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Reaction of laying hens to low phosphorus diets and addition of different phytase preparations

D. Jamroz[1], J. Orda[1], J. Skorupińska[1], A.Wiliczkiewicz[1], T. Wertelecki[1], R. Żyłka[2] and A.-M. Klünter[3]

Wrocław Agricultural University
[1]Department of Animal Nutrition and Feed Quality
Chełmońskiego 38D, 51-630 Wrocław, Poland
[2]Department of Physics and Biophysics
Norwida 25/27, 50-375 Wrocław, Poland
[3]CRNA, Societe Chimique Roche SA
Saint Louis Cedex, France

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ABSTRACT

The purpose of the presented study was to determine the reaction of hens to low-phosphorus diets and supplementation with different microbial phytases by assessing laying performance, feed intake, egg shell quality, as well as bone strength parameters and bone contents of Ca, P, Mg, and Zn. At the age of 16 weeks, 288 Lohmann Brown pullets were allocated to 6 dietary treatments, each treatment group consisting of 12 cages, 4 birds per cage. Before the laying period the pullets were fed with a standard diet containing 150 g crude protein (CP) 11.3 MJ ME, 4 g available P and 10.6 g of Ca per kg feed. The experimental diets fed from the first day of laying were based on wheat and barley and contained 165 g CP and 11.2 MJ ME/kg. In diets I to V the level of available P was lowered to 1.89 g/kg in the first period of laying and to 1.31 g/kg in second and third periods, while in the control (VI) diet the amount of available P was 3.09 g/kg. In diets II-V two kinds of phytase were applied at levels of 300 or 450 U/kg of diet. The phosphorus level did not affect the laying rate. The higher P level enhanced egg shell thickness. Supplementation with phytase did not affect the performance of hens. Improved feed conversion was noted only when the diet was supplemented with 450 U of phytase B per kg. Phytase supplementation of low-P diets significantly improved the strength and elasticity of the tibia and femur, which in groups II-V were on a similar level as in the positive control group.

KEY WORDS: microbial phytase, performance, egg shell quality, bone quality, laying hens

INTRODUCTION

Plant compounds that are used in formulating mixtures for poultry contain sufficient amounts of phosphorus to cover the birds' requirements. However, about 60-70% of total P exists in the form of phytic acid, which makes the phosphorus unavailable or hardly available for poultry (Oloffs et al., 2000; Liebert et al., 2001).

The presence of phytic acid in feed components worsens the absorption of both trace- and macroelements from the intestinal digesta (Van der Klis, 1991), which may lead to their deficit (De Groote and Huyghebaert, 1997). Phytic acid may bind some proteins and form stable phytate-protein complexes that can lower the utilization of proteins and amino acids. Furthermore, such complexes with intestinal proteases may inhibit protein degradation and digestion (Yi et al., 1996b; Szkudelski, 1997; Ravindran et al., 1999).

Microrganisms are able to hydrolyse phytates; phytase-myo-inositol hexaphosphate-phospho-hydrolase originating from microbes is an enzyme that releases phosphorus from phytate complexes (Żyła et al., 1989, 1996; Simons et al., 1990; Kornegay et al., 1996; Hadorn and Wiedmer, 1998). The activity of phytase produced by intestinal microorganisms is low, also the native phytases of wheat, barley or their by-products are not sufficient for significant improvement of phosphorus utilization (Oloffs et al., 2000; Liebert et al., 2001). Supplementation of diets with microbial phytase preparations can improve the bioavailability of phosphorus from dietary phytate (Sebastian et al., 1996; Leske and Coon, 1999; Saynaeve et al., 2000), increase P utilization and digestion, protein utilization, and enhance N retention in broilers and laying hens (Yi et al., 1996a; Szkudelski, 1997). Beneficial effects of phytases on phosphorus utilization, bone strength, and egg shell quality were also found in other experiments on hens (Um and Paik, 1999; Gordon and Short, 2001).

The purpose of the present study was to determine the response of laying hens to low-phosphorus diets supplemented with two levels of different phytase preparations. Laying performance, main parameters of egg shell quality, bone composition and strength parameters were investigated.

MATERIAL AND METHODS

The experiment was performed with 288 Lohmann Brown layers. At the age of 16 weeks pullets were transferred to cages, 4 pullets per cage and allocated to 6 treatments, each encompassing 12 cages. The average initial body weight of birds was similar in all groups. Feed and water were supplied *ad libitum*. The temperature in the laying house varied from 18 to 26°C during the warm summer days. The lighting program was 10 h darkness and 14 h light with an intensity of about 30 lx.

