. The phosphates were used in diets fed between days 1 and 35 of life to 300 Hubbard Flex male broilers, 6 replications (cages) of 10 chickens per treatment. Performance indices, Ca, P and Mg retention, and the concentration of these elements in blood and bone ash, as well as the physical parameters of femur and tibia bones were measured. The content of P in phosphates varied between 17.7% and 23%, their solubility in citric acid and HCl solutions ranged between 89–99%. The roentgenograms indicated that phosphate No. 1 contained pure MCP; No. 2, MCP with admixture of anhydrous dicalcium phosphate (DCP); No. 3, DCP with an admixture of MCP; No. 4, dicalcium phosphate dehydrate (DDCP); No. 5, DDCP with an admixture of DCP. The type of phosphate used in chicken diets did not influence body weight or feed intake. Phosphorous retention, Ca and P in serum, and some bone parameters were better in chickens fed the diet containing pure MCP ( $P < 0.01$ ). Lower concentrations of Ca and P in bones and worse parameters of bone elasticity were found in chickens fed diets containing DDCP with DCP. In the biological experiment, the overall best results were obtained in chickens fed diets containing pure hydrated monocalcium phosphate.

## Introduction

The low availability of native phosphorus from the phytates present in natural feeds, especially in grains (the main components of poultry diets), makes it necessary to add microbial phytases to diets (Leytem et al., 2008). Nonetheless, the

supplementation of phosphates, inorganic phosphorus sources, in broiler diets is still indispensable. The biological value of commercial phosphates depends on their natural origin (Fernandes et al., 1999; Gödöy and Chicco, 2001; Weiner et al., 2001; Rama Rao and Rammasubba Reddy, 2003), modifications during

their production process, chemical purity, and other factors (Ravindran et al., 1995; Wzorek and Kowalski, 1995; De Groote and Huyghebaert, 1997; Jamroz et al., 2001, 2010).

In our earlier extensive studies, 341 different production batches of mono-, di-, tri-, and Na and Mg phosphates were analysed. Their chemical composition, crystallography and physical characteristics, as well as solubility in four solvents were determined; the obtained results showed a great variability in their chemical quality (Gajda-Janiak et al., 2005; Jamroz et al., 2010, 2012).

In biological investigations performed on broiler chickens (Jamroz et al., 2012), five hydrated monocalcium phosphates (MCP) with natural admixtures of hydrated and dehydrated forms, differing in crystalline structure and chemical purity were examined. It was concluded that the type of phosphate used had a small effect on both performance indices and some mechanical parameters of bone quality. Significant differences were found, however, in Ca and P concentrations and activity of serum alkaline phosphatase. The small differences in the solubility of the MCP used, especially in citric acid, HCl, or ammonium citrate (but not in water) and its crystalline structure led to a significant diversification of Ca- and P- contents in blood and bones (Gajda-Janiak et al., 2005; Jamroz et al., 2010, 2012).

Monophosphates are characterized by different solubility, chemical composition, and purity as determined using the roentgenographic technique. Therefore, the objective of the investigations carried in this cycle of studies was to evaluate the quality of mono- and dicalcium phosphates with natural admixtures of hydrated and dehydrated forms based on the response of broiler chickens to rations containing them. Performance, retention of Ca, P, Mg, concentration of minerals in blood and in bones, and physical parameters of bone quality were considered as the indices of phosphate quality. The presented studies are a continuation of the investigations cited above.

## Material and methods

In the present study, commercial monocalcium phosphates (MCP) and dicalcium phosphates (DCP) with natural admixtures of hydrated and dehydrated forms were used (Table 1), selected from 341 different phosphates according to the criterion of frequency of similar P and Ca contents (Gajda-Janiak et al., 2005).

**Table 1.** Characteristics of commercial phosphates on the basis of crystalline phases determination

No. Treat-ments	Kind of phosphates used in the diets	Formula	Abbreviation
1	I	monocalcium phosphate monohydrate	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \times \text{H}_2\text{O}$ MCP
2	II	monocalcium phosphate monohydrate with admixture of anhydrous dicalcium phosphate	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \times \text{H}_2\text{O}$ $\text{CaHPO}_4$ MCP+ DCP
3	III	anhydrous dicalcium phosphate with admixture of monocalcium phosphate monohydrate	$\text{CaHPO}_4$ + $\text{Ca}(\text{H}_2\text{PO}_4)_2 \times \text{H}_2\text{O}$ DCP + MCP
4	IV	dicalcium phosphate dehydrate	$\text{CaHPO}_4 \times 2\text{H}_2\text{O}$ DDCP
5	V	dicalcium phosphate dihydrate with admixture of anhydrous dicalcium phosphate	$\text{CaHPO}_4 \times 2\text{H}_2\text{O}$ + $\text{CaHPO}_4$ DDCP + DCP

MCP – monocalcium phosphates; DCP – dicalcium phosphate; DDCP – dicalcium phosphate dehydrate

## Physical and chemical analytical methods of estimation of phosphate characteristics

The crystalline phases of phosphates were evaluated using X-ray irradiation, diffraction, and interference of waves on the crystalline walls using a Philips X Pert diffractometer combined with a graphite monochromator, PW 1752/00 with a Cu K $\alpha$  radiation at range of 2°–10°–60°. The roentgenographic images allow identification of the crystalline phases of the main phosphates and natural admixtures (Table 1, Figures 1–5).

