The effect of non-phytate phosphorus and phytase levels on performance, egg and tibia quality, and pH of the digestive tract in hens fed higher-calcium-content diets

M. Englmaierová, V. Skřivanová, M. Skřivan

Institute of Animal Science, Prague-Uhříněves, Czech Republic

ABSTRACT: The effect of three levels of non-phytate phosphorus (NPP) (3.0, 2.1, and 1.7 g/kg) and two levels of 3-phytase (F) (0 and 150 phytase units (FTU)/kg) together with a high dietary calcium concentration (approximately 41.0 g/kg) on the performance characteristics, egg quality, pH of the digestive tract, and tibia bone quality of ISA Brown hens housed in enriched cages was evaluated. The diets with 3.0 g/kg of NPP with and without F and 2.1 g/kg of NPP with 150 FTU of F significantly increased egg production ($P = 0.022$) and daily egg mass production ($P = 0.030$). A low level of NPP decreased ($P < 0.001$) the body weight of hens at the end of the experiment. The highest values of albumen height ($P < 0.001$), albumen index ($P = 0.004$), Haugh units ($P = 0.001$), and albumen percentage ($P = 0.004$) were associated with the diet with the low level of dietary NPP without F addition. The low level of NPP with 3-phytase increased the calcium and phosphorus contents in eggshells ($P = 0.002$ and $P = 0.050$). The lowest values of dry matter ($P < 0.001$) and ash content in tibia bone ($P = 0.040$) were measured for the hens fed the diet with 3.0 g/kg of NPP and 3-phytase supplementation. In addition, the low level of phosphorus without F addition increased ($P = 0.031$) the pH value in the small intestine to 6.21. In conclusion, 2.1 g of NPP with 150 FTU was found to be sufficient in the diet of hens in the middle of the laying cycle housed in enriched cages. But with respect to egg quality, higher calcium content decreased positive effect of F addition in diets with different levels of phosphorus.

Keywords: mineral nutrition; laying hen; egg production; egg quality; tibia; pH

Calcium (Ca) and phosphorus (P) are essential nutrients for various biochemical pathways and skeletal integrity in poultry. Reducing the dietary levels of Ca and P significantly decreased the bone quality (Swiatkiewicz and Arczewska-Wlosek, 2012). The physiological roles of these two macro minerals are intricately linked (Selle et al., 2009). There is a significant interaction between dietary Ca and P, and an improper ratio of Ca to P could depress growth performance in broilers (Hulan et al., 1985; Rao et al., 2003, 2006). This issue is more difficult to address in hens than in chickens because of hens' high calcium needs. An excess of Ca in hens' diets can cause antagonism of the absorption of minerals (P, magnesium, manganese, zinc), influencing the maintenance of the homeostasis of these minerals and, through the formation of Ca-phytate complexes, reducing the efficacy of phytase (F) (Driver et al., 2005; Selle et al., 2009). In addition, a high Ca content in the diet may reduce the energy value of the diet through the chelation of lipids (Driver et al., 2005). On the other hand, reducing the concentration of dietary Ca has been reported to improve F efficacy and phytate-P availability. However, this is often at the expense of optimal skeletal integrity (Wilkinson et al., 2011). For good growth performance and egg quality, Skřivan et al. (2010) recommend 2.7 g/kg of available phosphorus (AP) in a wheat-based diet and 3.0 g/kg of AP in a maize-based diet for hens with an intake of 115 g of feed containing 35 g/kg Ca. According to analyses of the model, Ahmadi and Rodehutscord (2012) showed that diets based on corn and soybean meal containing 2.2 g/kg

Supported by the Ministry of Agriculture of the Czech Republic (Projects No. QJ1310002 and No. MZE RO0714).

of nonphytate P (NPP) without supplemental F resulted in high egg production, egg mass, and feed efficiency in layers. In the presence of 150, 300, and 400 phytase units (FTU)/kg of feed, the dietary NPP level may be decreased, and optimal levels were calculated as being 1.8, 1.5, and 1.4 g/kg, respectively. In addition, Al-Massad et al. (2011) found that 1.2 g/kg NPP appears to be sufficient for maintaining production performance and eggshell quality in hens fed a corn-soybean meal diet containing 34 g/kg Ca and supplemented with F. On the other hand, Castillo et al. (2004) showed that the biological optimum levels of Ca for maximum egg production and specific gravity were 43.4 and 46.0 g/kg, respectively, and the economic optimum level for maximizing profits was 43.8 g/kg in diets containing 4.0 g/kg AP.

