Efficacy of contrast levels of non-phytate phosphorus and *Aspergillus niger* phytase in hens fed wheat-maize-based diets

M. Englmaierová, G. Dlouhá, M. Marounek, M. Skřivan

Institute of Animal Science, Prague-Uhříněves, Czech Republic

ABSTRACT: A 2 × 2 factorial design experiment examined the effect of dietary non-phytate phosphorus (NPP) (1.3 and 4.0 g/kg) and 3-phytase (F) (0 and 150 FTU/kg) on the performance indicators of hens, physical parameters of eggs, phosphorus (P) content of the eggshells, and the pH of the digestive tract of laying hens. Two hundred and forty hens (ISA Brown) were housed in enriched cages and fed a wheat-maize-based diet. A significant effect of both NPP and F was found for the yolk colour (P = 0.016) and shell thickness (P = 0.038). The F supplement or higher level of NPP alone and in combination increased the value of the yolk colour and shell thickness. The supplementation of the basal diet with F significantly increased the laying performance, especially with regard to the egg weight and feed conversion ratio. The higher dose of NPP had a negative effect on the egg production, feed intake, and feed conversion ratio. The lower level of NPP (1.3 g/kg) decreased the internal egg quality and shell quality, except for the shell strength. In contrast, the addition of 3-phytase to the diet increased the shell thickness and shell weight. The P content in the eggshells was not influenced by the dietary treatment. The higher level of NPP or F increased the pH in the gizzard to a value suitable for F activity. The hens fed a diet containing 1.3 g/kg NPP achieved a higher performance; moreover, the F supplement at 150 FTU/kg increased the external quality of the eggs.

Keywords: laying hen; egg production; feed intake; eggshell quality; pH

Mineral phosphorus (P) is important for proper bone development, formation of eggshells, and metabolism of laying hens. Approximately 20–50% of plant P is available in a poultry diet, and the rest is present in the form of phytate. Phytate P is biologically less available to poultry due to either insufficient quantity or the lack of secretion of phytase (F), which hydrolyses phytic acid in the digestive tract (Ravindran et al., 1995; Sebastian et al., 1998).

As shown by the results of Swiatkiewicz et al. (2010), reducing the dietary levels of Ca (from 3.70 to 3.25%) and P (from 0.65 to 0.60%) significantly decreased the percentage of the eggshell, thickness, density, and breaking strength of the eggshell. However, de Faria et al. (1999) observed decreases in the egg production, egg weight, and egg mass without alterations in the eggshell quality characteristics in hens fed diets containing 0.35% of total P as opposed to 0.55% of total P. Sohail et al. (2001) observed a reduction of egg production (by 8.5 and 6.8%) and feed consumption due to decreases in the non-phytate phosphorus (NPP) from 0.40 and 0.25% to 0.09%. Based on the findings of Skřivan et al. (2010), 0.27% of available phosphorus (AP) in a wheat-based diet and 0.30% of the AP in a maize-based diet are adequate for hens having an intake of 115 g of feed containing 3.5% Ca without a negative impact on the performance or egg quality. According to a study by Rodrigues et al. (1998), levels of 0.35 and 0.25% of AP for the initial and final laying phases were adequate for laying hen performance and egg quality during the second production cycle.

Phytase supplementation completely overcame the adverse effects associated with low dietary P

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and significantly reduced the impact of low dietary Ca on hen performance (Gordon and Roland, 1998). Furthermore, F improved bioavailability of Ca and eggshell quality at the marginal Ca level of 3.4% (Sohail and Roland, 2000). Keshavarz (2000) reported that F significantly increased phytate P retention by approximately 15%. The daily total P excretion was by 34–47% lesser for hens fed the lowest NPP levels using F than for the unsupplemented phytase control group. Supplementing F can improve the digestibility of P, Ca, and amino acids in layers fed corn-, soybean-, and by-product-based diets (Liu et al., 2007). Exogenous F are predominantly active under acid pH conditions, such as in the fore-stomach (crop, proventriculus, and gizzard) of poultry (Selle and Ravindran, 2007). Rather than adding high levels of inorganic P to layer diets, the safety margin currently included in the recommended dietary specifications could be provided by the addition of F. This addition would reduce the negative environmental effects of intensive poultry production that are associated with P excretion (Silversides and Hruby, 2009).

The objective of this study was to evaluate the effect of low 3-phytase supplementation into a diet consisting of a balanced representation of wheat and maize with different concentrations of NPP (1.3 or 4.0 g/kg) on the performance and pH values in the digestive tract of laying hens and the quality characteristics of the eggs. The same proportion of wheat and maize corrected the contrasting effect of the high or low activity of endogenous F in these two components.