The chemical composition of feed phosphates was assayed according to the method described by the European Chemical Industry Council (CEFIC), Brussels, Inorganic Feed Phosphates Quality. Total phosphorus content, expressed as P<sub>2</sub>O<sub>5</sub> (Regulation 2003/2003/EC, method 3.2) was determined by a gravimetric method using quinolone phosphomolybdate (dissolving the samples in a mixture of hydrochloric acid (HCl; 1.19 M · l<sup>-1</sup>) and nitric acid (HNO<sub>3</sub>; 1.4 M · l<sup>-1</sup>) 1:3 v:v at the boiling point). Calcium in phosphates was determined by complexometry after dissolution in nitric acid using disodium versenate (EDTA; 2.02 M · l<sup>-1</sup>) and the indicators, fluorexone with thymolphthalein. The solubility of phosphorus from inorganic phosphates was tested according to the methods recommended by the European Chemical Industry Council (Environmental Protection Agency, 2003). The following features were examined: solubility in water and solubility in 2% citric acid (Regulation 2003/2003/EC, method 3.1.3; temp. 20°C, 35–40 min, P content was determined by a gravimetric method using quinolone phosphomolybdate); solubility in 0.4% HCl; solubility in ammonium citrate pH 7 (Regulation 2003/2003/EC, method 3.1.5; temp. 65°C,