High calcium or phosphorus levels in the intestine reduce the absorption of both. Decreasing in solubility of minerals complexes as a result of increased ileal pH by the relatively high concentration of calcium was mentioned by Shafey (1993). Sheikhlar et al. (2009) showed that wide Ca : P ratio increased ($P < 0.05$) the retention of P. In addition, Ca and P of tibia linearly decreased as dietary NPP levels were reduced (Lim et al., 2001).

F addition has been found to overcome the adverse effects associated with low intake of inorganic P by animals and significantly reduce the effect of low levels of Ca in the diet on the performance of hens (Gordon and Roland, 1998). Moreover, F improves the bioavailability of Ca and improves eggshell quality at marginal levels of Ca (e.g. 34 g/kg) (Sohail and Roland, 2000), increases the digestibility of phytate P by approximately 20–45%, and increases retention by approximately 15% (Keshavarz, 2000). The question is what are the effects of F at higher levels of dietary Ca.

This study on laying hens was therefore performed to determine the effects of higher calcium contents in a diet of wheat and maize with a graded concentration of NPP, with or without microbial F addition, in terms of their performance characteristics, egg quality, pH values of digestive tract segments, and tibia bone quality.

MATERIAL AND METHODS

Hens, husbandry, and diets. Two hundred and forty 37-week-old ISA Brown hens were randomly assigned to 6 dietary treatments with 4 replicate cages at 10 hens per cage. The hens were housed in the same air-conditioned facility in three-floor enriched cages. The cages were equipped with a nest box, perch (150 cm), dust bath, and equipment for the abrasion of claws, conforming to the European Union Council Directive 1999/74/EC (1999). The cage provided 7560 cm^2 of floor area without the nest, 120 cm of feeder, and 3 nipple water dispensers. The room temperature was maintained at 20–22°C. A 16-h photoperiod was applied, and the light intensity was approximately 10 lx in the central storey.

Table 1. Ingredients and chemical composition of the diets used

Ingredient (g/kg)	High[1]	Medium[1]	Low[1]
Wheat	310	310	310
Maize	318.7	320.7	321.6
Wheat bran	20	20	20
Soybean meal	210	210	210
Lucerne meal	20	20	20
Rapeseed oil	25	25	25
Dicalcium phosphate	10.5	3.5	2.1
Sodium chloride	2	2	2
Limestone[2]	77	82	82.5
L-Lysine hydrochloride	0.6	0.6	0.6
DL-Methionine	1.2	1.2	1.2
Vitamin-mineral premix[3]	5	5	5
Calculated nutrient content (g/kg)			
AME_N (MJ/kg)	11.46	11.49	11.50
Crude protein	165.4	165.6	165.7
Lysine	8.24	8.25	8.25
Methionine	3.97	3.98	3.98
Methionine + cysteine	6.95	6.96	6.96

AME_N = apparent metabolizable energy

[1]levels of non-phytate phosphorus (NPP) in the diet were 3.0, 2.1, and 1.7 g/kg; other experimental diets were supplemented with 150 FTU/kg of phytase

[2]experimental diets contained 35% fine-grained limestone and 65% coarse-grained limestone

[3]vitamin-mineral premix provided per kg of diet: retinylacetate 3.0 mg, vitamin D_3 3000 IU, vitamin E 30 mg, niacin 25 mg, Ca pantothenate 8 mg, thiamine 2.0 mg, riboflavin 5 mg, pyridoxine 4 mg, folic acid 0.5 mg, biotin 0.075 mg, cobalamin 0.01 mg, choline Cl 250 mg, menadione 2.0 mg, betaine 100 mg, butylated hydroxytoluene 7.5 mg, ethoxyquin 5.6 mg, butylhydroxyanisole 1 mg, DL-methionine 0.7 g, Mn 70 mg, Zn 50 mg, Fe 40 mg, Cu 6 mg, I 1 mg, Co 0.3 mg, Se 0.2 mg

A completely randomized experimental design with a 3 × 2 factorial arrangement of treatments was employed: 3 non-phytate phosphorus (NPP) levels (3.0, 2.1, and 1.7 g/kg) and 2 phytase (F) levels (0 and 150 phytase units (FTU)/kg). Natuphos® (BASF, Ludwigshafen, Germany), a preparation of 3-phytase (EC 3.1.3.8) produced by *Aspergillus niger*, was chosen as the source of the F. The ingredients and nutrient composition of the wheat- and maize-based diets are listed in Table 1. Calculations of the nutrient contents were made using standard values (National Research Council, 1994). All of the diets contained a high calcium level – approximately 41 g/kg (Table 2). Fine limestone (0.09–0.50 mm grains) and coarse limestone (1.00–2.00 mm grains) was supplied at a ratio of 35 : 65. Feed and fresh water were supplied *ad libitum*. The experiment lasted for 12 weeks.