MATERIAL AND METHODS

A total of 240 hens (ISA Brown) at 44 weeks of age were randomly assigned to 4 dietary treatments with 6 replicate cages (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 the 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. The daily photoperiod consisted of 16 h of light and 8 h of darkness. The light intensity was approximately 10 lx in the central storey. The protocol was approved by the Ethical Committee of the Institute of Animal Science. A 2 × 2 full factorial design comprising 2 non-phytate phosphorus (NPP) levels (1.3 and 4.0 g/kg) and 2 phytase (F) levels (0 and 150 phytase units (FTU)/kg) was used. 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-maize-based diets are listed in Table 1. All of the diets contained (per kg): approximately 11.5 MJ of apparent metabolizable energy (AME_N), 165 g of crude protein, and 35 g of Ca. Calculations of the dietary NPP content were made using standard values (National Research Council, 1994). Fine- (0.09–0.50 mm) and coarse-grained (1.00–2.00 mm) limestone was supplied at a ratio of 65 : 35. Feed and fresh water were supplied *ad libitum*. The experiment lasted for 12 weeks.

Phytase activity of the feed was determined as described by Eeckhout and De Paepe (1994). Phytate P in feeds was determined by a capillary isotachophoretic method (Dušková et al., 2001).

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.

For the physical parameter determination, eggs were collected in the 47th, 52nd, and 55th week of the hens' age, and a total of 549 eggs were analyzed. Moreover, the egg weight was determined once a week. The albumen, yolk, and shell percentage was determined using the individual weight of each egg and weight of its components. The shell weight was assessed after drying at 105°C. The albumen and yolk height was measured using a digital micrometer head IP54 (Swiss Precision Instruments, Inc., Garden Grove, USA). 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, Canton, USA). The shell thickness (values from the sharp and blunt end and equator and the average of these 3 values) after removing the shell membranes was evaluated using the micrometer. The egg shell index was calculated after Ahmed et al. (2005) as:

$$SI = (SW/S) \times 100$$

where:

SW = shell weight

S = shell surface calculated as $S = 4.68 \times \text{egg weight (EW)}^{2/3}$

The formula for the albumen index is AI = {albumen height/[(long diameter of albumen + short diameter of albumen)/2]} × 100. The yolk index was calculated as YI = (yolk height/yolk diameter) × 100.

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

Analyses of the P content of the eggshells were conducted twice during the experiment in the 48th and 53rd week of age of the hens; a total of 384 eggs were analyzed (4 eggs per sample; 4 treatments; n = 24). Dry samples of the eggshells were ashed at 550°C. The total P in the dried eggshells and in feeds was assayed using a vanadate-molybdate reagent (AOAC, 2005; method 965.17). The calcium content in the diet was determined by atomic absorption spectrometry performed using a Solar M6 instrument (TJA Solutions, Cambridge, UK).

At the end of the experiment, eight hens from each treatment were slaughtered using the equipment for the euthanasia of poultry (CO_2-based device) Anieut G.d. (Hena s.r.o., Miličín, Czech Republic). The digestive tract was removed, and the pH in segments such as the crop, gizzard, and small intestine was immediately measured using a pH meter 3520 (Jenway, Staffordshire, UK).

The resulting values of the experiment with a 2 × 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, Verison 8.2, 2003). The main effects 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 significant at $P < 0.05$. The results in the tables are presented as the mean and standard error of the mean (SEM).

RESULTS

Low NPP and high NPP diet contained non-phytate P at 1.4 and 3.7 g/kg, respectively (Table 1). Phytase activity of the feed showed an average of 281 FTU/kg in non-supplemented diets and 397 FTU/kg in diets supplemented with 150 FTU.

The results regarding the performance of the hens are provided in Table 2. The reduction of NPP in the diet resulted in an increased egg production and decreased feed intake per egg, feed conversion ratio, and egg weight. Moreover, the addition of F (150 FTU/kg) improved the feed conversion ratio and increased the egg weight, compared with the treatments without F. With regard to the physical characteristics of egg quality (Table 3), a significant interaction between NPP and F was found for the yolk colour (P = 0.016) and shell thickness measured in the equatorial plane (P = 0.038) – the eggs from the hens fed the control diet had the lightest yolks and the thinnest shells compared with the

Table 1. Ingredients and chemical composition of the used diets

Ingredient (g/kg)	Low NPP[1]	High NPP[1]
Wheat	314.4	310
Maize	317	315.7
Wheat bran	20	20
Soybean meal	210	210
Lucerne meal	20	20
Rapeseed oil	25	25
Dicalcium phosphate	0.8	18.5
Sodium chloride	2	2
Limestone	84	72
L-Lysine	0.6	0.6
DL-Methionine	1.2	1.2
Vitamin-mineral premix[2]	5	5
Analyzed nutrient contents (g/kg)		
Dry matter	89.2	89.1
AME_N (MJ/kg)	11.5	11.4
Crude protein	165.8	165.2
Calcium	35.4	35.3
Total phosphorus	3.8	7.2
NPP (calculated)	1.3	4.0
NPP	1.4	3.7

