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PERFORMANCE AND BONE QUALITY OF LAYING HENS FED LOW-PHOSPHORUS DIETS BASED ON DIFFERENT CEREAL GRAINS AND SUPPLEMENTED WITH PHYTASE

PROIZVODNJA I KAKVOĆA KOKOŠI NESILICA HRANJENIH OBROCIMA S NISKIM FOSFOROM NA OSNOVI ŽITARICA S DODATKOM FITAZE

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SUMMARY

The purpose of the present study was to evaluate the response of laying hens to the low-phosphorus diets based on different kinds of cereal grains and supplemented with microbial phytase. Laying performance, feed intake, egg shell quality as well as bone strength parameters and Ca, P, Mg and Zn contents in the bones were involved as experimental parameters. At the age of 17 weeks, 288 Lohmann Brown pullets were assigned to the six dietary treatments, each treatment consisting of 12 cages with 4 birds per one. Until the beginning of the laying period the pullets were fed a standard diet containing 145 g of crude protein and 11.3 MJ ME, 4 g non-phytate P and 10.6 g of Ca per kg feed. The experimental diets provided from the first day of laying were based on maize (diets I, II, III) or on a combination of maize, barley and wheat (diets IV, V, VI) and contained about 165 g CP and 11.2 MJ ME/kg. In diets I, III or IV and VI the level of non-phytate phosphorus was decreased to 1.41 g/kg while in control diets (III or V) the amount of non-phytate P was 3.0 g/kg. In low-P diets III and VI microbial 6-phytase was included at the level of 450 FTU/kg diet.

The phosphorus level in diets did not affect the laying rate during the experimental period of 53 weeks. The higher P-level in feed enhanced egg shell strength only ($P<0.01$). Supplementation of low-P-diets with phytase did not affect the performance of hens, such as feed intake and egg quality, however it improved the egg shell strength as compared to low-P unsupplemented groups. Regarding strength and elasticity of the tibia, the P-level and phytase supplement were without visible effects. The best parameters that characterize the bone quality were obtained by addition of 3 g of non-phytate P level per kg of diets.

Key words: laying hens, microbial phytase, performance, egg shell quality, bone quality

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INTRODUCTION

Cereal grains are rich in phosphorus, however, its overall availability is limited to the level about 60-70 % only. Phosphorus bound in the form of phytates (phytate P) is available for poultry at a very low extent (Kornegay et al., 1996; Jamroz et al., 1998; Ollofs et al., 2000; Hempel et al., 2004a, 2004b; Liebert et al., 2005).

Both microbial phytase (myo-inositol hexaphosphate phosphohydrolase) produced by the intestinal microorganisms and native phytase present in cereal grains allow only partial utilization of dietary phosphorus (Simons et al., 1990; Żyła et al., 1996; Kornegay et al., 1996; Hadorn and Wiedmer, 1998). In spite of being higher than in other cereal grains, the activity of native phytase present in wheat and barley is not sufficient for beneficial utilization of their own phosphorus. Thus supplementation of cereal grains-based diets with microbial phytase preparations may increase the phosphorus availability, protein digestibility and conversion and could improve the utilization of some trace elements as the result of degradation of phytate-protein and phytate-mineral complexes (Sebastian et al., 1996; Yi et al.,1996a, Szkudelski, 1997; Leske and Coon,1999; Musapour et al., 2005). However, in some publications only limited beneficial effect of phytase supplementation on the laying hens performance was registered (Jamroz et al., 2003; Panda et al., 2005). On the other hand, beneficial effects of phytase on utilisation of phosphorus, improved egg shell and bone quality were reported by Saveur (1991), Zhang and Coon (1997), Um and Paik (1999), Gordon and Short (2001).

The purpose of the present study was to evaluate the influence of dietary phosphorus level and efficacy of phytase supplementation of diets based on different cereal grains, either maize (with very low activity of native phytase) or a combination of maize, barley and wheat. Laying rate, egg weight, quality of egg shells and bird's bones as well as the quality and Ca, P, Mg and Zn content were involved as experimental parameters.

MATERIAL AND METHODS

The experiment was carried out with a total of 288 Lohmann Brown layers. At the age of 17 weeks the pullets were allocated into battery cages at 4 birds per cage and divided into 6 treatments, each consisting of 12 cages as replications. The average initial body weight of birds was similar in all treatment groups. Feed and water were supplied *ad libitum*. The lighting program was 10 hours darkness and 14 hours light with an intensity of about 30 lx/m^2.

Before the start of the laying period, pullets were fed the standard diet containing 145 g crude protein and 11.3 MJ ME/kg. The experimental diets were based on maize (diets I, II, III) or maize-barley-wheat (diets IV, V, VI). The total P content was different and was calculated to reach 4.0 or 5.9 g per kg diet; the level of non-phytate phosphorus in low-phosphorus and standard diets reached 1.41 or 3.0 g P per kg (Table 1). The composition and nutrient contents of the used diets are summarized in Table 2.