The number of eggs and hens and their health status were monitored daily. The hen-day egg production and feed intake were calculated weekly on a per-cage basis. Egg weights were determined once per week. The hens were weighed at the beginning and at the end of the experiment, i.e. at the age of 37 and 49 weeks.

Sampling and analyses. The total P in the diets was assayed using a vanadate-molybdate reagent (AOAC, 2005; method 965.17). The Ca content in the diets was determined by atomic absorption spectrometry performed using a Solar M6 instrument (TJA Solutions, Cambridge, UK). The phytate P contents of the diets were determined by a capillary isotachophoretic method (Dušková et al., 2001). The phytase activity of the diets was determined as described by Eeckhout and De Paepe (1994).

To determine physical parameters, eggs were collected at weeks 40, 43, and 46 of hens' age (Tůmová and Gous, 2012). All daily egg production was analyzed. A total of 606 eggs were analyzed in the experiment. The albumen, yolk, and shell percentages were determined based on the individual weight of each egg and the weights of its components. The shell weight was measured after drying at 105°C. The albumen and yolk heights were measured using a digital micrometer head IP54 (Swiss Precision Instruments, Inc., Garden Grove, USA). The Haugh units (HU) were calculated as indicated by Haugh (1937). The shell breaking strength and shell deformation were determined on the vertical axis using an Instron 3360 apparatus (Instron, Norwood, USA). The shell thickness (values measured at the sharp and blunt ends and the equator, and the three obtained values were averaged) was measured using a micrometer after removing the shell membranes. The egg shell index (SI) was calculated as follows (Ahmed et al., 2005):

$$SI = (SW/S) \times 100, \quad S = 4.68 \times EW^{2/3}$$

where:
SW = shell weight
S = shell surface
EW = egg weight

The albumen index was determined using the formula:

$$AI = \{\text{albumen height}/[(\text{long diameter of albumen} + \text{short diameter of albumen})/2]\} \times 100$$

The yolk index was calculated as

$$YI = (\text{yolk height}/\text{yolk diameter}) \times 100$$

The colour of the yolk was measured using the DSM Yolk Colour Fan (DSM Nutritional Products, Basel, Switzerland).

Analyses of the P and Ca contents of the eggshells were conducted twice during the experiment, at weeks 38 and 44 of hens' age. A total of 576 eggs were analyzed (4 eggs per sample; 6 treatments; $n = 24$). Dry samples of the eggshells were ashed at 550°C. The P and Ca contents of the dried egg-

Table 2. Analyzed calcium and phosphorus content and phytase activity of the diets used

Levels of NPP	High		Medium		Low	
Phytase (FTU/kg)	0	150	0	150	0	150
Calcium (g/kg)	40.9	40.9	41.1	41.1	41.2	41.2
Total P (g/kg)	5.5	5.4	4.5	4.5	3.9	3.9
Phytate P (g/kg)	2.5	2.4	2.4	2.4	2.3	2.2
Non-phytate P (g/kg)	3.0	3.0	2.1	2.1	1.6	1.7
Phytase activity (FTU/kg)	144	313	160	300	165	255

NPP = non-phytate phosphorus, FTU = phytase units

shells were determined in a manner similar to that described previously for analysis of these elements in the hens' diets.

At the end of the experiment, eight hens from each treatment were slaughtered using a CO_2-based equipment for the euthanasia of poultry (Anieut G.d., Hena s.r.o., Miličín, Czech Republic). The digestive tract of each hen was removed, and the pH in segments such as the crop, gizzard, small intestine, and caecum was immediately measured using a pH meter 3520 (Jenway, Staffordshire, UK). The right tibia bones were excised from the carcasses, cleaned of all tissue, and frozen at –20°C until processing. The tibia breaking strengths were measured using an Instron 3342 apparatus (Instron) with a 50-kg load cell with a crosshead speed of 50 mm/min. Each tibia was supported on a 5.75-cm span. The broken tibias were later used for other measurements. The tibias underwent a 48-h defattening process under the action of finally evaporating hexan. The bones were dried at 105°C for 24 h, placed in a desiccator, and weighed to determine their fat-free dry weight. Then, the bones were placed in a muffle furnace at 600°C for 24 h and cooled in a desiccator, and the ash weight was recorded.

Statistical analysis. The data from the experiment, with a 3 × 2 full factorial design, were analyzed using Two-Way Analysis of Variance (ANOVA) with the General Linear Models (GLM) procedure of SAS (Statistical Analysis System, Version 8.2, 2003). The main effects considered were the concentration of non-phytate phosphorus (NPP), phytase supplementation (F), and the interaction between these two factors (NPP × F). All of the differences were considered to be significant at $P < 0.05$. The results in the tables are presented as the mean and standard error of the mean (SEM).