Before the laying period, pullets were fed a standard diet containing 150 g crude protein and 11.3 MJ ME/kg

Three diets were prepared to contain 4.01 or 3.29 total P per kg (low-phosphorus diet LP-1 and LP-2) and 5.5 g P per kg (control diet) (Table 1).

TABLE 1

Composition of diets, g/kg

Components	Diets		
	LP-1	LP-2	control
Barley var. Rudzik	300	300	300
Winter wheat var. Cobra	414	414	366
Soya oil	16	16	30
Soyabean oilmeal	178	178	189
Dicalcium phosphate	4	-	13
Limestone	74	78	88
Mineral-vitamin premix[1]	14	14	14
Calculated			
metabolizable energy, MJ/kg	11.20	11.20	11.20
crude protein (N x 6.25)	165.0	165.0	165.2
methionine	3.53	3.53	3.52
lysine	8.95	8.95	9.18
Ca	30.00	31.47	37.00
P	4.01	3.29	5.50
P available[2]	1.89	1.31	3.09
Mg	1.32	1.32	1.32
Zn, mg/kg	83.1	84.1	80.3

[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; methionine 1 g and NaCl 4 g

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

Diets LP-1 and LP-2 were fed either unsupplemented or supplemented with two phytase preparations at a level of 300 or 450 phytase units per kg (Table 2). All diets were fed *ad libitum* in mash form. Group VI was fed the control diet from week 19 of life to the end of the experiment (298 days). From week 1 to 14 week of the laying period groups I to V were fed diet LP-1 that was either unsupplemented or supplemented, thereafter until the end of the experiment respective diets LP-2 containing 1.31 g of available P per kg.

Laying performance was determined during 298 days of laying. The number of eggs was recorded daily, all eggs from each cage were weighed once a week. Feed intake, cleanness and abnormalities of the egg shell and mortality of birds were recorded during the laying period.

TABLE 2

Design of experiment

Group	Diet	Phytase[2], U/kg diet
I	LP-1 and LP-2	No supplement
II	LP-1 and LP-2	+ A[1] 300
III	LP-1 and LP-2	+ A 450
IV	LP-1 and LP-2	+ B[1] 300
V	LP-1 and LP-2	+ B 450
VI	Control diet	No supplement

[1] A (NATUPHOS® 5000 G, lot NHPG 159 6740 phytase U/g
B (RONOZYME® P (CT), lot HB 900003 3133 phytase U/g

[2] 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

During weeks 13, 19, and 33 from the start of the experiment (measurement periods 1, 2, and 3) the quality of eggs and bones and the content of Ca, P, Mg and Zn in bones were estimated.

Thirty eggs were randomly selected from each group to determine the weight of whole eggs. Egg analyses were performed using standard procedures with a PM-600 PX-processor apparatus (Technical Service and Supplies Ltd., York, England). Shell thickness was measured in three points using a Modul with Micrometric screw (Mitutoys). Each measurement was carried out in duplicate. Shell strength was determined under a maximum pressure of 3.5 kg.

In each measurement period one hen from each cage was randomly chosen, weighed and killed. From five selected animals per group the tibia and femur bones from the left leg were prepared for evaluation of strength and elasticity. Analysis of physical properties was done on fresh bones that had been stored in a refrigerator at a temperature of 4-5°C. Bone deflection (h) was measured by a standard method, in which the force (Fn) was applied to the shaft of the bone supported at both epiphyses, taking into account Hook's law concerning bone elasticity, where the vector of deflection is proportional to the supported force ($Fn = \alpha \times h$), and α is the coefficient of elasticity.

At the breaking point, the inner and the outer diameter as well as the cortex thickness of the bone were measured using a slide calliper assuming that the bone cross section was circular. Measurements were taken from ten different, randomly chosen points. On the basis of the obtained data, the surface of bone and the cross section were determined. The elasticity of the bones was estimated by a mediation model:

$$\alpha = E \quad \frac{\pi (D^4 - d^4)}{6 L^3}$$

where:
D - external diameter
d - internal diameter
L - distance between support points
E - Young's Modulus.

The parameters were evaluated according to variable force (from low to high pressure) up to the bone breaking point. Maximal loading was compared with the strength coefficient of long bones (W). Finally, the coefficient of elasticity was estimated by comparing two factors: deflection and breaking force: $\alpha = \Delta F n / \Delta h$. All estimated parameters of bone quality were expressed in relation to 100 g of metabolic body weight.

The chemical composition of the diets was determined according to standard methods (AOAC, 1990). The Ca, P, Mg and Zn contents were determined in dried 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.

The energy value of the diets (Table 1) was calculated according to the European Table (1989).