Table 1. Design of experiment
Tablica 1. Plan pokusa

Experimental groups Pokusne skupine	Kind of cereals in diets Vrsta žitarica u obroku	Non-phytate phosphorus in feed Nefitatni fosfor u hrani (g/kg)	Phytase supplement* Dodatak fitaze (FTU/kg)
I	Maize - Kukuruz (65 %)	1.41	-
II control		3.00	-
III		1.41	450
IV	Maize - Kukuruz (20 %) Barley - Ječam (35 %) Wheat - Pšenica (10 %)	1.41	-
V control		3.00	-
VI		1.41	450

*RONOZYME® P (CT), Batch HB 955001, declared phytase activity: 5000 U/g
One unit of phytase activity is defined as the amount that releases 1 μmol of inorganic phosphate from 5.0 mmol sodium phytate per minute at pH 5.5 in 37 °C

Table 2. Composition and nutrient content of diets
Tablica 2. Sastav i sadržaj hranjivih tvari u obroku

Feed components (in %)	Diets - treatments - Obroci - tretmani					
	I	II	III	IV	V	VI
Barley var. Rudzik Ječam var. Rudzik	-	-	-	35.0	35.00	35.0
Winter wheat var. Cobra Zimska pšenica var. Cobra	-	-	-	10.0	10.0	10.0
Maize - Kukuruz	65.70	65.10	65.70	21.2	20.70	21.20
Soya oil - Sojino ulje	-	0.20	-	2.60	2.70	2.60
Soyabean meal - Sojina sačma	23.70	23.8	23.70	20.8	20.80	20.80
Monocalcium phosphate Monokalcijev fosfat	0.21	0.90	0.21	-	0.69	-
Limestone - Vapno	8.95	8.53	8.59	9.07	8.65	9.07
Mineral-vitamin premix[1] Mineralno-vitaminski premiks[1]	1.00	1.00	1.00	1.00	1.00	1.00
Salt - Sol	0.31	0.31	0.31	0.32	0.33	0.32
Calculated metabolizable energy Izračunata metabolička energija (MJ/kg)	11.20	11.20	11.20	11.20	11.20	11.20
Analysed or estimated levels of nutrients - Analizirane ili procijenjene vrijednosti hranjivih tvari (g/kg)						
Crude protein - Sirove bjelančevine (Nx6.25)	164.60	165.40	165.10	164.90	164.80	165.30
Methionine - Metionin	3.55	3.54	3.44	3.46	3.60	3.44
Lysine - Lizin	7.77	7.82	8.10	7.84	7.81	7.79
Ca	35.80	36.20	35.3	36.30	35.40	36.30
P	4.28	5.92	4.33	4.00	5.66	4.05
P non-phytate[2] - P nefitinski[2]	1.41	3.00	1.41	1.41	3.00	1.41
Soluble NSP[3] - Topivi NSP[3]	18.63	18.64	18.63	32.05	32.08	32.05
Unsoluble NSP - Netopivi NSP	58.13	57.87	58.13	63.31	63.18	63.31
β-glucans - β-glukani	1.16	1.16	1.16	14.16	14.17	14.16

[1] supplied per kg diet: retinol acetate 10.000 IU; cholekalciferol 2.000 IU; (mg) tocopherol 15; menadion 1.5; thiamine 2; riboflavin 4; pyridoxine 1.0; cyanocobalamin 0.01; nicotinic acid 30; folic acid 0.5; panthothenic acid 4; choline chloride 250; Mn 60; Zn 50; Fe 30; Cu 5; I 0.5; Se 0.12 and NaCl 4 g

[2] calculated on the basis of P-availability from Nutrient Requirements (1996)

[3] calculated according to Bach Knudsen,1997

Diets containing 1.41 g of non-phytate phosphorus (P-1.4) per kg were fed either unsupplemented (diets I, IV) or supplemented (diets III, VI) with microbial 6-phytase at the dose of 450 phytase units per kg (see Table 1). All diets in mash form were provided *ad libitum* from week 19 of life until the end of experiment.

Laying performance was monitored during 371 days of the laying period. The number of laid eggs was recorded daily, all eggs obtained from each cage were weighed once a week. Feed intake, abnormalities of the egg shell and mortality of birds were registered constantly during the whole experimental period.

During weeks 4, 17, 30, 43 and 52 after the start of the experiment the quality of egg shells was estimated. Thirty eggs from each treatment group and every term were randomly selected in order to determine the average weight of the whole eggs. The eggs shell parameters were evaluated using standard procedures with a PM-600 PX-processor apparatus (Technical Service and Supplies Limited, York England). Shell thickness was measured on three points using a Modul with Micrometric screw (Mitutoys). Every measurement of eggs was done twice. Shell strength was determined under a maximum pressure of 3.5 kg.