RESULTS

As is evident from Table 2, all of the diets contained high levels of calcium (40.9–41.2 g/kg). The phytase activity of the diets ranged 144–165 FTU/kg for the non-supplemented diets and 255–313 FTU/kg for the diets supplemented with 150 FTU.

With regard to the performance characteristics (Table 3), the interaction between NPP and F was found to be significant for egg production ($P = 0.022$) and egg mass ($P = 0.030$). The highest values were obtained for hens fed the diet with the high level of NPP, with and without F, and the diet with the medium level of NPP with F. F addition significantly decreased egg weight, feed intake, and the feed conversion ratio, compared with treatments without F. The high level of NPP increased egg weight and feed intake. The highest mortality was associated with the treatment with the low NPP level without 3-phytase addition. The body weights at the end of the experiment were considerably lower for hens fed diets with a level of NPP of approximately 1.7 g/kg.

Table 4 summarises the results of the analysis of physical egg quality parameters. NPP and F were found to have a significant combined effect on

Table 3. Performance characteristics of laying hens fed different levels of phosphorus and phytase

Levels of NPP	High		Medium		Low		SEM	Probability		
Phytase (FTU/kg)	0	150	0	150	0	150		NPP	F	NPP × F
Hen-day egg production (%)	89.5^{ab}	89.1^{ab}	86.3^{c}	90.1^{a}	86.7^{c}	87.8^{bc}	0.32	0.034	0.021	0.022
Egg weight (g)	62.0	61.8	61.8	60.9	61.7	60.9	0.09	0.003	< 0.001	ns
Egg mass (g/hen/day)	55.3^{a}	55.1^{a}	52.8^{b}	55.0^{a}	53.4^{b}	53.0^{b}	0.22	0.001	ns	0.030
Feed intake (g/day/bird)	122.0	118.7	117.8	117.0	118.3	116.8	0.46	0.011	0.040	ns
Feed intake (g/egg)	136.5	133.8	137.2	130.2	137.3	133.5	0.71	ns	0.001	ns
FCR (g/g)	2.21	2.17	2.24	2.14	2.23	2.21	0.012	ns	0.018	ns
Mortality (%)	2.6	2.6	0.0	0.0	7.7	0.0				
BW at 37th week of hens' age (g)	1974	1994	1942	1995	2007	2002	17.0	ns	ns	ns
BW at 49th week of hens' age (g)	1895	1769	1871	1972	1657	1630	31.1	< 0.001	ns	ns

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, BW = body weight, FCR = feed conversion ratio, ns = non-significant

$^{a–c}$means in the same row with different superscripts differ significantly

Table 4. Effect of phosphorus and phytase levels on physical characteristics of eggs and calcium and phosphorus content in eggshells

Levels of NPP	High		Medium		Low		SEM	Probability		
Phytase (FTU/kg)	0	150	0	150	0	150		NPP	F	NPP × F
Yolk and albumen ratio (%)	40.6^{a}	39.3^{ab}	39.7^{ab}	39.4^{ab}	38.4^{b}	40.6^{a}	0.23	ns	ns	0.007
Albumen height (mm)	6.7^{c}	7.0^{bc}	7.0^{b}	6.8^{bc}	7.4^{a}	6.8^{bc}	0.05	ns	ns	< 0.001
Albumen index (%)	8.1^{c}	8.4^{bc}	8.7$^{a.b}$	8.2^{bc}	9.2^{a}	8.2^{bc}	0.07	ns	0.008	0.004
Haugh units	80.5^{c}	82.0^{bc}	82.6^{b}	81.6^{bc}	85.0^{a}	81.3^{bc}	0.29	ns	ns	0.001
Albumen weight (g)	40.0	40.6	40.0	39.5	40.6	39.5	0.13	ns	ns	ns
Albumen percentage (%)	64.1^{bc}	64.6^{ab}	64.3^{abc}	64.5^{abc}	64.9^{a}	63.9^{c}	0.96	ns	ns	0.004
Yolk height (mm)	18.1	18.2	18.1	17.8	18.2	18.0	0.04	ns	ns	ns
Yolk index (%)	44.3	44.0	44.1	43.3	43.9	43.6	0.12	ns	ns	ns
Yolk weight (g)	15.9^{ab}	15.9^{abc}	15.7^{abc}	14.5^{c}	15.5^{bc}	16.0^{a}	0.06	ns	ns	0.049
Yolk percentage (%)	25.7^{ab}	25.3^{bc}	26.0^{abc}	25.4^{abc}	24.9^{c}	25.9^{a}	0.08	ns	ns	0.002
Yolk colour (La Roche)	10.5	10.9	10.8	10.9	10.8	11.0	0.05	ns	0.010	ns
Shell thickness										
Blunt end (μm)	347	341	350	338	345	340	1.1	ns	< 0.001	ns
Equator (μm)	350	350	356	347	357	353	1.2	ns	ns	ns
Sharp end (μm)	366	359	366	359	362	358	1.1	ns	0.007	ns
Average (μm)	354	350	358	348	355	350	1.0	ns	0.001	ns
Shell deformation (mm)	0.479	0.470	0.481	0.480	0.468	0.474	0.0019	ns	ns	ns
Shell breaking strength (N)	42.2	41.2	43.2	40.0	41.6	41.2	0.27	ns	0.005	ns
Shell index (g.100/cm^2)	8.6	8.6	8.8	8.5	8.6	8.6	0.02	ns	0.029	ns
Shell weight (g)	6.3^{a}	6.3^{a}	6.4^{a}	6.2^{b}	6.4^{a}	6.3^{ab}	0.01	ns	0.017	0.021
Shell percentage (%)	10.2	10.1	10.4	10.1	10.2	10.2	0.03	ns	ns	ns
Shell Ca content (g/kg DM)	384^{bc}	383^{c}	382^{c}	387^{ab}	382^{c}	390^{a}	0.6	ns	< 0.001	0.002
Shell P content (g/kg DM)	1.37^{ab}	1.32^{b}	1.31^{b}	1.30^{b}	1.32^{b}	1.46^{a}	0.017	ns	ns	0.050