Statistical calculations were conducted using the Statgraphics Plus ver. 7.0 software. Data was compared using one-way and two-way ANOVA (Brandt, 1997). A split-plot ANOVA was used to test for period x treatment interactions. The significance of differences between 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

In the first measurement period, the body weight of hens averaged 1855 g, except in group I (2052 g); in the second period it averaged 1984 g, in the third, 2072 g. The low phosphorus level in the diets of groups I to V did not result in significant changes of body weight during the 298 days of the experiment (Table 3). The health status of the birds was very good.

Phosphorus level and phytase addition did not significantly affect laying rate, egg weight, number of laid eggs, or total egg mass, although slightly better results (by 3%) in total egg mass were noted in group V, in which the diet was supplemented with phytase B at a rate of 450 U/kg feed and in group III (phytase A at 450 U/kg)

TABLE 3

Body weight of hens, g[1]

Item	Group						SEM
	I	II	III	IV	V	VI	
Measurement 1	2052	1827	1855	1851	1902	1840	34.2
Measurement 2	1877^{a}	1907^{a}	2216^{b}	1893^{a}	1994^{ab}	2017^{ab}	39.4
Measurement 3	2128	2078	2000	2154	2028	2042	43.6
Mean for measurement	1		2		3		
	1888^{a}		1973^{ab}		2065^{b}		23.3

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b}P<0.05; ^{A,B} P<0.01

[1] means in measurements 1, 2 and 3 were calculated for 12 cages x 4; 12 x 3 and 12 x 2 birds, respectively

TABLE 4

Laying performanc (mean for 298 days)

Parameters	Group						SEM
	I	II	III	IV	V	VI	
Laying rate, %	92.8	92.6	93.1	92.8	93.2	91.9	0.36
Egg weight, g	61.0	59.6	60.9	60.7	62.2	61.2	0.36
Total egg mass, kg/hen	16.9	16.4	16.9	16.7	17.3	16.9	0.12
Feed intake, g/bird /day	119.0^{A}	124.3^{AB}	129.4^{B}	117.0^{A}	119.9^{A}	122.4^{AB}	0.74
Feed intake, kg/kg egg mass	2.11^{A}	2.26^{A}	2.29^{A}	2.11^{A}	2.07^{B}	2.15^{A}	0.02
Cracked eggs, %	2.16	1.97	2.52	2.58	2.77	1.65	0.24
Deformed eggs, %	0.01^{a}	0.01^{a}	0.14^{ab}	0.01^{a}	0.01^{a}	0.26^{b}	0.04

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} P<0.05; ^{A,B} P<0.01

(Table 4). Feed conversion calculated per kg of egg mass was significantly lower in group V than in other groups. Differences in the percentage of cracked or deformed eggs among groups were relatively low and statistically not significant (Table 4).

Egg shell thickness in group VI was higher than in group I; in the first and second periods these differences were highly significant (P≤0.01), however in the third period (in older hens) no significant difference was found. In young hens (in the first period) egg shell thickness was significantly improved by addition of phytase A at 300 U/kg to the diet and in the second period, by phytase B at 450 U/kg (Table 5).

The strength and elasticity of the tibia and femur are presented in Tables 6 and 7. Similarly as egg shell quality, large individual variation of the analysed bone parameters were observed. The most important parameters of bone quality, i.e. strength (N) and strength to surface ratio, indicate that the phosphorus content in