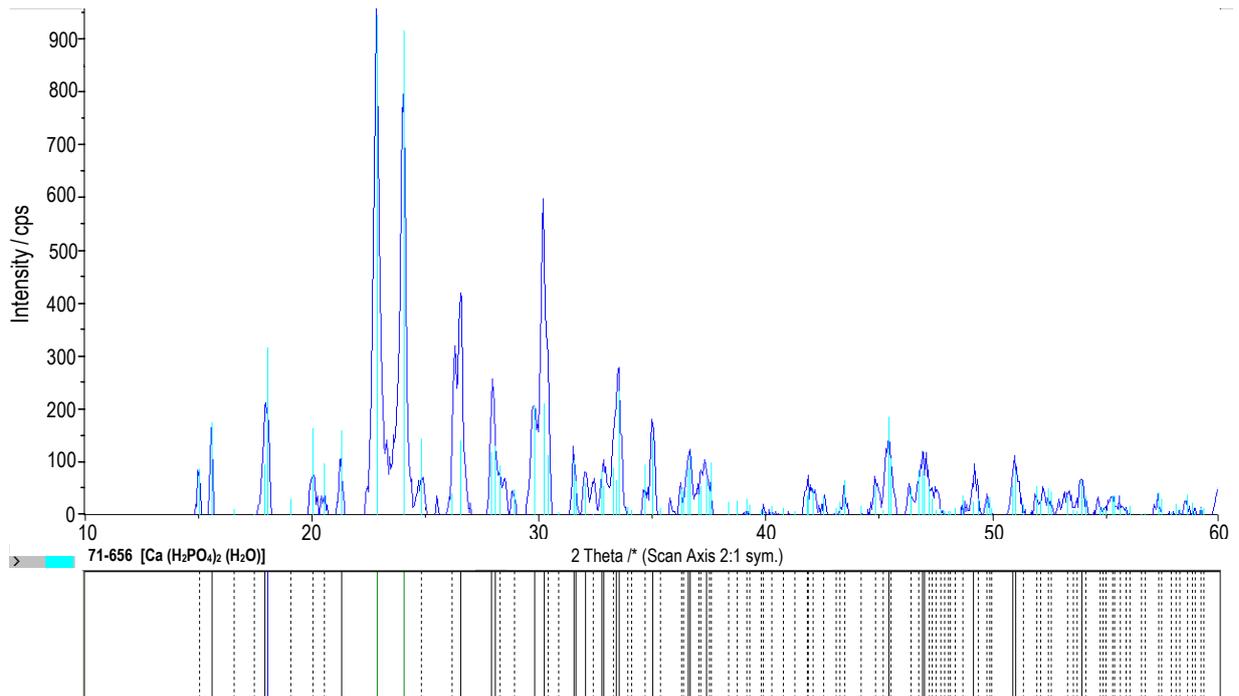


Figure 1. Roentgenogram of phosphate No. 1 with main phase of MCP

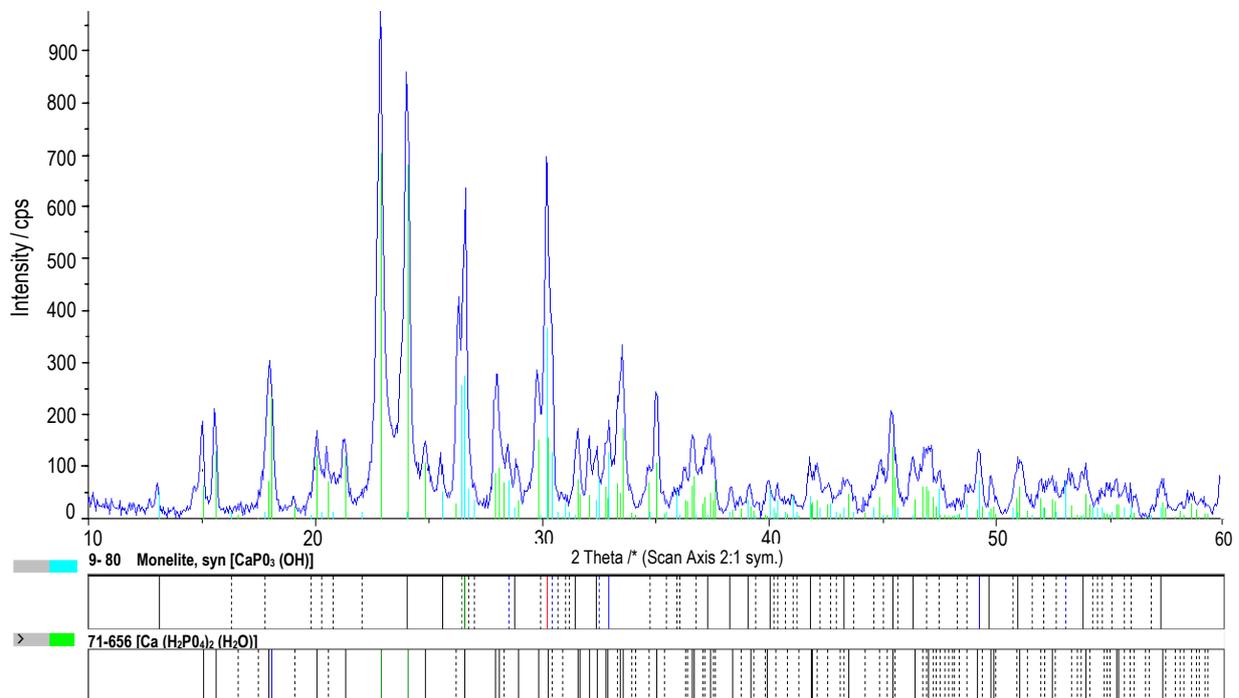


Figure 2. Roentgenogram of phosphate No. 2 with main phase of MCP with admixture of DCP

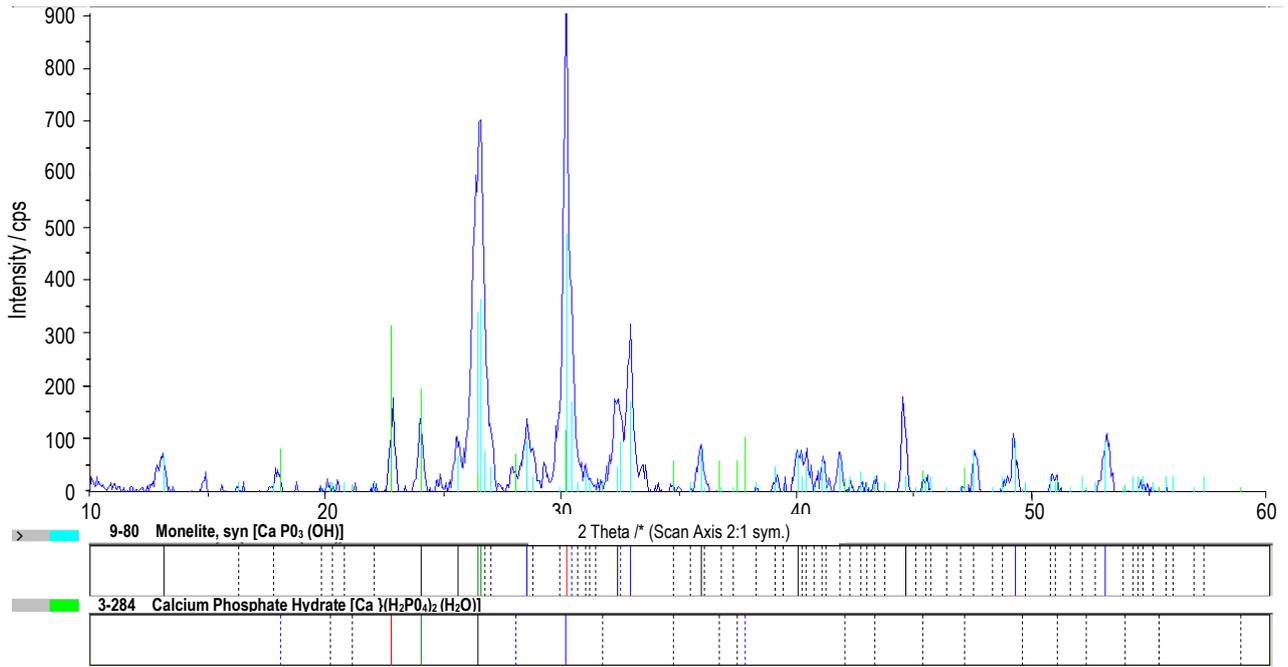


Figure 3. Roentgenogram of phosphate No. 3 with main phase of DCP with admixture of MCP