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, DM = dry matter, ns = non-significant
$^{a–c}$means in the same row with different superscripts differ significantly

albumen quality characteristics such as albumen height (P < 0.001), albumen index (P = 0.004), Haugh units (P = 0.001), and albumen percentage (P = 0.004). The highest values were measured for eggs laid by the hens fed the diet with the low level of NPP without F addition. The percentage and weight of yolk (P = 0.002 and P = 0.049) and yolk and albumen ratio were the highest in the eggs laid by hens fed the diet with 1.7 g/kg of NPP with 3-phytase. Higher shell weights (P = 0.021) were measured for eggs laid by hens fed the diet with the high level of NPP and hens fed the diets

Table 5. Breaking strength, dry matter, and ash content of tibia bone in hens fed different levels of phosphorus and phytase

Levels of NPP	High		Medium		Low		SEM	Probability		
Phytase (FTU/kg)	0	150	0	150	0	150		NPP	F	NPP × F
Breaking strength (N)	200	194	197	183	175	174	5.0	ns	ns	ns
DM (g/kg)	921^{a}	895^{b}	918^{a}	922^{a}	918^{a}	925^{a}	2.2	0.006	ns	< 0.001
Ash (g/kg)	506^{a}	457^{d}	497^{ab}	480^{bc}	486^{abc}	470^{cd}	3.6	ns	< 0.001	0.040
Ash (g/kg DM)	549	510	541	521	530	508	3.3	ns	< 0.001	ns

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, DM = dry matter, ns = non-significant
$^{a–d}$means in the same row with different superscripts differ significantly

Table 6. Effect of phosphorus and phytase levels on pH of the digestive tract of hens

Levels of NPP	High		Medium		Low		SEM	Probability		
Phytase (FTU/kg)	0	150	0	150	0	150		NPP	F	NPP × F
Crop	4.66	4.56	4.67	4.52	4.92	4.46	0.045	ns	0.006	ns
Gizzard	4.49	4.44	4.45	4.43	4.03	3.73	0.069	< 0.001	ns	ns
Small intestine	5.75^{c}	5.73^{c}	5.74^{c}	5.95^{b}	6.21^{a}	6.05^{ab}	0.040	< 0.001	ns	0.031
Caecum	5.83	5.94	5.97	6.03	6.54	5.76	0.056	ns	ns	ns

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, ns = non-significant

$^{a–c}$means in the same row with different superscripts differ significantly

with the medium and low levels of dietary NPP without F supplementation. The highest Ca and P contents (P = 0.002 and P = 0.050) were measured in the shells of eggs laid by hens fed the diet with 1.7 g/kg of NPP with 150 FTU of 3-phytase. A separate effect of phosphorus on internal and external egg quality parameters was not observed.

As shown in Table 5, the breaking strength of the tibia bone was not influenced by NPP or F. The high level of NPP with F addition significantly decreased the dry matter (P < 0.001) and ash content in the tibia bones (P = 0.040). Phytase supplementation decreased the pH in the crop segment of the digestive tract (P = 0.006), whereas P addition increased the pH in the gizzard segment (P < 0.001) (Table 6). The highest value of pH in the small intestine (P = 0.031) was measured in the hens fed the diet with the low level of NPP without the addition of F.