AME_N = apparent metabolisable energy, NPP = non-phytate phosphorus

[1]levels of NPP in the diet; other experimental diets were supplemented with 150 FTU/kg of phytase

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

Table 2. The performance characteristics of laying hens

Phytase (FTU/kg)	Low NPP		High NPP		SEM	Probability		
	0	150	0	150		NPP	F	NPP × F
Hen-day egg production (%)	82.7	85.3	79.4	79.4	0.48	< 0.001	ns	ns
Egg weight (g)	62.5	63.6	63.6	64.5	0.12	< 0.001	< 0.001	ns
Egg mass (kg/hen)	4.3	4.6	4.2	4.4	0.05	ns	ns	ns
Feed intake (g/day/bird)	120.7	120.1	119.4	117.3	0.60	ns	ns	ns
Feed intake (g/egg)	146.3	141.3	151.1	150.0	1.02	0.001	ns	ns
FCR (kg feed/kg egg mass)	2.34	2.24	2.39	2.35	16.110	0.020	0.019	ns
Mortality (pcs)	2	0	2	0				

NPP = non-phytate phosphorus, F = phytase, FCR = feed conversion ratio, ns = not significant

other treatments. The content of NPP in the diet particularly influenced the albumen and shell quality: the higher level of NPP (4.0 g/kg) increased the albumen height, albumen index, Haugh units, yolk height, shell thickness, shell index, shell weight, and shell percentage. The eggs laid by the hens in the treatments with 3-phytase addition showed lower values of yolk index and higher values of yolk

Table 3. Physical characteristics of eggs and Ca and P contents in eggshells

Phytase (FTU/kg)	Low NPP		High NPP		SEM	Probability		
	0	150	0	150		NPP	F	NPP × F
Eggshell surface (cm^2)	73.6	74.2	74.3	75.0	0.19	0.049	ns	ns
Albumen height (mm)	6.4	6.4	7.0	7.2	0.05	< 0.001	ns	ns
Albumen index (%)	7.7	7.6	8.6	8.7	0.08	< 0.001	ns	ns
Haugh units	77.9	78.0	82.1	83.0	0.36	< 0.001	ns	ns
Albumen weight (g)	40.5	40.9	41.1	41.5	0.19	ns	ns	ns
Albumen percentage (%)	64.5	64.4	64.4	64.3	0.10	ns	ns	ns
Yolk height (mm)	18.2	17.9	18.3	18.3	0.05	0.007	ns	ns
Yolk index (%)	44.0	43.3	44.1	43.7	0.12	ns	0.033	ns
Yolk colour – La Roche	10.5	10.9	10.9	10.9	0.06	ns	ns	0.016
Yolk weight (g)	16.2	16.4	16.3	16.7	0.07	ns	0.050	ns
Yolk percentage (%)	26.0	25.9	25.8	25.9	0.09	ns	ns	ns
Yolk and albumen ratio (%)	40.4	40.4	40.2	40.5	0.19	ns	ns	ns
Shell deformation (mm)	0.486	0.477	0.482	0.476	0.0025	ns	ns	ns
Shell breaking strength (N)	37.26	38.40	38.50	38.94	0.291	ns	ns	ns
Shell thickness, blunt end (mm)	0.322	0.333	0.335	0.338	0.0013	< 0.001	0.006	ns
Shell thickness, equator (mm)	0.328	0.338	0.343	0.342	0.0013	< 0.001	ns	0.038
Shell thickness, sharp end (mm)	0.336	0.345	0.349	0.350	0.0014	0.002	ns	ns
Shell thickness, average (mm)	0.329	0.339	0.342	0.343	0.0012	< 0.001	0.022	ns
Shell index ($g.100/cm^2$)	8.1	8.3	8.4	8.4	0.03	0.003	ns	ns
Shell weight (g)	6.0	6.2	6.2	6.3	0.03	< 0.001	0.010	ns
Shell percentage (%)	9.5	9.7	9.8	9.8	0.03	0.025	ns	ns
Shell P content (g/kg ash)	1.31	1.29	1.34	1.29	0.012	ns	ns	ns

NPP = non-phytate phosphorus, F = phytase, ns = not significant

Table 4. pH of the digestive tract

Phytase (FTU/kg)	Low NPP		High NPP		SEM	Probability		
	0	150	0	150		NPP	F	NPP × F
Crop	4.95	5.02	4.91	4.86	0.037	ns	ns	ns
Gizzard	4.02	4.44	4.50	4.90	0.107	0.035	0.018	ns
Small intestine	5.83	5.67	5.59	5.65	0.077	ns	ns	ns

NPP = non-phytate phosphorus, F = phytase, ns = not significant

weight, shell thickness, and shell weight. The shell strength and P content in the eggshells was not negatively influenced by the dietary treatment. As shown in Table 4, the lowest values for the digestive tract pH were recorded in the gizzard. In addition, the higher level of dietary NPP and F supplementation increased the pH in the gizzard segment.