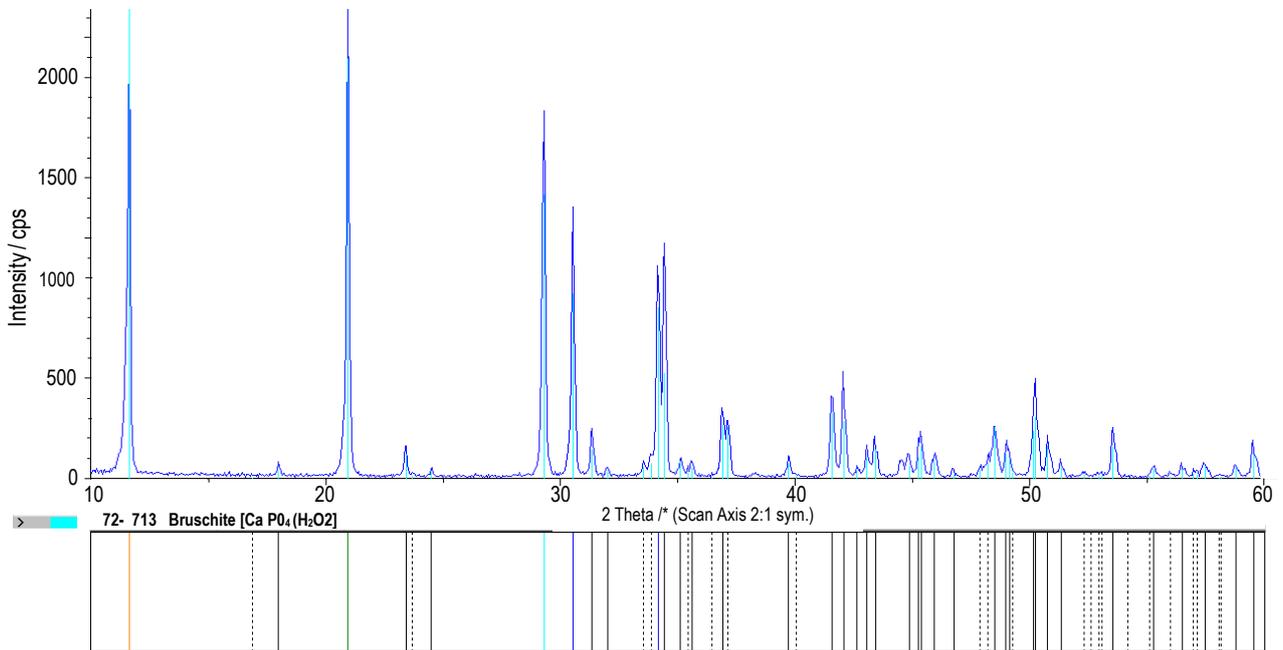
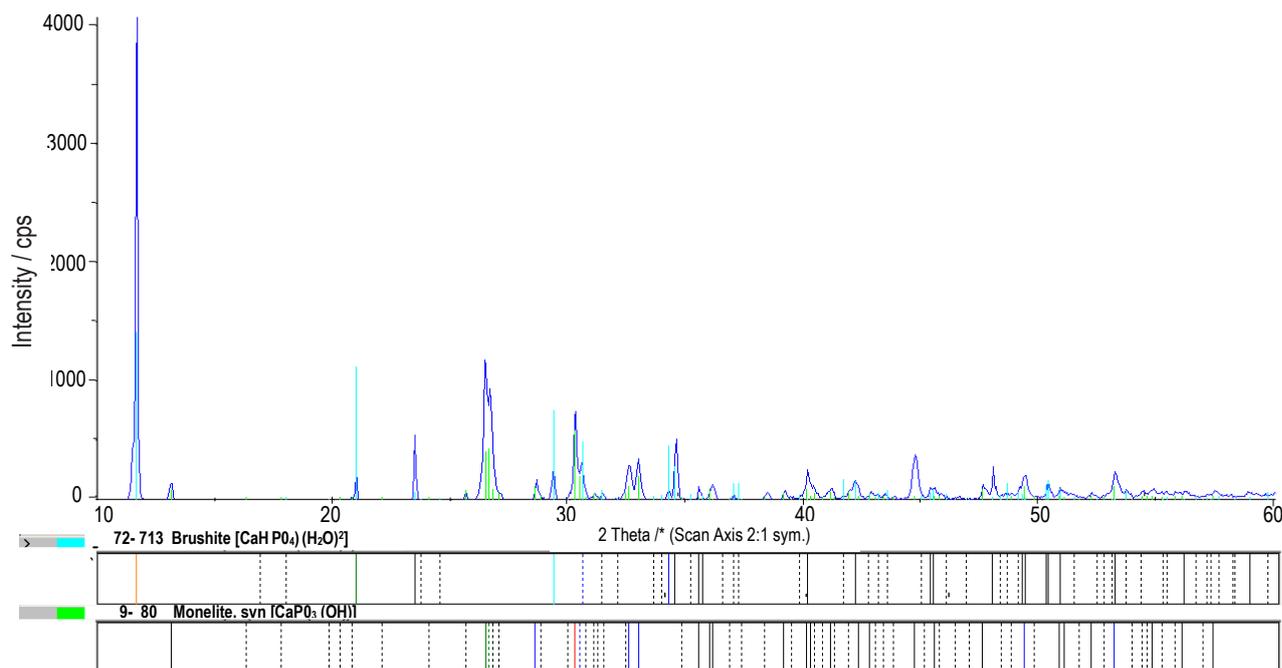


Figure 4. Roentgenogram of phosphate No. 4 with main phase of DDCP



**Figure 5.** Roentgenogram of phosphate No. 5 with main phase of DDCP with admixture of DCP

alkaline citrate from diammonium citrate pH 9.4–9.7; 30 min + 60 min, P content was determined by a gravimetric method using quinoline phosphomolybdate).

### Birds and diets

All procedures were approved by the Local Animal Care and Use Committee in Wrocław.

Three hundred one-day-old Hubbard Flex male chicks, with an average body weight of  $47.3 \pm 1.0$  g, were randomly assigned to five dietary treatments (Table 1). The chickens were reared in battery cages and each treatment had six replicates (cages) with 10 birds per cage. The environmental temperature was reduced from 32°C to 21°C, the lighting programme was 24 h light per day in the first week, then during 8 to 35 days, 16 h light per day. The birds had free access to drinking water served *via* a nipple system and to feeders containing feed mixtures in mash form.