TABLE 5

Parameters of egg shell quality in three measurement periods

Parameters	Group						SEM
	I	II	III	IV	V	VI	
			Measurement 1				
Laying rate, %	95.0	97.0	96.3	95.5	94.0	95.7	
Egg weight, g	61.7^{ab}	59.6^{Aa}	61.8^{ab}	61.3^{ab}	62.3^{b}	62.5^{B}	0.31
Egg shell strength, kg	3.14^{A}	3.18^{A}	3.04^{A}	2.99^{AB}	2.82^{AB}	2.56^{B}	0.05
Egg shell thickness, mm	0.326^{A}	0.342^{B}	0.326^{A}	0.324^{A}	0.319^{A}	0.340^{B}	0.01
			Measurement 2				
Laying rate, %	95.0	92.5	94.6	96.7	94.2	95.2	
Egg weight, g	64.1^{ab}	62.5^{a}	64.6^{ab}	63.5^{ab}	65.0^{b}	65.1^{b}	0.32
Egg shell strength, kg	3.12^{A}	3.05^{a}	2.88^{ab}	2.79^{ab}	2.62^{Bb}	3.08^{A}	0.05
Egg shell thickness, mm	0.289^{A}	0.286^{A}	0.298^{A}	0.303^{A}	0.367^{B}	0.359^{B}	0.01
			Measurement 3				
Laying rate, %	90.6	86.3	86.5	88.6	93.1	92.0	
Egg weight, g	64.9	64.4	64.4	64.9	65.3	65.4	0.34
Egg shell strength, kg	2.57	2.61	2.46	2.60	2.31	2.53	0.05
Egg shell thickness, mm	0.358^{a}	0.357^{a}	0.377^{b}	0.355^{a}	0.357^{a}	0.362^{a}	0.02
			Means for three measurements				
Egg weight, g	63.6^{ab}	62.2^{Aa}	63.6^{ab}	63.2^{ab}	64.2^{Bb}	64.3^{B}	0.31
Egg shell strenght, kg	2.94^{A}	2.95^{A}	2.79^{AB}	2.79^{AB}	2.58^{B}	2.72^{AB}	0.05
Egg shell thickness, mm	0.324^{A}	0.328^{A}	0.337^{ABC}	0.327^{AB}	0.347^{BC}	0.354^{C}	0.01
Means for measurements	1		2		3		
egg weight, g	61.5^{A}		64.1^{B}		64.9^{B}		0.20
egg shell strength, kg	2.95^{A}		2.92^{B}		2.51^{C}		0.03
egg shell thickness, mm	0.33^{A}		0.32^{B}		0.37^{C}		0.00

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} $P<0.05$; ^{A,B} $P<0.01$

the diet and supplementation with phytase significantly affected these parameters in the first and third periods (strength to surface ratio). A clear tendency towards improvement of bone strength was observed particularly in the positive control group (VI) in comparison with groups I-V.

The parameters of bone strength (N/mm^2) and elasticity were improved in hens fed diets supplemented with phytases in comparison with the low-P group (I). Addition of phytases to the low phosphorus diet improved both tibia and femur strength and elasticity in nearly all cases (except group II). The effects were slightly better with higher phytase dosages. The best tibia quality was found in hens from group V and femur quality in groups III and V.

The average values of the analysed parameters for the tibia and femur in subsequent measurement periods indicate that in older birds these parameters were higher in both the low P (I) and the positive control group (VI).

TABLE 6

Parameters of tibia bones of hens calculated per 100 g of metabolic body weight ($BW^{0.67}$)

Parameters	Group						SEM
	I	II	III	IV	V	VI	
				Measurement 1			
Cortex thickness, mm	0.49	0.52	0.47	0.46	0.57	0.56	0.02
Surface, mm^2	10.23	11.70	10.17	9.27	13.23	12.86	0.59
Strength, N	69.16^{a}	78.67^{ab}	74.10^{a}	74.91^{a}	79.59^{ab}	98.99^{b}	3.32
Strength to surface ratio, N/mm^2	4.17^{abc}	4.44^{abc}	4.82^{abc}	5.46^{b}	3.97^{a}	5.25^{b}	0.18
Elasticity alpha - coefficient, N/mm	95.35^{a}	120.07^{ab}	113.60^{ab}	111.25^{ab}	121.84^{ab}	131.61^{b}	5.40
				Measurement 2			
Cortex thickness, mm	0.49^{bc}	0.40^{a}	0.43^{ab}	0.43^{ab}	0.48^{bc}	0.51^{c}	0.01
Surface, mm^2	9.71^{ab}	8.20^{a}	8.45^{a}	9.03^{ab}	9.92^{ab}	10.82^{b}	0.31
Strength, N	62.49^{a}	60.15^{a}	68.24^{a}	64.27^{a}	74.60^{b}	73.78^{b}	2.38
Strength to surface ratio, N/mm^2	4.14^{a}	4.75^{b}	4.71^{b}	4.46^{ab}	4.65^{b}	4.25^{a}	0.14
Elasticity alpha - coefficient, N/mm	103.59	94.76	111.83	101.53	118.60	116.84	3.90
				Measurement 3			
Cortex thickness, mm	0.51	0.43	0.49	0.43	0.47	0.45	0.01
Surface, mm^2	10.02	9.28	9.75	9.07	9.85	9.01	0.21
Strength, N	68.42^{a}	61.55^{a}	79.36^{b}	82.40^{b}	70.54^{a}	81.01^{b}	2.96
Strength to surface ratio, N/mm^2	4.04^{a}	3.99^{a}	5.06^{b}	4.52^{ab}	4.41^{a}	5.59b	0.17
Elasticity alpha - coefficient, N/mm	116.54^{a}	113.31^{a}	169.81^{b}	118.90^{ab}	119.78^{ab}	140.97^{ab}	6.60
				Means for three measurements			
Cortex thickness, mm	0.49	0.45	0.46	0.44	0.51	0.51	0.01
Surface, mm^2	9.99	9.73	9.51	9.14	11.00	10.90	0.25
Strength, N	66.69^{a}	66.79^{a}	73.91^{ab}	73.94^{ab}	74.91^{ab}	84.59^{b}	1.76
Strength to surface ratio, N/mm^2	4.12^{a}	4.39^{ab}	4.86^{ab}	4.87^{ab}	4.34^{ab}	5.03^{b}	0.09
Elasticity alpha - coefficient, N/mm	105.07	109.48	130.37	110.66	120.07	130.67	3.30