At the end of the experiment, 12 animals were randomly chosen from each dietary treatment, weighed and sacrificed. Tibia bones from the left legs were prepared for evaluation of strength and elasticity. Right tibia as well as femur bones were used for determination of chemical composition. Analysis of physical properties was done on bones, which were stored in a refrigerator at temperature of 4-5 °C for 10 hours.

The tibia bones maximal deflection (h) (mm), breaking force (F) (N), coefficient of elasticity (F/h) (N/m) and total breaking energy of pressure (W) (mJ) were estimated and calculated as physical parameters. The parameters were evaluated according to variable force (from low to high pressure) up to the bone breaking point. The bone maximal deflection under pressure force was measured with standard method in which the force (F) (N) (speed of pressure was 1.2 mm/min) was applied at the shaft of the bone supported at both epiphyses. The end of this estimation was a breaking force point and from this value was calculated total breaking energy.

The elasticity coefficient ($\alpha = \Delta F/\Delta h$) was estimated by comparing it to factors of maximal breaking force and maximal deflection.

The chemical composition of diets was determined according to standard methods (AOAC, 2000). The Ca, P, Mg and Zn contents were determined in dried fat free tibia bones prepared from the right legs. After previous mineralization of diets and bones, phosphorus was analysed by the vanadomolybdate method, using a Specol 11 (Carl Zeiss Jena) spectrophotometer at a wave length of 470 nm. Ca, Mg and Zn were determined by atomic absorption spectrophotometry using an AAS-3 EA 30, Carl Zeiss Jena apparatus.

Dietary non-starch polysaccharides content was calculated on the basis of chemical composition of cereal grains (Bach Knudsen,1997). The energy value of the diet (see Table 2) was calculated according to the European Tables of Energy Values of Feeds for Poultry, WPSA (1989).

Statistical evaluations were conducted using the Statgraphics Plus ver. 7.0 software. Data were compared using one-way and two-way ANOVA (Brandt,1997). A split-plot ANOVA was used to test the periods x treatment interactions. The significance of differences among treatment groups was examined after data transformation (arcsin) for percentages and $\log_{10}$ for other variables. Tukey's Multiple Range Test was used to determine the significance of differences between treatment means ($P \leq 0.05$ and $P \leq 0.01$).

RESULTS

The initial **body weight** of hens varied between 1743 and 1786 g, with maximal differences of about 50 g. Final weight reached 1928-1998 g, and body weight gain 180-210 g (Table 3).

Average laying rate calculated for 371 days of experiment is presented in Table 4. It varied from 85.0 (diet IV) to 88.9 % (diet I) ($P<0.05$). Significantly reduced **egg production** was observed in low-phosphorus treatment groups fed diets containing maize, barley and wheat (IV and VI). Different dietary phosphorus level and phytase supplementation, irrespective of kind of diets, had no significant effect on the laying rate (Table 4), however, when feeding the maize-based diets significantly better results were obtained.

Egg weight varied between 61.7-62.2 g, without differences related to the kind of cereal grain, phosphorus level or phytase supplementation.

Table 3. Body weight of hens (means, ±SD)
Tablica 3. Tjelesna masa kokoši (srednja, ±SD)

	Diets - treatments - Obroci - tretmani						Non-phytate P level Razina nefitinskog P (g/kg)			Cereal grains Žitarice	
	I	II	III	IV	V	VI	P-1.4	P-3.0	P-1.4 + phytase	Maize Kukuruz	Maize Barley Wheat Kukuruz Ječam Pšenica
Initial body weight (kg) Početna tjelesna masa (kg)	1.748	1.786	1.780	1.754	1.743	1.761	1.751	1.765	1.770	1.771	1.753
	55	54	58	36	64	41	45	62	50	56	48
Final body weight (kg) Konačna tjelesna masa (kg)	1.928	1.992	1.980	1.964	1.998	1.942	1.946	1.995	1.961	1.967	1.968
	156	206	134	177	153	229	164	177	184	166	185
Weight gain (g) Prirast tjelesne mase (g)	180	206	200	210	254	181	195	230	190	195	215
	149	184	128	170	159	225	157	170	179	151	184

All differences among groups and interactions were found to be insignificant - Razlike među skupinama i interakcijama nisu značajne.