On days 1–10 the birds were fed the prestarter mix, on days 11–12, the starter, and on days 22–35, the grower mixes differing in the content of mono- and dicalcium phosphates. The birds of all treatments were fed isoproteinous and isoenergetic diets based on similar amounts of wheat, maize and soyabean meal (Table 2). The crude protein content amounted to about  $222 \text{ g} \cdot \text{kg}^{-1}$  in the prestarter and starter, and to  $202 \text{ g} \cdot \text{kg}^{-1}$  in the grower diets. The contents of Ca and P were equalized. In the diet offered in treatment I, pure MCP was used; in treatment II, MCP with an admixture of DCP; in treatment III, DCP + MCP; in IV, pure dicalcium

**Table 2.** Composition of experimental diets,  $\text{g} \cdot \text{kg}^{-1}$

Specification	Prestarter <sup>1</sup> 1–10 days	Starter <sup>1</sup> 11–20 days	Grower <sup>2</sup> 21–35 days
Wheat	214–226	217–227	250–269
Maize	353	353	353
Soyabean meal	336–345	315–337	270–297
Soya oil	32–37	39–41	50–54
Vitamin-mineral premix	10.0 <sup>1</sup>	10.0 <sup>1</sup>	10.0 <sup>2</sup>
DL-methionine 98%	3.0–3.1	2.9–3.0	2.8–2.9
L-lysine 98%	0.7–1.0	0.5–1.0	1.0–1.9
NaCl	2.8	2.8	2.9
Phosphate	15.9–21.0	13.8–18.4	12.0–16.0
Limestone	13.6–20.7	15.4–21.7	14.3–19.8
ME, MJ·kg <sup>-1</sup>	12.2	12.4	12.8
Crude protein	224.4	220.7	201.9
Crude fat	62.2	69.8	79.8
Crude fibre	33.7	33.4	32.9
Met + Cys	9.31	9.10	8.9
Lysine	12.52	12.01	11.5
Ca	10.5	10.5	9.5
P total	7.8	7.3	6.7
P available <sup>3</sup>	5.0	4.5	4.0
Mg	1.8	1.7	1.6

<sup>1</sup> provided per kg of diet, IU: vit. A 12000, vit. D<sub>3</sub> 3000; mg: vit. E 35, vit. K<sub>3</sub> 2.5, vit. B<sub>1</sub> 3, vit. B<sub>2</sub> 7, vit. B<sub>6</sub> 5, vit. B<sub>12</sub> 0.02, biotin 0.15, nicotinic acid 40, calcium pantothenate 14, folic acid 1.5, choline 600, Mn 70, Fe 70, Cu 15, Zn 60, Se 0.2, Co 0.3, J 1, coccidiostat salinomycin (Sacox 120) 60; <sup>2</sup> provided per kg of diet; IU: vit. A 10000, vit. D<sub>3</sub> 2000; mg: vit. E 30, vit. K<sub>3</sub> 1.5, vit. B<sub>1</sub> 2, vit. B<sub>2</sub> 5, vit. B<sub>6</sub> 3, vit. B<sub>12</sub> 0.015, biotin 0.15, nicotinic acid 25, calcium pantothenate 10, folic acid 0.8, choline 500, Mn 60, Fe 50, Cu 15, Zn 50, Se 0.2, Co 0.2, J 0.7;

<sup>3</sup> calculated according to Smulikowska and Rutkowski (2005)

phosphate dehydrate (DDCP); and in treatment V, DDCP with an admixture of DCP was used. The vitamin-mineral premixes used in this study were free of antibiotic growth promoters or alternative substances and contained a coccidiostat.

### Measurements and sample collection

The body weight of chickens of each replicate was determined on days 1, 21 and 35. Feed intake was recorded for the periods of 1–21 and 22–35 days of life, as well as for the whole experimental period. The feeds were given twice a day in quantities enabling the consumption of the entire portion in a short time. Mortality and the causes of death were recorded. The retention of minerals was determined between days 31–35 of life. Feed intake was registered, excrements were collected twice a day and stored at +4°C, then lyophilized for chemical assays. The averages for replication were calculated. On day 35 post-hatch, all chickens were individually weighed, then sixteen birds from each treatment (2 or 3 birds per replication) were randomly selected on the basis of average weight within a replication.

The birds were killed by cervical dislocation, then both legs were cut off. The muscles were removed, the femurs and tibias were cleaned and stored for assessment of quality parameters (32 bones per treatment). For mechanical measurements, 16 left femurs and 16 left tibias were used. The same number of the right bones was designated for chemical analysis using the procedures described by Kim et al. (2004) and Jamroz et al. (2004, 2007). Such mechanical parameters as breaking strength and deflection were determined in fresh bones using the INSTRON 5544 (USA) device. Bone deflection was measured by a standard method, in which the force (F) was applied at a distance of  $L=13$  mm to the shaft of a bone supported on both epiphyses. The advance value of the head during estimation of breaking force on an INSTRON apparatus was  $0.8 \text{ mm} \cdot \text{min}^{-1}$  for bones of 35-day-old birds. Force (F) was increased up to the moment that the bone broke. The elasticity coefficient,  $F \cdot \text{h}^{-1}$ , was also calculated. On the basis of mechanical measurements, the maximum loading force  $F_n$  (force at break point), maximum bone deflection  $h_n$  (deflection at break point), maximum bone deflection  $U_n = h_n \cdot l^{-1}$ , and breaking work (work needed to break the bone) were calculated. The measurement techniques were presented in detail by Jamroz et al. (2004). Sixteen right bones were defatted in ether extract for 24 h according to the Soxhlet method and dried at 45°C. Crude ash and Ca, P and Mg content were determined in each of them.