Means for measurements	1	2	3
Cortex thickness, mm	0.51	0.46	0.46
Surface, mm^2	11.24^{A}	9.40^{B}	9.50^{B}
Strength, N	79.24^{A}	67.33^{B}	73.38^{AB}
Strength to surface ratio, N/mm^2	4.68	4.49	4.59
Elasticity alpha - coefficient, N/mm	115.95^{AB}	108.01^{A}	128.90^{B}

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} $P<0.05$; ^{A,B} $P<0.01$

TABLE 7

Parameters of femur bones of hens calculated per 100 g of metabolic body weight (BW $^{0.67}$)

Parameters	Group						SEM
	I	II	III	IV	V	VI	
				Measurement 1			
Cortex thickness, mm	0.52	0.50	0.49	0.44	0.51	0.52	0.01
Surface, mm^2	10.77	11.71	10.67	10.16	12.49	11.46	0.38
Strength, N	74.63^{a}	78.73^{ab}	80.43^{ab}	82.52^{ab}	86.89^{ab}	97.44^{b}	2.81
Strength to surface ratio, N/mm^2	4.24^{a}	4.49^{ab}	4.98^{ab}	5.37 ^{b}	4.46 ^{ab}	5.64^{b}	0.18
Elasticity alpha - coefficient, N/mm	96.82^{a}	105.95^{ab}	114.19^{ab}	99.77^{a}	102.42^{ab}	123.31^{b}	4.20
				Measurement 2			
Cortex thickness, mm	0.50b^{c}	0.40^{a}	0.42^{a}	0.48^{abc}	0.50^{b}c	0.52^{c}	0.01
Surface, mm^2	10.65^{a}	8.92^{b}	9.52^{ab}	10.48^{a}	10.92^{a}	10.96^{a}	0.24
Strength, N	77.60^{a}	70.95^{ab}	76.33^{a}	58.73^{b}	83.49^{a}	73.37^{ab}	2.46
Strength to surface ratio, N/mm^2	4.65^{ab}	5.09^{a}	4.67^{ab}	3.54^{b}	4.74^{ab}	4.13^{ab}	0.14
Elasticity alpha - coefficient, N/mm	84.46^{ab}	83.88^{ab}	95.65^{ab}	75.64^{a}	103.01^{b}	91.23^{ab}	3.00
				Measurement 3			
Cortex thickness, mm	0.47	0.47	0.47	0.44	0.54	0.46	0.02
Surface, mm^2	10.40	10.77	10.53	10.36	11.86	10.18	0.32
Strength, N	72.41	73.18	95.28	89.58	97.48	84.75	3.97
Strength to surface ratio, N/mm^2	4.10^{a}	4.06^{a}	5.62^{b}	5.06^{ab}	5.04^{ab}	5.46^{b}	0.19
Elasticity alpha - coefficient, N/mm	94.18^{a}	99.47^{ab}	129.49^{ab}	134.50^{b}	130.96^{b}	120.96^{ab}	6.90
				Means for three measurements			
Cortex thickness, mm	0.50	0.46	0.46	0.45	0.51	0.50	0.01
Surface, mm^2	10.61	10.47	10.27	10.34	11.75	10.87	0.20
Strength, N	74.88	74.29	83.74	76.94	88.70	85.19	1.98
Strength to surface ratio, N/mm^2	4.33	4.55	5.08	4.66	4.73	5.08	0.10
Elasticity alpha - coefficient, N/mm	91.82	96.53	113.01	103.30	110.66	111.83	3.00

Means for measurements	1	2	3
Cortex thickness, mm	0.49	0.47	0.47
Surface, mm^2	11.21	10.27	10.65
Strength, N	83.44^{a}	73.31^{b}	84.67^{a}
Strength to surface ratio, N/mm^2	4.86	4.46	4.86
Elasticity alpha - coefficient, N/mm	107.13^{a}	88.88^{Ab}	117.43^{B}