Table 4. Laying performance (mean for 371 days) (means, ±SD)
Tablica 4. Nesenje (prosjek za 371 dan)

	Diets - treatments - Obroci - tretmani						Non-phytate P level Razina nefitatnog P (g/kg)			Cereal grains Žitarice	
	I	II	III	IV	V	VI	P-1.4	P-3.0	P-1.4 + phytase	Maize Kukuruz	Maize Barley Wheat Kukuruz Ječam Pšenica
Laying rate (%) Postotak nesivosti (%)	88.9a	87.6ab	87.6ab	85.0b	88.1ab	85.4b	87.0	87.8	86.5	88.0a	86.2b
	3.8	3.2	3.0	4.5	3.6	2.9	4.5	3.3	3.1	3.3	3.9
Egg weight (g) Težina jaja (g)	62.2	61.9	61.3	61.8	62.1	61.7	62.0	62.0	61.5	61.8	61.8
	1.0	1.3	1.1	1.1	1.4	0.9	1.1	1.3	1.0	1.2	1.1
Total egg mass (kg/hen) Ukupna masa jaja (kg/kokoš)	20.6a	20.1ab	19.9ab	19.5b	20.3ab	19.5b	20.0	20.2	19.7	20.2	19.8
	0.9	0.8	0.6	1.0	1.0	0.6	1.1	0.8	0.6	0.8	0.9
Feed intake (g/bird/day) Unos hrane (g/kokoš/dan)	130.4	130.6	130.5	130.0	130.0	129.6	130.2	130.3	130.1	130.5a	129.8b
	1.7	1.0	1.5	0.6	0.4	0.6	1.3	0.8	1.2	1.4	0.5
Feed intake (kg/kg egg mass) Unos hrane (kg/kg mase jaja)	2.36a	2.41ab	2.43ab	2.48b	2.38a	2.46b	2.42	2.40	2.45	2.40	2.44
	0.09	0.10	0.07	0.13	0.11	0.08	0.13	0.10	0.07	0.09	0.12
Cracked and deformed eggs (%) Napukla i deformirana jaja (%)	2.68	2.66	2.80	2.76	2.79	2.46	2.63	2.60	2.55	2.66a	2.55b
	1.41	1.41	2.14	1.33	1.32	1.19	1.84	1.30	1.71	1.92	1.20

a, b Means in the same line not sharing a common superscript are significantly different ($P<0.05$); interactions - insignificant

The lowest (P<0.05) total egg mass was noted in low-phosphorus treatment groups fed the maize-barley-wheat diet, while addition of phytase did not exert any effect on this parameter. Two-factorial analysis of variance for this parameter indicated no significant differences, irrespective of dietary phosphorus level and the kind of cereal grains used.

Concerning feed intake (on average 130 g per hen/day), only the kind of cereal grains used in diets significantly affected this parameter, while **feed conversion** was similar, irrespective of dietary phosphorus level, phytase addition and kind of cereal grains (Table 4). Feed conversion calculated per kg of egg mass was significantly lower in hens of treatment groups I and V than in other groups.

Differences in the percentage of **cracked or deformed eggs** among groups were relatively low and statistically insignificant, however the kind of cereal grains used in diets significantly influenced this parameter.

In five measurements conducted during weeks 4, 17, 30, 43 and 52 of laying period, the **egg mass, egg shell strength and thickness** were significantly different. The best egg shell quality (P<0.01) was observed in hens fed the control (3.0 g av P) and low-phosphorus diets supplemented with microbial phytase, but the egg shell thickness was significantly higher in low-P treatment groups fed diets supplemented with phytase (see Table 5). The analysis of the changes in these parameters during five measurement cycles indicates the significant (P<0.01) increase of egg mass with an increasing age of hens and in the same way a decrease of egg shell strenght (Table 6).

Great individual variation in **tibia quality** parameters was noted, however, the strenght of this bone showed significant differences among teratment groups (Table 7). The best strength of egg shell was noted for the hens of groups II and V (3.0 g of non-phytate P/kg) and group I (1.4 g of non-phytate P/kg), in other parameters of bone quality only insignificant variability occurred.

Considering the described factors, the evaluation of chemical composition of bone ash showed variability of mineral content in bones. The average composition of femur was about 51 % of crude ash, 285 g Ca, 158 g P, 5.6 g Mg and 393 mg of Zn per kg ash and of tibia about 52.5 % of crude ash, 252 g Ca, 159 g P, 5.6 g Mg and about 370 mg of Zn per kg ash (see Tables 8 and 9).

Table 5. Egg shell quality related to diets, phosphorus level or cereal grains (means, ±SD)
Tablica 5. Kakvoća ljuske jaja u vezi s obrocima, razinom fosfora i žitarica

	Diets - treatments - Obroci - tretmani						Non-phytate P level Razina nefitatnog P (g/kg)			Cereal grains Žitarice	
	I	II	III	IV	V	VI	P-1.4	P-3.0	P-1.4 + phytase	Maize Kukuruz	Maize Barley Wheat Kukuruz Ječam Pšenica
Egg mass (g) Masa jaja (g)	63.9	63.0	63.0	63.9	63.2	63.1	63.9	63.1	63.1	63.3	63.4
	4.92	4.89	5.74	5.01	6.22	5.36	4.9	5.6	5.5	5.2	5.5
Egg shell strenght (kg) Čvrstoća ljuske jaja (kg)	2.95Aa	3.25Bb	3.00Aa	3.04Aa	3.12AB	3.00Aa	2.99A	3.18B	3.02B	3.06	3.05
	0.78	0.67	0.81	0.85	0.95	0.98	0.82	0.82	0.89	0.77	0.93
Egg shell thickness (mm) Debljina ljuske jaja (mm)	0.351A	0.334B	0.329B	0.324B	0.333B	0.353A	0.337ab	0.334a	0.341b	0.338	0.337
	0.044	0.044	0.043	0.055	0.044	0.047	0.052	0.044	0.046	0.045	0.050

a, b Means in the same line not sharing a common superscript a,b or A,B are significantly different (P<0.05);