DISCUSSION

From previous experiments by Skřivan et al. (2010) and Englmaierová et al. (2012) it is evident that a higher concentration of NPP (4.1 and 3.7 g/kg) in the diet has a negative effect on the hen-day egg production, feed intake, and feed conversion ratio. Thus, in the present study, lower levels of NPP were chosen. The high AP level of 3.0 g/kg (unlike the lower levels of 2.1 and 1.7 g/kg) increased the egg production and egg mass production. This finding is consistent with the findings of Skřivan et al. (2010), who showed that AP levels of 2.7 g/kg in a wheat-based diet and 3.0 g/kg in a maize-based diet are adequate for hens with an intake of 115 g of feed with 35 g/kg of Ca and without F addition and do not have a negative impact on performance or egg quality. The treatment with 2.1 g/kg NPP yielded results for hen-day egg production and egg mass production similar to those obtained with the diet with 3.0 g of NPP/kg, with or without F. Ahmadi and Rodehutscord (2012) stated that on the basis of an evaluation using a full quadratic model, maize-soybean meal-based diets containing 2.2 g/kg of NPP without supplemental F resulted in high egg production, egg mass, and feed efficiency in hens. In the presence of 150, 300, and 400 FTU/kg of feed, the dietary NPP level may be decreased, and the optimal levels were calculated as 1.8, 1.5, and 1.4 g/kg, respectively. At the end of the experiment, the heaviest hens were those from the group with 2.1 g/kg of NPP. The NPP level of 3.0 and 1.7 g/kg decreased live weight by about 100 and 300 g. A low and higher level of NPP in diets with higher Ca contents was the cause of body weight decrease. Garlich et al. (1975) found that body weight gain in hens was significantly reduced by a phosphorus-deficient diet (3.9 g of total P/kg) fed for 21 days. Boling et al. (2000) showed that a 1.0 g of AP/kg diet resulted in significantly lower (P < 0.05) body weights. In addition, live weight of hens fed the 2.0, 2.5, and 4.5 g of AP/kg diets did not significantly differ throughout the experiment from the 21st to the 70th week of hens' age (Boling et al., 2000). On the other hand, Mikaelian and Sell (1981) and Liebert et al. (2005) did not observe an effect of P on body weight.

In this experiment, egg weight increased with increasing NPP in the diet (P = 0.003), while supplementation with 3-phytase decreased egg weight (P < 0.001). Heavier eggs being laid after feeding hens a diet with a higher content of P were also reported by Gordon and Roland (1998) and by Francesch et al. (2005). The opposite results concerning the effect of F on egg weight were reported by Cabuk et al. (2004), who noted that F supplementation increased egg weight. Neither Carlos and Edwards (1998) nor Berry et al. (2003) found any significant effect of F on egg weight. In

the present experiment, lower egg weight due to F addition was probably caused by lower feed intake and higher egg production in these treatments.

In contrast to the performance results, the highest albumen quality was associated with the treatment with the low level of dietary NPP without F supplementation. Skřivan et al. (2010) stated that a high level of AP (4.1 g/kg) in a wheat-based diet significantly ($P < 0.001$) decreased albumen height, albumen index, and Haugh units (HU). Conversely, Um and Paik (1999) did not detect significant differences in Haugh units or the shell strength of egg laid by hens fed diets containing different levels of P and F. Phytase addition into diets with higher Ca content significantly decreased values of shell thickness ($P = 0.001$), shell breaking strength ($P = 0.005$), and ash content ($P < 0.001$) in tibia bone. These results are not consistent with those of Al-Sharafat et al. (2009), who reported that F supplementation significantly increased tibia ash and Ca in tibia ash. The negative effect of F observed in the present study could be due to the greater Ca : P ratios (from 7.5 : 1 to 10.5 : 1) in all of the evaluated diets, and F supplementation may have exacerbated this effect by releasing Ca from the phytate complex. Keshavarz (2003) showed that the presence of F reduced several indices of shell quality. On the other hand, 150 FTU of 3-phytase improved the pigmentation of the egg yolk. Similar observations were reported by Kozlowski and Jeroch (2011). The low content of NPP with 3-phytase significantly increased the P and Ca contents in the shell. The higher storage of the minerals in the shell was probably related to a lower egg production and lower egg weight.