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} $P<0.05$; ^{A,B} $P<0.01$

TABLE 8

Content of crude ash in tiba bone and content of minerals in crude ash, %

Parameters	Group						SEM
	I	II	III	IV	V	VI	
				Measurement 1			
Crude ash, %	45.5^{a}	47.2^{ab}	48.7^{b}	47.0^{ab}	46.8^{ab}	47.3^{ab}	0.34
In crude ash							
Ca	52.3	49.2	50.6	51.4	51.1	51.7	4.86
P	36.5	34.9	34.7	34.9	35.5	35.8	2.84
Mg	1.18	1.15	1.05	1.07	1.05	1.14	0.17
Zn, ppm	684.2	710.2	700.1	654.6	627.4	748.8	19.4
				Measurement 2			
Crude ash, %	51.6^{ab}	48.4^{a}	51.3^{ab}	49.3^{ab}	52.1^{b}	50.3^{ab}	0.39
In crude ash							
Ca	52.6^{ab}	57.0^{a}	52.7^{ab}	52.3^{ab}	50.1^{ab}	47.2^{b}	9.25
P	31.8^{ab}	35.0^{a}	32.8^{ab}	33.4^{ab}	31.0^{b}	33.0^{ab}	4.06
Mg	0.93	0.97	0.88	0.97	0.90	0.90	0.14
Zn, ppm	708.5	773.6	743.5	746.5	684.3	780.5	13.07
				Measurement 3			
Crude ash, %	50.0^{ab}	46.7^{ab}	50.9^{a}	49.0^{ab}	46.4^{b}	49.9^{ab}	0.59
In crude ash							
Ca	48.1^{a}	53.2^{b}	48.0^{a}	49.8^{ab}	53.7^{b}	50.7^{ab}	6.66
P	32.2^{a}	34.4^{ab}	31.1^{a}	34.0^{ab}	38.5^{b}	34.6^{ab}	7.13
Mg	0.90^{ab}	1.01^{Ac}	0.82^{Ba}	0.94^{b}	0.98^{Abc}	0.98^{bc}	0.16
Zn, ppm	658.4^{a}	768.2^{b}	717.1^{ab}	723.9^{ab}	807.4^{b}	792.1^{b}	15.7
				Means for three measurements			
Crude ash, %	49.0^{AB}	47.4^{A}	50.3^{B}	48.4^{AB}	48.4^{AB}	49.2^{AB}	0.10
Ca	51.0	53.2	50.4	51.2	51.6	49.8	0.30
P	33.5	34.8	32.9	34.1	35.0	34.4	4.13
Mg	1.00^{a}	1.04^{Aa}	0.92^{Bb}	0.99^{ab}	0.98^{ab}	1.01^{a}	3.06
Zn, ppm	683.7	750.7	720.2	708.3	706.4	773.8	9.68

Means for measurements	1	2	3
Crude ash, %	47.1^{Aa}	50.5^{Bb}	48.8^{c}
Ca	51.0	52.0	50.6
P	35.4^{A}	32.8^{B}	34.1^{AB}
Mg	1.11^{A}	0.92^{B}	0.94^{B}
Zn, ppm	687.6	739.5	744.5

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} $P<0.05$; ^{A,B} $P<0.01$

Similarly to tibia quality, supplementation of feed with phytases also positively influenced femur quality in all cases. The reduction of the phosphorus level in diet I decreased the bone quality parameters only in the second and third experimental periods. All calculated interactions were insignificant.

The content of dry matter in the bones was similar in all groups (average 92%). The crude ash content ranged from about 44 to 52% and significant differences between groups were noted (Tables 8 and 9), therefore the concentration of minerals was calculated in relation to crude ash. Tibia and femur ash contained from 48 to 57% Ca, and from 31 to 36.6% P. Only in some cases were the differences between groups significant. Relatively high variability was found in the Mg and Zn contents.