A, B Means in the same line not sharing a common superscript are significantly different (P<0.01); interactions - insignificant

Table 6. Egg shell quality in subsequent measurement cycles (means, ±SD)
Tablica 6. Kakvoća ljuske jaja u ciklusima mjerenja

	Measurements in weeks - Mjerenja u tjednima				
	4	17	30	43	52
Egg mass (g) Masa jaja (g)	59.9A	62.6B	63.1B	64.9C	66.4D
	4.0	4.8	4.9	5.5	5.2
Egg shell strenght (kg) Čvrstoća ljuske jaja (kg)	3.82A	3.29B	2.87C	2.66D	2.65D
	0.68	0.72	0.67	0.72	0.82
Egg shell thickness (mm) Debljina ljuske jaja (mm)	0.317A	0.353C	0.349C	0.332B	0.335B
	0.070	0.034	0.035	0.037	0.041

A, B, C Means in the same line not sharing a common superscript are significantly different (P<0.01)

Table 7. Parameters of tibia bones of hens calculated per 100 g metabolic body weight (BW $^{0.67}$) (means, ±SD)
Tablica 7. Parametri kosti goljenice (tibijalnih kosti) kokoši izračunati na 100 g metaboličke težine tijela

	Experimental groups - Pokusna grupa						Non-phytate P level Razina nefitatnog P (g/kg)			Cereal grains Žitarice	
	I	II	III	IV	V	VI	P-1.4	P-3.0	P-1.4 + phytase	Maize Kukuruz	Maize Barley Wheat Kukuruz Ječam Pšenica
Maximum deflection in breaking point (mm) Maksimalna defleksija na prijelomu (mm)	0.59	0.56	0.63	0.60	0.58	0.58	0.59	0.57	0.61	0.60	0.59
	0.09	0.07	0.07	0.09	0.08	0.14	0.09	0.08	0.12	0.09	0.10
Strength Snaga (N)	75.4ab	80.8b	70.4a	71.1a	74.1ab	69.9a	73.3	77.4	70.1	75.5	71.7
	20.6	21.4	30.6	23.8	24.9	31.4	21.9	23.0	30.3	24.1	26.2
Elasticity coefficient (N/m) Koeficijenti elastičnosti (N/m)	186218	201189	176363	185560	175408	192810	185889	187738	184944	187873	184592
	49780	42245	47330	34831	45531	67096	42018	44957	57790	46367	50001
Break energy (mJ) Snaga prijeloma (mJ)	0.43	0.46	0.44	0.43	0.43	0.42	0.43	0.44	0.43	0.44	0.43
	0.09	0.09	0.10	0.08	0.07	0.14	0.08	0.08	0.12	0.09	0.10
Percentage of deflection in breaking point (%) Postotak otklona na mjestu loma (%)	1.18	1.12	1.27	1.20	1.16	1.17	1.19	1.14	1.22	1.19	1.17
	0.19	0.15	0.19	0.18	0.16	0.27	0.18	0.15	0.23	0.18	0.21

a, b Means in the same line not sharing a common superscript are significantly different (P<0.05); interactions - insignificant

Table 8. Chemical composition of crude ash of femur bones of hens (means, ±SD)
Tablica 8. Kemijski sastav sirovog pepela bedrene kosti kokoši