The small intestine is the main part of the digestive tract for Ca and P absorption and is highly affected by intestinal pH. The 3-phytase from *Aspergillus niger* has two optimal pH levels, 2.5 and 5.0 (Lassen et al., 2001; Tamim et al., 2004). Significant interaction between NPP and F was found in the pH of the small intestine. The low level of P without F addition was found to have a negative effect on the pH in the small intestine, which increased to 6.21. Shafey et al. (1991) similarly showed that the pH in the small intestine of broilers ranges from 5.5 to 6.6. However, because the pH was within this range, the dietary Ca complexes with the phytate made the phytate unavailable for F activity (Wilkinson et al., 2011). Nelson (1976) observed negative effects of the Ca level on F activity in the gut. Coincidently, Wilkinson et al. (2011) showed that dietary Ca reduced the efficacy of F, which resulted in decreased phytate-P and increased phosphorus excretion and facilitating the formation of mineral-phytate complexes. Consistently with results of Englmaierová et al. (2012), the high and medium levels of P increased the pH in the gizzard to a level suitable for F activity.

CONCLUSION

The performance and egg quality of hens was found to depend on the ratio of minerals in the feed mixture. The presence of a higher Ca content negatively affected the impact of F in the diets with different levels of P. With respect to performance, 2.1 g of NPP with 150 FTU was found to be sufficient in the diet of hens housed in enriched cages. A high level of dietary NPP with F addition, in combination with a high concentration of Ca in the diet, negatively affected the chemical composition of the tibia. The highest albumen quality was associated with the diet with 1.7 g of NPP without F addition.

REFERENCES

Ahmadi H., Rodehutscord M. (2012): A meta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. Poultry Science, 91, 2072–2078.

Ahmed A.M.H., Rodriguez-Navarro A.B., Vidal M.L., Gautron J., Garcia-Ruiz J.M., Nys Y. (2005): Changes in eggshell mechanical properties, crystallographic texture and in matrix proteins induced by moult in hens. British Poultry Science, 46, 268–279.

Al-Massad M., Al-Shdefat R., Khashroum A. (2011): The effects of microbial phytase and dietary calcium level on the performance and eggshell quality in laying hens fed marginal phosphorus diets. Asian Journal of Animal Sciences, 5, 118–126.

Al-Sharafat A., Al-Desiet B., Al-Kouri S. (2009): The effect of calcium level, microbial phytase and citric acid on performance parameters and eggshell quality of laying hens fed corn soybean meal diet. Journal of Animal and Veterinary Advances, 8, 1829–1837.

AOAC (2005): Official Methods of Analysis. 18th Ed. AOAC International, Gaithersburg, USA.

Berry W.D., Hess J.B., Lien R.J., Roland D.A. (2003): Egg production, fertility, and hatchability of breeder hens receiving dietary phytase. Journal of Applied Poultry Research, 12, 264–270.

Boling S.D., Douglas M.W., Shirley R.B., Parsons C.M., Koelkebeck K.W. (2000): The effects of various dietary levels

of phytase and available phosphorus on performance of laying hens. Poultry Science, 79, 535–538.

Cabuk M., Bozkurt M., Kirkpinar F., Ozkul H. (2004): Effect of phytase supplementation of diets with different levels of phosphorus on performance and egg quality of laying hens in hot climatic conditions. South African Journal of Animal Science, 34, 13–17.

Carlos A.B., Edwards H.M. (1998): The effects of 1,25-dihydroxycholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. Poultry Science, 77, 850–858.

Castillo C., Cuca M., Pro A., Gonzalez M., Morales E. (2004): Biological and economic optimum level of calcium in white leghorn laying hens. Poultry Science, 83, 868–872.

Driver J.P., Pesti G.D., Bakalli R.I., Edwards H.M. (2005): Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. Poultry Science, 84, 1406–1417.

Dušková D., Marounek M., Březina P. (2001): Determination of phytic acid in feeds and faeces of pigs and poultry by capillary isotachophoresis. Journal of the Science of Food and Agriculture, 81, 36–41.

Eeckhout W., De Paepe M. (1994): Total phosphorus, phytate phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology, 47, 19–29.

Englmaierová M., Dlouhá G., Marounek M., Skřivan M. (2012): Efficacy of contrast levels of non-phytate phosphorus and *Aspergillus niger* phytase in hens fed wheat-maize-based diets. Czech Journal of Animal Science, 57, 499–505.

European Union Council Directive (1999): Council Directive 1999/74/EC of July 19th, 1999. Official Journal of the European Communities, L 203, 53–57.

Francesch M., Broz J., Brufau J. (2005): Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on maize- or barley-based diets. British Poultry Science, 46, 340–348.

Garlich J.D., James R.L., Ward J.B. (1975): Effect of short-term phosphorus deprivation on laying hens. Poultry Science, 54, 1193–1199.

Gordon R.W., Roland D.A. (1998): Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. Poultry Science, 77, 290–294.

Haugh R.R. (1937): The Haugh unit for measuring egg quality. US Egg and Poultry Magazine, 43, 552–555, 572–573.

Hulan H.W., De Groote G., Fontaine G., De Munter G., McRae K.B., Proudfoot F.G. (1985): The effect of different totals and ratios of dietary calcium and phosphorus on the performance and incidence of leg abnormalities of male and female broiler-chickens. Poultry Science, 64, 1157–1169.