TABLE 9

Content of crude ash in femur bone and content of minerals in crude ash, %

Parameters	Group						SEM
	I	II	III	IV	V	VI	
				Measurement 1			
Crude ash, %	44.0^{a}	45.5^{ab}	46.7^{ab}	46.8^{ab}	47.9^{ab}	50.3^{b}	0.80
In crude ash							
Ca	53.7	54.2	52.2	54.0	52.4	49.4	0.76
P	36.6	36.1	35.6	36.1	36.1	34.0	0.53
Mg	1.23	1.23	1.15	1.12	1.15	1.13	0.02
Zn, ppm	800.0	827.4	774.6	781.5	714.6	771.6	15.0
				Measurement 2			
Crude ash, %	50.0	47.0	48.9	48.1	49.1	49.6	0.51
In crude ash							
Ca	52.7	56.9	54.1	55.0	52.9	51.9	0.80
P	33.8	35.8	33.5	34.7	32.9	32.8	0.43
Mg	1.00	1.02	1.02	1.01	1.01	0.94	0.02
Zn, ppm	802.8	870.9	850.8	819.9	831.9	853.6	11.49
				Measurement 3			
Crude ash, %	47.1	46.6	50.5	48.7	48.1	50.7	0.62
In crude ash							
Ca	50.3	51.2	48.3	50.6	49.5	50.2	0.59
P	34.5	34.8	31.1	33.9	34.7	34.2	0.52
Mg	1.00^{AB}	1.04^{A}	0.86^{B}	0.99^{AB}	0.97^{AB}	1.01^{AB}	0.02
Zn, ppm	817.7^{AB}	829.8^{AB}	757.4^{A}	796.0^{AB}	834.2^{AB}	890.5^{B}	13.19
				Means for three measurements			
Crude ash, %	47.0	46.4	48.7	47.9	48.4	50.2	0.39
Ca	52.5	54.1	51.5	53.2	51.6	50.5	0.45
P	35.3	35.6	33.4	34.9	34.6	33.7	0.30
Mg	1.07	1.01	1.01	1.04	1.04	1.03	0.01
Zn, ppm	806.9	842.7	794.3	799.1	793.6	838.6	8.06

Means for measurements	1	2	3
Crude ash, %	46.8^{A}	48.8^{B}	48.6^{B}
Ca	52.6^{a}	53.9^{A}	50.0^{Bb}
P	35.7^{a}	33.9^{b}	34.0^{b}
Mg	1.17^{A}	1.00^{B}	0.98^{B}
Zn, ppm	778.3^{A}	838.3^{B}	820.9^{B}

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} $P<0.05$; ^{A,B} $P<0.01$

Phytase caused significant diversification of Mg and Zn content only in the third measurement period. The reasons for the lower concentration of P and the higher Mg concentration in the bones, which were observed in the second experimental period, are unclear and difficult to explain.

The coefficients of correlation that were calculated for bone strength and elasticity and Ca and P content in dry matter of bones varied insignificantly between 0.36 and 0.46.

There were some significant differences between groups in Ca, P, Mg and Zn concentrations in crude ash, but they are inconclusive; however, the content of Mg in ash increased and the content of Zn declined with the age of the hens (Tables 8 and 9).

DISCUSSION

In the present study the low-phosphorus diet for layers was supplemented with two different phytase preparations, each of them was applied at two levels. The amount of available P was reduced to 1.89 g and then to 1.31 g per kg of feed as compared with 3.09 g/kg in the control diet and it did not lead to a decrease of body weight or laying rate. These basic performance parameters were also not improved by phytase supplementation. The average laying rate for the total period of egg production was 92.7%. The number and weight of eggs obtained were also not affected by the P level and did not depend on the kind or level of added phytase. Adult hens usually show a slight response to feed supplementation with biologically active substances (Jamroz et al., 1998). The results of the present study are in agreement with the reports of Htoo and Liebert (2001).

The daily feed intake in hens fed the low-P diets or diets supplemented with phytase B was similar; a significantly higher feed intake was recorded only in the group fed the diet with 450 U/kg phytase A. However, feed conversion was significantly better in the group fed the diet with 450 U/kg phytase B. The possibility of improvement of feed conversion by phytase addition has been described by Hadorn and Wiedmer (1998) and Oloffs et al. (2000). One of the possible explanations of the low phytase efficacy in hens in the present experiment may be the high amounts of wheat and barley in the diet; these grains are characterized by relatively high native phytase activity (Liebert et al., 2001).

The phosphorus level or addition of phytase did not affect the percentage of cracked eggs. The highest shell thickness was found in eggs from the positive control (VI) and groups II and III in the first and third experimental period. Only in individual cases was a slight enhancement in this parameter registered, particularly when phytase B at a level of 450 U or phytase A at level of 300 or 450 U/kg were applied.

Phytases, enzymes degrading phytate in feeds, release mineral elements and improve P but also Ca conversion. This makes better egg shell mineralization possible and consequently improves the quality of eggs (Sauveur, 1991; Um and Paik, 1999). The averages, calculated for three measurements, show that a higher egg shell thickness was found in the positive control group and in groups fed low P diets supplemented with 450 U phytase B or with 450 U phytase A. Generally, the strength of egg shells was lower in comparison with results reported by Jamroz et al. (1998), which might be related to the genotype of the hens.