	Experimental groups - Pokusna grupa						Non-phytate P level Razina nefitinskog P (g/kg)			Cereal grains Žitarice	
	I	II	III	IV	V	VI	P-1.4	P-3.0	P-1.4 + phytase	Maize Kukuruz	Maize Barley Wheat Kukuruz Ječam Pšenica
Weight of dried bone (g)	5.01	5.23	5.13	5.24	5.22	5.21	5.13	5.23	5.17	5.13	5.22
Težina suhe kosti (g)	0.64	0.68	0.83	0.69	1.02	0.64	0.66	0.85	0.73	0.71	0.78
Dry matter (%)	93.0	93.8	93.9	93.7	93.1	94.0	93.4	93.4	94.0	93.6	93.6
Suha tvar (%)	2.51	1.43	1.04	1.36	1.15	1.85	2.01	1.32	1.47	1.77	1.49
Crude ash (%)	52.0	52.6	50.8	50.0	51.3	50.7	51.0	51.9	50.8	51.8	50.7
Pepeo (%)	3.66	3.52	4.31	3.01	4.47	3.06	3.43	4.00	3.65	3.81	3.51
Ca (g/kg of crude ash)	284.3	285.4	284.7	288.1	282.8	284.3	286.2	284.1	284.5	284.8	285.0
Ca (g/kg pepela)	6.65	5.36	7.29	7.63	8.97	10.07	7.26	7.35	8.60	6.31	8.97
P (g/kg of crude ash)	157.3	161.5	156.7	159.4	158.3	156.1	158.3	159.9	156.4	158.5	157.9
P (g/kg pepela)	5.57	4.05	4.37	4.20	2.83	4.76	4.94	3.78	4.48	5.06	4.13
Mg (g/kg of crude ash)	5.48	5.74	5.51	5.52	5.64	5.58	5.50	5.69	5.54	5.58	5.58
Mg (g/kg pepela)	0.23	0.36	0.30	0.33	0.32	0.28	0.28	0.34	0.29	0.32	0.31
Zn (mg/kg of crude ash)	393.4	395.2	392.9	381.1	403.0	392.5	387.3	399.1	392.7	393.8	392.2
Zn (mg/kg pepela)	25.66	22.48	23.11	23.84	19.49	29.85	25.02	20.96	26.10	23.12	25.69

All differences among groups are insignificant

Table 9. Chemical composition of crude ash of tibia bones of hens (means, ±SD)
Tablica 9. Kemijski sastav pepela kosti goljenice kokoši

	Experimental groups - Pokusna grupa						Non-phytate P level Razina nefitinskog P (g/kg)			Cereal grains Žitarice	
	I	II	III	IV	V	VI	P-1.4	P-3.0	P-1.4 + phytase	Maize Kukuruz	Maize Barley Wheat Kukuruz Ječam Pšenica
Weight of dried bone (g)	6.07	6.41	6.21	5.92	6.32	6.22	6.00	6.36	6.21	6.23	6.15
Težina suhe kosti (g)	0.78	1.14	0.75	0.73	0.76	0.81	0.75	0.95	0.76	0.89	0.76
Dry matter (%)	93.3	93.8	93.9	93.7	93.2	93.7	93.5	93.5	93.8	93.7	93.6
Suha tvar (%)	2.01	1.40	0.84	1.34	1.07	1.76	1.69	1.25	1.35	1.47	1.40
Crude ash (%)	52.8	53.0	52.2	52.2	52.6	51.8	52.5	52.8	52.0	52.7	52.2
Pepeo (%)	3.53	3.42	3.38	2.82	3.56	2.66	3.14	3.42	2.98	3.36	2.97
Ca (g/kg of crude ash)	252.5	252.6	254.9	253.1	247.1	254.6	252.8	249.9	254.8	253.3	251.6
Ca (g/kg pepela)	18.95	16.42	19.06	20.23	14.41	22.55	19.17	15.37	20.42	17.69	19.09
P (g/kg of crude ash)	156.4	159.1	160.7	159.5	157.5	163.2	157.9	158.3	161.9	158.7	160.1
P (g/kg pepela)	9.16	9.74	5.57	8.35	12.66	5.25	8.72	11.07	5.44	8.32	9.30
Mg (g/kg of crude ash)	5.57	5.61	5.74	5.58	5.61	5.66	5.57	5.61	5.70	5.64	5.62
Mg (g/kg pepela)	0.46	0.36	0.57	0.53	0.54	0.53	0.49	0.45	0.54	0.47	0.52
Zn (mg/kg of crude ash)	369.0	368.0	376.5	359.0	373.1	373.0	364.0	370.5	374.8	371.2	368.3
Zn (mg/kg pepela)	36.35	31.58	45.80	33.96	29.45	27.98	34.78	29.98	37.16	37.45	30.44

All differences among groups are insignificant

DISCUSSION

In previous studies a reduced dietary level of non-phytate phosphorus from about 3.0 g/kg to 1.9 or 1.3 g/kg did not decrease the performance of hens and egg shell quality (Htoo and Liebert, 2001; Jamroz et al., 2003). Similar results were observed in the present long-term experiment, when using the diets with non-phytate phosphorus at the level of 1.41 or 3.0 g/kg, however, significantly ($P<0.05$) lower laying rate and egg weight were obtained only in treatment group fed the low-P maize-barley-wheat-based diet. For other experimental parameters, such as feed intake ($P<0.05$) and feed conversion, as well as the percentage of cracked eggs ($P<0.05$), the kind of cereal grains used in the diet formulation showed greater effect than phosphorus level or phytase supplementation of low phosphorus diets. These results are in contrast to the effects described by Hadorn and Wiedmer (1998) and Oloffs et al. (2000) and Al-Kouri et al. (2004). Low phytase efficacy in diets containing a combination of maize, wheat and barley in the present experiment was in opposition to the effect found by Liebert et al. (2001).