### Chemical analyses

Feed components and complete diets were chemically analysed according to standard AOAC (2005) methods: the nitrogen content by the Kjeldahl method using a Kjeltac 2300 apparatus produced by Foss Tecator (Sweden); crude protein by multiplication of the N content by 6.25; crude fat by ether extraction; crude fibre by the Henneberg-Stohmann method using a Fibertec Tecator apparatus (Sweden); phosphorus content in experimental diets and bones was analysed after mineralization with nitric acid ( $\text{HNO}_3$ ) and perchloric acid ( $\text{HClO}_4$ ) by the ammonium vanadomolybdate method using a Spekol 11 (Carl Zeiss, Jena) spectrophotometer at a wave length of 470 nm; calcium and magnesium in diets and bones were determined by atomic absorption spectrophotometry using an AA 240 FS type apparatus (Candela).

Serum Ca was determined photometrically with a CPC test; P by UV, and Mg using LCF tests (EMAPOL, Poland). Alkaline phosphatase (E.C. 3.1.3.1) was determined by a Biosystems method using di-sodium 4-nitrophenylphosphate (CAS 4264-83-9). Nutrients and amino acids were chemically determined in the separate components. On the basis of the obtained data, the diets were calculated using a simplex optimization, and then, the nutritive value in complete feed mixtures was controlled again and the averages for each diet were calculated. The energy value of diets was calculated on the basis of determined nutrients and according to the formula published in the European Tables of Energy Values of Feeds for Poultry (1989) and amounted to  $12.2/12.8 \text{ MJ} \cdot \text{kg}^{-1}$ .

### Statistical analysis

All data were statistically evaluated by one-factorial ANOVA using StatSoft Statistica® software (2005). The differences for all parameters were tested according to the following statistical model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where:  $Y_{ij}$  – means the variance associated with parameter  $a$ ,  $\mu$  – the overall mean,  $a_i$  – the treatment effect (kind of phosphate),  $e_{ij}$  – the error.

The replication measurements or individual data for blood and bone parameters were treated as the experimental units and differences between treatment means were analysed for significance ( $P < 0.01$  or  $p < 0.05$ ) using Tukey's test. The data are presented as averages and are accompanied by  $\pm$  SD.

## Results

The examined phosphates were characterized by large differences in solubility in water (1.1%–79.7%). When citric acid and HCl solutions were used to simulate the conditions in the animal stomach, the solubility of phosphates was similar and varied between 89%–99% (Table 3). Phosphates Nos. 1 and 2 had a similar Ca and P contents and solubility parameters. The roentgenograms of phosphates Nos. 1 and 2 show only relatively similar crystalline phase images resulting from different presences of MCP and DCP (Figures 1 and 2), however, the presence of the main phase of DDCP (Figures 4 and 5) clearly differentiates the crystalline image of phosphates Nos. 4 and 5.

**Table 3.** Content of P-total, Ca and solubility of phosphates used in experiment

Treatments – phosphates with admixture	P-total in P <sub>2</sub> O <sub>5</sub> , %	Ca, %	Phosphate solubility expressed as P <sub>2</sub> O <sub>5</sub> , %			
			H <sub>2</sub> O	2% citric acid	0.4% HCl	ammonium citrate
I MCP	23.0	17.0	79.7	96.2	96.2	93.2
II MCP with DCP	22.9	17.2	78.1	97.6	99.6	92.7
III DCP with MCP	20.2	22.3	18.7	18.7	89.8	71.3
IV DDCP	17.7	22.8	1.2	97.8	99.5	61.1
V DDCP with DCP	18.3	25.7	1.1	97.1	98.6	33.6

MCP, DCP, DDCP – see Table 1

The average body weight of chickens in the treatments on days 21 and 35 post hatch did not differ significantly. The average daily feed intake calculated for the period of days 1–35 amounted to 77–79 g; FCR, about 1.55 kg per kg BW (Table 4). In all of the treatments, bird mortality was low (single cases only). The considerable differences in the solubility of the analysed phosphates did not correlate with performance parameters, except for P retention, which was significantly higher ( $P < 0.01$ ; 61.3%) in the group fed the diet containing pure MCP.

**Table 4.** Body weight and feed intake of chickens (means)

Item	Treatments					SEM
	MCP	MCP with DCP	DCP with MCP	DDCP	DDCP with DCP	
Body weight, g						
on day 21	721	736	735	737	734	1.95
on day 35	1995	1987	1968	1992	2005	5.40
Average feed intake in the period						
1–35. day of life, g · head · day <sup>-1</sup>	77.6	79.7	79.0	79.0	77.2	0.606
FCR, kg · kg <sup>-1</sup> BW	1.539	1.587	1.573	1.570	1.525	0.772

All differences between treatments were insignificant; MCP, DCP, DDCP – see Table 1