In contrast to the indistinct effects on egg shell quality, the tibia and femur strength was improved by phytase addition. The differences between groups were statistically significant. The average values for bone strength varied from 3.97 to 5.59 N/mm^2 (60-99 N) for the tibia and from 3.54 to 5.62 N/mm^2 (58-97 N) for the femur. However, Fleming et al. (1994) and Gordon and Short (2001) reported values ranging from 115 to 249 N. Phosphorus utilization depends on nutrition, housing systems and strains of hens or broilers (Simons et al., 1990). The bone parameters can also be differentiated by the kind of determined indices (Quian et al., 1996; Zhang and Coon, 1997). The strength indices relating to the tibia and femur, expressed in N/mm^2, were lower in hens from group I and the best in group VI, groups supplemented with 450 U/kg phytase A and 300 U/kg phytase B, respectively. The elasticity of bones, low in group I, was clearly higher in the positive control, group VI, and in groups supplemented with phytase.

In contrast to the assessed egg parameters, only a small decrease in shell quality was observed (in the second period) as an effect of the level of available P/kg of the diet being reduced to 1.89 and then to 1.31 g from 3.09 g. In the tibia and femur in the control group only a slight reduction in the elasticity coefficient in the third measurement period was found. It is clear that in older hens the bone parameters were better than in younger ones at the beginning of egg production. The same pattern was observed in the case of low P (I) and enzyme-supplemented diets. There was no tendency towards higher accumulation of Ca in bones. In contrast to Ca, the phosphorus content of bones of hens fed low-P diets with phytases was higher in the first and third measurement periods. In the present study the variability of Mg and Zn content in bones caused some difficulties in interpretation of the obtained results, however, in the opinion of Yi et al. (1996a) the concentration of Zn in bones can be improved by phytase supplementation.

Comparison of some parameters of bone quality and chemical composition (Table 10) points to significant differences between the tibia and femur. The femur is significantly stronger and more elastic than the tibia. The content of Ca, P, Mg and Zn in the femur is higher, too. This indicates that the tibia is a weaker bone. It is much more exposed to mechanical injury, especially since it is positioned at an angle to the base.

TABLE 10
Comparison of some parameters of bone quality, calculated per 100 g metabolic body weight ($BW^{0.67}$) and content of minerals in crude ash of bones

Item	Femur	Tibia	SEM
Cortex thickness, mm	0.48	0.48	0.006
Surface, mm^2	10.71^A	10.08^B	0.158
Strength, N	80.46^A	73.45^B	1.310
Strength to surface ratio, N/mm^2	4.73	4.59	0.068
Elasticity alpha coefficient, kg/mm^2	3.54^A	3.99^B	0.076
Content of minerals in crude ash, %			
Ca	52.2	51.2	0.372
P	34.56	34.11	0.239
Mg	1.05^A	0.99^B	0.097
Zn, ppm	812.5^A	723.8^B	6.834

In conclusion it can also be stated that the bone quality parameters of laying hens are more rational and useful indices for estimation of phosphorus availability and phytase efficiency than egg shell characteristics. A similar conclusion was drawn by Orban et al. (1999) and Um and Paik (1999).

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STRESZCZENIE

Reakcja kur niosek na obniżony poziom fosforu oraz dodatek różnych preparatów fitazy do mieszanek

Celem badań było określenie wpływu dodatku dwóch preparatów fitazy do mieszanek o obniżonej zawartości dostępnego fosforu na produkcję jaj, pobranie paszy, jakość skorupy jaj, wytrzymałość i elastyczność kości i zawartość w nich Ca, P, Mg i Zn. Kurki Lohmann Brown w wieku 16 tygodni przydzielono do 6 grup, z których każda obejmowała 12 klatek po 4 ptaki. Przed rozpoczęciem nieśności ptaki żywiono mieszanką standardową zawierającą 150 g białka ogólnego, 11,3 MJ EM i 4,0 g P przyswajalnego w kg. Mieszanki doświadczalne sporządzono z pasz roślinnych, w tym około 70% stanowiły pszenica i jęczmień. Mieszanki zawierały 165 g białka ogólnego, 11,2 MJ EM/kg. W dietach I-V ilość P dostępnego wynosiła 1,89 g do 14 tygodnia nieśności, a następnie 1,31 g/kg, dieta kontrolna VI zawierała 3,09 g P dostępnego/kg. Dwa rodzaje fitazy (A i B) dodano w ilości 300 lub 450 jedn./kg mieszanki do diet II-V. Dodatek fitazy do mieszanek niskofosforowych nie wpłynął na wskaźniki produkcji jaj. Tylko zastosowanie 450 jedn./kg fitazy B poprawiło wykorzystanie paszy. W odróżnieniu od parametrów jakości skorupy jaj, dodatek fitazy istotnie poprawił wskaźniki wytrzymałości i elastyczności kości podudzia i uda, które były wyższe lub podobne jak w grupie kontrolnej (VI).