The specificity of our experiment was the long-time monitoring of laying performance as affected by feeding of low-phosphorus diets. During more than 12 months of egg production the average laying rate reached about 87 %, average egg weight was 61.8 g and total egg mass about 20 kg per hen. In our opinion the results obtained with such low non-phytate P level (1.41 g/kg) and during such a long experiment, cannot be recognized and evaluated as negative.

A clear response of laying hens to the low-P level was observed with regard to egg shell strenght and bone strength which was higher in both positive control groups (P 3.0 g/kg) as compared with negative controls (1.41 g available P, without supplementation).

Better bone mineralization obtained as an effect of mineral-phytic acid bonds degradation by the added phytase and further release of minerals (Sauveur,1991; Quian et al., 1996; Zhang and Coon, 1997; Um and Paik, 1999; Jamroz et al., 2003) was not observed in our experiment.

Calcium concentration in bones was very stable and reached about 285 (femur) and about 252 g/kg (tibia) of crude ash (CA), P content was about 158 and 159 g/kg CA, respectively. Magnesium content in ash was 5.6 g/kg and Zn – 393 and 370 mg/kg CA, respectively. These indices are similar to the data obtained in experiment realized by Jamroz et al. (2003).

The overall bone quality of hens was comparable to the data reported by Fleming et al. (1994) and Zhang and Coon (1997). Improved retention of zinc in the bones of hens as an effect of phytase application that was suggested by Sebastian et al. (1966) and Yi et al. (1996) was not observed in the present experiment.

REFERENCES

1. Al-Kouri, S., Liebert, F., Sünder, A. (2004). Effect of citrate and phytase supplementation on laying performance, egg shell quality and tibia mineralization of layers with marginal P-supply. Proceed. 8. Tagung Schweine- und Geflügelernährung. Lutherstadt Wittenberg,13-18.
2. AOAC,2000. Official Methods of Analysis. Association of Official Analytical Chemists. Internat. 17th Ed, Gaithersburg, MD.
3. Bach Knudsen, K. C., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci.Technol.,67,319-338.
4. Brandt S., 1997. Statistical and Computational Methods in Data Analysis. Ed.3. Springer Verlag, New York, USA.
5. European Tables of Energy Values of Feedstuffs for Poultry, 1989. WPSA, Beekbergen (The Netherlands).
6. Gordon, S. H., Short, F. J., 2001. The effect of phytase supplementation and limestone granules on tibia breaking strength of laying hens in cage systems. Proceedings 13th Europ.Symp.Poultry Nutr., Blankenberge, pp. 242-243.
7. Hadorn, R., Wiedmer, H., 1998. Effekte einer Phytasezulage zu einem Phosphor reduzierten Legehennenfutter. Arch. Geflügelk., 62,3,97-103.
8. Hempel, P., Elwert, Ch., Rodehutscord, M. (2004a). Praeceacale Nettoabsorption von Phosphor aus mineralischen Quellen bei Legehennen. Proceed.

8. Tagung Schweine- und Geflügelernährung. Lutherstadt Wittenberg,173-176.

9. Hempel, P., Strobel, E., Elwert, Ch., Rodehutscord, M. (2004b). Untersuchungen zum Phosphorbedarf von Legehennen in verschiedenen Abschnitten der Legeperiode. Proceed. 8.Tagung Schweine- und Geflügelernährung. Lutherstadt Wittenberg, 84-86.

10. Htoo, J. K., Liebert, F., 2001. Efficiency of phytate in layer diets without phosphorus supplementation. Proceed.8th Symp. Witamine u.Zusatzstoffe in der Ernährung von Mensch und Tier, Jena, pp. 477-480.

11. Jamroz, D., Orda, J., Skorupińska, J., Wiliczkiewicz, A., Wertelecki, T., Żyłka, R., Klünter, A-M. (2003). Reaction of laying hens to low-phosphorus diets and addition of different phytase preparations. J. Anim. Feed Sci.,12,95-110.

12. Jamroz, D., Skorupińska, J., Orda, J., Wiliczkiewicz, A.,1998. Influence of antibiotica promotors on the egg production and quality as well as nitrogen and phosphorus utilization in laying hens. Arch. Geflügelk.,62,2,1-9.

13. Kornegay, E. T., Denbow, D. M., Yi, Z., Ravindran V.,1996. Response of broilers to graded levels of microbial phytase added to maize-soya bean-based diets containing three levels of non-phytate phosphorus. Brit.J.Nutr.,75,839-852.

14. Leske, K. L., Coon, C. N., 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poult. Sci.,78, 1151-1157.

15. Liebert, F., Htoo, J. K., Sünder, A. (2005). Performance and nutrients utilization of laying hens fed low-phosphorus corn-soybean and wheat-soybean diets supplemented with microbial phytase. Poult. Sci.,84,1576-1583.