**Table 5.** Balance of minerals in 35 days old chickens (means)

Specification	Treatments					SEM
	MCP	MCP with DCP	DCP with MCP	DDCP	DDCP with DCP	
P – intake, g · kg <sup>-1</sup>	1.82 <sup>A</sup>	1.65 <sup>B</sup>	1.62 <sup>B</sup>	1.50 <sup>B</sup>	1.62 <sup>B</sup>	0.017
P – excreted, g · day <sup>-1</sup>	0.70	0.79	0.75	0.75	0.77	0.015
P – retained, % of intake	61.3 <sup>A</sup>	51.9 <sup>Ba</sup>	53.9 <sup>Bb</sup>	50.2 <sup>Ba</sup>	52.2 <sup>Bb</sup>	0.507
Ca – intake, g · day <sup>-1</sup>	1.81 <sup>a</sup>	1.80 <sup>a</sup>	1.74 <sup>ab</sup>	1.66 <sup>b</sup>	1.74 <sup>ab</sup>	0.018
Ca – excreted, g · day <sup>-1</sup>	0.70 <sup>b</sup>	0.80 <sup>Aa</sup>	0.73 <sup>Bc</sup>	0.68 <sup>b</sup>	0.61 <sup>Bd</sup>	0.012
Ca retained, % of intake	61.3 <sup>A</sup>	55.4 <sup>B</sup>	58.2 <sup>AB</sup>	59.4 <sup>AB</sup>	65.1 <sup>C</sup>	0.552
Mg – intake, g · day <sup>-1</sup>	0.37	0.37	0.38	0.36	0.37	0.004
Mg – excreted, g · day <sup>-1</sup>	0.25 <sup>ab</sup>	0.27 <sup>a</sup>	0.27 <sup>a</sup>	0.24 <sup>b</sup>	0.25 <sup>ab</sup>	0.004
Mg retained, % of intake	33.6 <sup>b</sup>	27.4 <sup>a</sup>	28.2 <sup>c</sup>	32.7 <sup>bc</sup>	33.4 <sup>b</sup>	0.711

<sup>a,b,A,B</sup> means in the rows with different letters are different at:

<sup>a,b</sup>  $p < 0.05$ ; <sup>A,B</sup>  $P < 0.01$ ; MCP, DCP, DDCP – see Table 1

In other treatments these values varied between 50.2% and 53.9% (Table 5). Ca-retention varied between 55.4%–65.1%. In birds fed diets containing pure MCP or DDCP with a DCP admixture, the retention of Ca amounted to 61% and 65%, respectively ( $P < 0.01$ ). The best Mg retention was found in birds fed diets containing pure MCP, DDCP + DCP, or DDCP.

The serum parameters reflect the variability caused by the feed phosphates used (Table 6). Significantly higher Ca and P concentrations were found in chickens fed the diet containing pure MCP ( $P < 0.01$ ). The Mg concentration was higher in chickens fed the diets with pure MCP and DCP with an admixture of MCP ( $P < 0.01$  and  $p < 0.05$ ). The kind of phosphate used did not cause any significant differences in serum alkaline phosphatase activity. Some mechanical parameters, i.e. maximum force needed to break the femur and tibia, were similar in all treatments (Table 7). A significantly lower elasticity of femur bones was found in chickens fed the diet containing DDCP+DCP ( $P < 0.01$ ). Tibia elasticity was similar in all treatments. The young module was significantly lower only in the femur ( $p < 0.05$ ) of birds fed the diet with DDCP+DCP. In the tibia, similar values of this parameter were found. The kind of phosphate used significantly ( $p < 0.05$ ) affected the crude ash content only in the tibia (Table 8). The highest amount of ash was found in the tibia of chickens fed the diet with pure MCP ( $p < 0.05$ ).

**Table 6.** Ca, P, Mg concentration and alkaline phosphatase activity in blood serum in 35 days old chickens (means)

Item	Treatments					SEM
	MCP	MCP with DCP	DCP with MCP	DDCP	DDCP with DCP	
Ca, mmol <sup>-1</sup>	2.59 <sup>A</sup>	2.14 <sup>B</sup>	2.18 <sup>B</sup>	2.22 <sup>B</sup>	2.22 <sup>B</sup>	0.021
P, mmol <sup>-1</sup>	3.56 <sup>Aa</sup>	3.13 <sup>b</sup>	3.27 <sup>ab</sup>	3.05 <sup>B</sup>	3.01 <sup>B</sup>	0.026
Mg, mmol <sup>-1</sup>	1.10 <sup>ab</sup>	1.06 <sup>b</sup>	1.17 <sup>Aa</sup>	1.04 <sup>B</sup>	1.08 <sup>ab</sup>	0.006
Alkaline phosphatase activity, U · l <sup>-1</sup>	4112	4508	3666	3972	4483	181.4

<sup>a,b,A,B</sup> means in the rows with different letters are different at: <sup>a,b</sup>  $p < 0.05$ ; <sup>A,B</sup>  $P < 0.01$ ; MCP, DCP, DDCP – see Table 1

**Table 7.** Physical parameters of femur and tibia bones in 35 day old chickens (means)

Specification	Treatments					SEM
	MCP	MCP with DCP	DCP with MCP	DDCP	DDCP with DCP	
<b>Femur</b>						
maximum breaking force, N	207.1	200.1	204.7	204.9	203.3	1.259
elasticity, N · m <sup>-1</sup>	1.54 × 10 <sup>5A</sup>	1.50 × 10 <sup>5A</sup>	1.30 × 10 <sup>5AB</sup>	1.28 × 10 <sup>5AB</sup>	1.11 × 10 <sup>5B</sup>	0.182 × 10 <sup>5</sup>
young module, N · m <sup>-2</sup>	6.57 × 10 <sup>9a</sup>	6.43 × 10 <sup>9ab</sup>	5.74 × 10 <sup>9ab</sup>	5.74 × 10 <sup>9ab</sup>	4.53 × 10 <sup>9b</sup>	0.715 × 10 <sup>8</sup>
<b>Tibia</b>						
maximum breaking force, N	388.9	353.3	362.3	24.1	382.3	2.256
elasticity, N · m <sup>-1</sup>	2.29 × 10 <sup>5</sup>	2.34 × 10 <sup>5</sup>	2.15 × 10 <sup>5</sup>	2.16 × 10 <sup>5</sup>	2.53 × 10 <sup>5</sup>	0.337 × 10 <sup>5</sup>
young module, N · m <sup>-2</sup>	1.22 × 10 <sup>10</sup>	1.19 × 10 <sup>10</sup>	1.06 × 10 <sup>10</sup>	1.31 × 10 <sup>10</sup>	1.15 × 10 <sup>10</sup>	0.800 × 10 <sup>5</sup>