DISCUSSION

The complete diets for hens in the Czech Republic have unnecessarily high P contents. This fact results in increased feed costs, often decreased performance, and environmental pollution by the unused P that is excreted. The recommendation for laying hen diets is 0.25% AP or 250 mg/hen per day (National Research Council, 1994), but much higher levels than this amount are commonly used in the industry. The AP or NPP requirement found in the literature varies from 0.13 to 0.30 (Mayer and Parsons, 2011).

Phytases are also used as dietary feed additives in order to increase the digestibility of phytate P and positively influence the digestibility of other nutrients. However, at higher doses (300–500 FTU/kg), F emphasises the negative effect of high levels of P in the diet. In the current study, a higher concentration of P in the diet decreased the hen-day egg production, feed intake, and feed conversion ratio. The higher level of P that we chose was the same as in the experiments of Skřivan et al. (2010), in which an identical negative effect of excessive P was observed. In addition, F mobilises phytate, Ca, and other nutrients. It is known that a higher concentration of Ca at the end of the laying period can improve the eggshell quality and increase the hen-day egg production. In our case, the addition of F (150 FTU/kg) non-significantly increased the egg production only in those hens fed the diet with the lower level of AP. Moreover, our results indicate that the lower level of AP (1.3 g/kg) significantly ($P < 0.001$) decreased the egg weight in comparison to 4.0 g of AP per kg of diet. Some authors have reported that a low dietary AP without supplemental F decreased performance characteristics, such as egg production, feed consumption or egg weight (Summers, 1995; Gordon and Roland, 1998; Boling et al., 2000; Francesch et al., 2005). In contrast, an increase in egg production, feed consumption, and egg weight (Um and Paik, 1999; Cabuk et al., 2004) and an improvement in the feed conversion (Jalal and Scheideler, 2001; Liebert et al., 2005) were found after F addition. The reduction of the feed conversion ratio and increase of the egg weight due to the F supplement is also evident from our results. The opposite results were reported by Carlos and Edwards (1998) and Berry et al. (2003) who did not find any significant effect of F on the egg weight. Accordingly, Keshavarz (2003) showed that the presence of F did not have an effect on performance but reduced several indices of shell quality. In addition, Lim et al. (2003) recorded a significant interaction between the NPP level and F for egg production. The high NPP level (0.25%) and F supplementation (300 FTU/kg) increased egg production only in the second 10-week period (weeks 31–41).

The values of the albumen height, albumen index, Haugh units, and yolk height were significantly decreased at the lower concentration of NPP. There were no observed effects of F addition on these parameters. A low P content could reduce the formation of phosphoproteins in egg yolk and albumen. In contrast, the F added to the low-NPP feed improved the pigmentation of the egg yolk, a result that was also observed by Kozlowski and Jeroch (2011). The lower values of eggshell quality without decreases in its strength can be explained by a significant increase in egg production at the lower content of NPP. The addition of F to a low dietary content of NPP positively influenced the eggshell quality versus a high content of NPP.

The main sites of microbial F activity in the digestive tract of laying hens are the crop and caeca (Al-Sharafat et al., 2009), and F is more efficient at a lower pH. The 3-phytase from *Aspergillus niger* has two optimal pH levels (2.5 and 5.0), while the 6-phytase from *Peniophora lycii* is between 4.5–5.0 (Lassen et al., 2001; Tamim et al., 2004). In this experiment, the higher level of P or addition of F increased the pH in the gizzard to a value suitable for F activity. Mucosal F have also been identified in the small intestine of poultry. However, their efficacy is largely thought to be rendered insignificant due to the levels of Ca present in poultry diets (Tamin et al., 2004). Because the pH in the small intestine of broilers is between 5.5 and 6.6 (Shafey et al., 1991), the dietary Ca complexes with the phytate thus make the phytate unavailable for F activity (Wilkinson et al., 2011).

CONCLUSION

A diet for hens with no added F, containing only 1.3 g/kg of NPP, was shown to lead to a higher egg production than a diet with a concentration of 4.0 g/kg NPP; however, the egg weight did not decrease. From the point of view of albumen quality and certain eggshell quality indicators (with the exception of eggshell strength), a diet without added F is deficient in NPP. The supplementation of 150 FTU/kg F to a low-P diet increased the characteristics of performance and egg quality.

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Corresponding Author

Prof. Miloš Skřivan, Institute of Animal Science, Přátelství 815, 104 01 Prague 10-Uhříněves, Czech Republic
Tel. +420 267 009 720, fax +420 267 711 448, e-mail: skrivan.milos@vuzv.cz