16. Musapour, A., Pourreza, J., Samie, A., Moradi-Shahrbak, H. (2005). The effects of phytase and different level of dietary calcium and phosphorus on phytate phosphorus utilization in laying hens. Intern.Poult. Sci.,4(8), 560-562.

17. Nutrient Requirements of Poultry. Nutritive Value of Feedstuffs. The Kielanowski Institute of Animal Physiology and Nutrition, Jabłonna, 2005, 4th Ed., (in Polish).

18. Oloffs, K., Cossa, J., Jeroch, H., 2000. Phosphorus utilisation from different vegetable feedstuffs by laying hens. Arch. Geflügelk., 64,1,24-28.

19. Panda, A. K., Rama Rao, S.V., Raju, M. V. L. N., Bhanja, S. K. (2005). Effect of microbial phytase on production performance of White Leghorn layers fed on a diet low in non-phytate phosphorus. Brit.Poult. Sci., 46,6,467-469.

20. Sauveur, B. 1991. Phosphorus metabolism in laying hens: effects of intermittent calcium and phosphorus supplies. Proceedings 8th Europ.Symp.Poultry Nutr., Venezia-Mestre,Italy, pp. 213-228.

21. Sebastian, S., Touchburn, S. P., Chavez, E. R., Laque, P. C., 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-soybean diets. Poult. Sci., 75,729-736.

22. Simons, P. C. M., Versteegh, H. A. J., Jongbloed, A. W., Kemme, P. A., Slump, P., Bos, K. D., Wolters, M. G. E., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr., 64, 525-540.

23. Statgraphics Plus ver.7 for DOS,1993, Statistical Graphics System.

24. Szkudelski, T., 1997. Phytic acid – its influence on organism. J.Anim.Feed Sci.,6, 427-438.

25. Quian, H., Kornegay, E. T., Veit, H. OP., 1996. Effects of supplemental phytase and phosphorus on histological, mechanical and chemical traits of tibia and performance of turkey fed on soya bean meal-based semi-purified diets high in phytate phosphorus. Brit.J.Nutr.,76,263-272.

26. Um, J. S.. Paik, J. K., 1999. Effects of microbial phytase supplementation on egg production, egg shell quality and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci.,78,75-79.

27. Yi, Z., Kornegay, E. T., Denbow, D. M., 1996 Supplemental microbial phytase improves zinc utilization in broilers. Poult. Sci.,75,540-546.

28. Zhang, B., Coon, C. N., 1997. The relationship of various tibia bone measurements in hens. Poult. Sci.,76,1698-1701.

29. Żyła, K., Ledoux, D. R., Kujawski, M., Veum, T. L., 1996. The efficacy of an enzymatic coctail and a fungal mycelium in dephosphorylating corn-soybean meal-based feeds fed to growing turkeys. Poult. Sci.,75,381-387.

SAŽETAK

Svrha ovog istraživanja bila je ocijeniti odgovor kokoši nesilica na obroke s niskim fosforom na bazi raznih vrsta žitarica s dodatkom mikrobijelne fitaze. Nesenje, unos hrane, kakvoća ljuske jaja kao i parametri čvrstoće kosti i sadržaj Ca, P, Mg i Zn u kostima bili su obuhvaćeni kao pokusni parametri. U dobi od 17 tjedana 288 pilenki Lohmann Brown izabrano je za šest hranidbenih tretmana, a svaki tretman sastojao se od 12 kaveza po 4 pilenke. Do početka nesenja pilenke su hranjene standardnim obrocima koji su sadržavali 145 g sirovih bjelančevina i 11.3 MJ ME, 4 g nefitinskog P i 10.6 g Ca po kilogramu hrane. Pokusni obroci su se prvog dana nesenja temeljili na kukuruzu (obroci I, II, III) ili na kombinaciji kukuruza, ječma i pšenice (obroci IV, V, VI) i sadržavali su oko 165 g CP i 11.2 Mj ME/kg. U obrocima I, III ili IV i VI razina nefitinskog fosfora smanjena je na 1.41 g/kg dok je u kontrolnim obrocima (III ili V/) količina nefitinskog P bila 3.0 g/kg. U obrocima s niskim P (III i VI) uključena je mikrobijelna 6-fitaza na razini od 450 FTU/ kg obroka.

Razina fosfora u obrocima nije djelovala na postotak nesivosti u pokusu od 54 tjedna. Više razine P u hrani povećale su samo čvrstoću ljuske jajeta. (P < 0,01). Dodavanje fitaze u obroke s niskim P nije djelovalo na unos hrane i kakvoću jaja, međutim poboljšalo je čvrstoću ljuske jajeta u usporedbi sa skupinama s niskim P bez dodataka. Što se tiče čvrstoće i elastičnosti goljenice, razina P i dodavanje fitaze nije imalo vidljivih učinaka. Najbolji parametri koji karakteriziraju kakvoću kosti dobiveni su dodavanjem 3 g nefitinskog P na kg obroka.