Keshavarz K. (2000): Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poultry Science, 79, 748–763.

Keshavarz K. (2003): A comparison between cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus. Poultry Science, 82, 1415–1422.

Kozlowski K., Jeroch H. (2011): Efficacy of different levels of *Escherichia coli* phytase in hens fed maize-soyabean meal based diets with a decreased non-phytase phosphorus content. Journal of Animal and Feed Sciences, 20, 224–235.

Lassen S.F., Breinholt J., Ostergaard P.R., Brugger R., Bischoff A., Wyss M., Fuglsang C.C. (2001): Expression, gene cloning, and characterization of five novel phytases from four basidiomycete fungi: *Peniophora lycii*, *Agrocybe pediades*, a *Ceriporia* sp., and *Trametes pubescens*. Applied and Environmental Microbiology, 67, 4701–4707.

Liebert F., Htoo J.K., Sunder A. (2005): Performance and nutrient utilization of laying hens fed low-phosphorus corn-soybean and wheat-soybean diets supplemented with microbial phytase. Poultry Science, 84, 1576–1583.

Lim H.S., Namkung H., Um J.S., Kang K.R., Kim B.S., Paik I.K. (2001): The effects of phytase supplementation on the performance of broiler chickens fed diets with different levels of non-phytate phosphorus. Asian-Australian Journal of Animal Science, 14, 250–257.

Mikaelian K.S., Sell J.L. (1981): Performance of laying hens fed various phosphorus levels continuously or phase fed decremental phosphorus levels. Poultry Science, 60, 1916–1924.

National Research Council (1994): Nutrient Requirements of Poultry. 9th Ed. National Academies Press, Washington, USA.

Nelson T.S. (1976): The hydrolysis of phytate phosphorus by chicks and laying hens. Poultry Science, 55, 2262–2264.

Rao S.V.R., Raju M.V.L.N., Reddy M.R., Pavani P., Sunder G.S., Sharma R.P. (2003): Dietary calcium and non-phytin phosphorus interaction on growth, bone mineralization and mineral retention in broiler starter chicks. Asian-Australasian Journal of Animal Science, 16, 719–725.

Rao S.V.R., Raju M.V.L.N., Reddy M.R., Pavani P. (2006): Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. Animal Feed Science and Technology, 131, 133–148.

Selle P.H., Cowieson A.J., Ravindran V. (2009): Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock Science, 124, 126–141.

Shafey T.M., McDonald M.W., Dingle J.G. (1991): Effects of dietary calcium and available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. British Poultry Science, 32, 185–194.

Shafey T.M. (1993): Calcium tolerance of growing chickens: effect of ratio of dietary calcium to nonphytate phosphorus. Worlds Poultry Science Journal, 49, 5–18.

Sheikhlar A., Kasim A.B., Chwen L.T., Bejo M.H. (2009): Effect of varying ratios of dietary calcium and phosphorus on performance, phytate P and mineral retention in Japanese quail (*Coturnix cotnurnix Japonica*). International Journal of Poultry Science, 8, 692–695.

Skřivan M., Englmaierová M., Skřivanová V. (2010): Effect of different phosphorus levels on the performance and egg quality of laying hens fed wheat- and maize-based diets. Czech Journal of Animal Science, 55, 420–427.

Sohail S.S., Roland D.A. (2000): Influence of phytase on calcium utilization in commercial layers. Journal of Applied Poultry Research, 9, 81–87.

Swiatkiewicz S., Arczewska-Wlosek A. (2012): Bone quality characteristics and performance in broiler chickens fed diets supplemented with organic acids. Czech Journal of Animal Science, 57, 193–205.

Tamim N.M., Angel R., Christman M. (2004): Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poultry Science, 83, 1358–1367.

Tůmová E., Gous R.M. (2012): Interaction between oviposition time, age, and environmental temperature and egg quality traits in laying hens and broiler breeders. Czech Journal of Animal Science, 57, 541–549.

Um J.S., Paik I.K. (1999): Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poultry Science, 78, 75–79.

Wilkinson S.J., Selle P.H., Bedford M.R., Cowieson A.J. (2011): Exploiting calcium-specific appetite in poultry nutrition. Worlds Poultry Science Journal, 67, 587–598.

Received: 2013–07–23
Accepted after corrections: 2013–12–06

Corresponding Author

Ing. Michaela Englmaierová, Ph.D., Institute of Animal Science, Přátelství 815, 104 01 Prague 10-Uhříněves, Czech Republic
Phone: +420 267 009 547, fax: +420 267 711 448, e-mail: englmaierova.michaela@vuzv.cz