<sup>a,b,A,B</sup> means in the rows with different letters are different at: <sup>a,b</sup>  $p < 0.05$ ; <sup>A,B</sup>  $P < 0.01$ ; MCP, DCP, DDCP – see Table 1

**Table 8.** Content of crude ash, Ca, P and Mg in bone ash in 35 days old chickens (means)

Specification	Treatments					SEM
	MCP	MCP with DCP	DCP with MCP	DDCP	DDCP with DCP	
<b>Femur</b>						
crude ash, %	45.5	45.2	43.2	45.2	42.6	0.360
content in ash, g · kg <sup>-1</sup>						
Ca	224.1 <sup>A</sup>	219.6 <sup>A</sup>	218.8 <sup>A</sup>	208.4 <sup>B</sup>	200.5 <sup>C</sup>	0.742
P	170.1 <sup>A</sup>	172.7 <sup>ab</sup>	171.3 <sup>a</sup>	172.5 <sup>a</sup>	176.1 <sup>Bb</sup>	0.472
Mg	6.4 <sup>B</sup>	5.5 <sup>C</sup>	5.7 <sup>A</sup>	6.2 <sup>Ba</sup>	6.7 <sup>Bb</sup>	0.054
<b>Tibia</b>						
crude ash, %	45.8 <sup>Aa</sup>	42.0 <sup>b</sup>	43.0 <sup>ab</sup>	41.0 <sup>B</sup>	41.8 <sup>b</sup>	0.389
content in ash, g · kg <sup>-1</sup>						
Ca	217.6 <sup>A</sup>	215.1 <sup>A</sup>	215.5 <sup>A</sup>	206.8 <sup>BC</sup>	196.4 <sup>D</sup>	0.563
P	170.5	172.9	173.0	170.9	172.9	0.443
Mg	6.2 <sup>Ab</sup>	5.5 <sup>B</sup>	5.4 <sup>B</sup>	6.1 <sup>Ab</sup>	6.6 <sup>Aa</sup>	0.049

<sup>a,b,A,B,C,D</sup> means in the rows with different letters are different at: <sup>a,b</sup>  $p < 0.05$ ; <sup>A,B,C,D</sup>  $P < 0.01$ ; MCP, DCP, DDCP – see Table 1

The Ca concentration in crude ash was higher in bones from birds fed diets with MCP or phosphates containing MCP ( $P < 0.01$ ). Differences in phosphorus concentrations in bone ash among treatments were found to be significant in the femur (the highest for DDCP + DCP) and in significant in the tibia. The highest Mg contents in femur and tibia ash were found in birds fed MCP, DCP, and DDCP + DCP ( $P < 0.01$  and  $p < 0.01$ , respectively). Unclear convergence was observed between physical parameters and the chemical composition of bones.

## Discussion

Despite much research on the development of the best methods for assaying the biological value of phosphates and on the degree of phosphorus utilization from different chemical bonds, the problems related to phosphorus utilization in animal organisms have still not been well elucidated (Anselme, 2003; Rodehutsord and Dieckmann, 2005; Nancy et al., 2009; Rodehutsord, 2009). According to some opinions, determination of phosphate solubility is sufficient for estimating their usefulness for animals (Sullivan et al., 1992; Ravindran et al., 1995;

De Groote and Huyghebaert, 1997). The results of our studies show that no direct relation between phosphate solubility and chemical composition and chicken performance can be found. A more distinct response of chickens to the kind of phosphate administered was seen in blood Ca, P and Mg concentrations and the content of these elements in bone ash (Jamroz et al., 2012). Similar results were also obtained in the current investigations.

Research carried out by Rath et al. (2000), Jamroz et al. (2001, 2004, 2007), Shapiro and Heaney (2003) and Hemme et al. (2004) shows that the Ca and P contents in bones and bone mineralization indices may be good parameters reflecting phosphate quality. In the present study, the best chemical composition (albeit, not for all mineral elements) was found in the bones of chickens fed the diet containing pure hydrated monocalcium phosphate. These results were accompanied by the highest serum Ca, P and Mg concentrations. The physical parameters of bones, strength and elasticity estimated for the femur and tibia, do not give unambiguous information about the dependence between phosphate characteristics and quality and bone mechanical indices. The best mechanical parameters of the femur were found in chickens fed diets with MCP. For the tibia, all of the differences among treatments were insignificant. Significant differences among treatments in Ca, P and Mg contents in bone ash were recorded. The highest values of Ca and Mg were determined in chickens fed diets containing MCP, but the P level in ash measured in the same treatment was the lowest.

## Conclusions

On the basis of results reported by Jamroz et al. (2012) as well as in the present study, it can be concluded that the quality of phosphates influences the performance of broilers and their mechanical bone parameters to a small degree. The higher P retention found in the present study, however, and higher concentration of Ca, P and Mg in blood and in bone ash in chickens fed diets containing pure monocalcium phosphates (MCP) in comparison with birds fed dicalcium phosphate dehydrate (DDCP) with dicalcium phosphate (DCP) admixture, point to the superior value of MCP in comparison with DDCP with a DCP admixture. The roentgenographic analysis of phosphate crystalline characteristics could be good supplemental information, mainly for the producers of phosphates, but the usefulness of this kind of quality estimation of phosphates in biological evaluation on broilers was not confirmed.

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