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Negative Effect of Phytase Superdosing in Laying Hens

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ABSTRACT

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Hisex hens, aged 24 weeks, were divided into 6 groups. Each group consisted of 4 cages with 10 hens per cage with dimensions and equipment meeting the EU directives. This 2 × 3 factorial experiment included two levels of calcium (Ca; 35 or 42 g/kg) and 3 different additions of 6-phytase OptiPhos® (Ph; 0, 300, and 1500 phytase units (FTU)/kg) to the diet. The content of nonphytate phosphorus (NPP) in all diets was the same (1.8 g/kg). The experiment lasted 12 weeks. A significant interaction of Ph × Ca (P = 0.029) was observed in hen-day egg production. A diet with 35 g/kg Ca and the highest dose of Ph (1500 FTU/kg) resulted in a lower hen-day egg production (84.1%) than did the other treatments (91.5–88.6%). Phytase superdosing negatively influenced egg mass production (P = 0.001) and the feed conversion ratio (P = 0.018). Neither Ph nor Ca influenced eggshell ash content. Both additions of Ph into mixed feed decreased Haugh units (P < 0.001). A higher content of Ca in the diet increased shell thickness (P = 0.024) and shell breaking strength (P = 0.039), while Ph addition increased shell percentage (P = 0.004) and shell breaking strength (P = 0.009). The results of this experiment demonstrate the unsuitability of Ph superdosing in mixed feed for laying hens.

Keywords: Hisex; dietary calcium; OptiPhos®; performance

Phytates, which are salts of phytic acid, are the main form of phosphorus (P) in plants. Due to insufficient phytase activity in the digestive tract of monogastric animals, phytate P bioavailability is limited (Simons et al. 1990). Therefore, inorganic phosphorus is added to mixed feed. Excretion of undigested P leads to environmental pollution. One of the most effective ways of protecting the environment is the addition of microbial phytases (Ph) to feed mixtures with low P concentration (Dilger et al. 2004). Dietary supplements of Ph for poultry are recommended in amounts up to 500 phytase units (FTU)/kg (Selle and Ravindran 2007). The addition 500 FTU/kg of Ph hydrolyzes 35% phytate P (Camden et al. 2001). Recently, higher dietary additions of Ph (more than 1000 FTU/kg) have been recommended (Zeng et al. 2014; Manobhavan et al. 2016). Information about Ph superdosing in feed mixtures for laying hens is rare. We found 3 sources, none of which discussed Ph superdosing at various dietary calcium (Ca) concentrations. Meyer and Parsons (2011) added 6-phytase OptiPhos® at 0, 150, 250, and 15 000 FTU/kg to feed mixtures for laying hens; these additions contained 38 g/kg Ca and 1.05 g/kg nonphytate phosphorus (NPP). The effect of superdosing of Phyzyme XP, at 10 000–30 000 FTU/kg, on the productive performance of laying hens was observed by Kim et al. (2017). The concentration of Ca in all dietary treatments was 39.1 g/kg, and the NPP concentration in experimental treatments was 2.6 g/kg. Phytase releases not only P but also Ca as well as other cations from the phytate complex. Laying hens metabolize a large amount of Ca for

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egg shell formation, and an increasing Ca : P ratio can lead to a reduction in performance (Roland and Gordon 1996).

The issue of Ph superdosing in relation to two dietary Ca concentrations is the subject of this experiment.

MATERIAL AND METHODS

Hens, husbandry, and diets. Two hundred forty 24-week-old Hisex Brown hens were randomly assigned to 6 treatments with 4 replicate cages at 10 hens per cage. The hens were housed in three-floor cages in the same air-conditioned facility. Each cage was 7560 cm^2. A nest box, feeder (120 cm), and 3 nipple water dispensers were included in each cage. In addition, the cages were equipped with a perch (150 cm), a dust bath, and equipment for claw abrasion; this equipment conformed to the European Council Directive 1999/74 EC (Council Directive 1999). Room temperature was maintained at 20–22°C, and the light cycle was 16 h of light and 8 h of darkness. The light intensity was approximately 10 lx in the central storey. Feed and fresh water were supplied *ad libitum*. The 2 × 3 factorial experiment included two levels of calcium (Ca; 35 or 42 g/kg) and 3 different additions of 6-phytase OptiPhos® (Ph; 0, 300, and 1500 FTU/kg) in the diet. OptiPhos® (Huvepharma, Bulgaria) is an *Escherichia coli* derived 6-phytase (EC 3.1.3.26) produced by a genetically modified strain of *Komagataella pastoris* (DSM 23036; formerly known as *Pichia pastoris*) in a submerged fermentation process. Phytase was added into the vitamin-mineral premix. The ingredients and nutrient composition of the diets are listed in Table 1. The content of NPP in all diets was the same (1.8 g/kg). The experiment lasted 12 weeks. The study protocol was approved by the Ethical Committee of the Institute of Animal Science, Prague-Uhříněves.

The numbers of eggs and hens and their health status were monitored daily. Hen-day egg production and feed intake were calculated weekly on a per-cage basis. Egg weights were determined three times per week. Hens were weighed at the beginning and the end of the experiment, i.e. at 24 and 36 weeks of age.

Diet analyses. The feed crude protein content was measured using a Kjeltec Auto 1030 instrument (Tecator, Sweden). The fat content in the diet was determined by extraction with petroleum ether using a Tecator 1045 Soxtec Extraction Unit (Tecator). Dry homogenized diets were ashed at 550°C, and the ash was dissolved in 3 M hydrochloric acid. The total P in the solution was determined using a vanadate-molybdate reagent (AOAC International 2005; method No. 965.17). The Ca concentration in the hydrochloric acid extract was measured by atomic absorption spectrometry using a Solaar M6 instrument (TJA Solutions, UK). The phytate P contents of the diets were determined by the capillary isotachophoretic method (Duskova et al. 2001). The phytase activity of the diets was determined as described by Eeckhout and De Paepe (1994).

Egg quality analyses. For physical parameters determination, eggs were collected three times during the experiment (Tumova et al. 2017). Once within each collection period, whole-day egg production was analysed. A total of 662 eggs were analysed. The albumen, yolk, and shell percentages were determined based on the individual weight of each egg and the weights of its components. The albumen height was measured using an IP54 digital micrometer (Swiss Precision Instruments, Inc., USA). Haugh units (HU) were calculated according to the methods of Haugh (1937). Shell breaking strength was determined on the vertical axis using an Instron 3360 apparatus (Instron, USA). After removing the shell membranes, shell thickness (i.e. the average of 3 values from the sharp and blunt ends and equator of the shell) was measured using a micrometer.

Analyses of the ash, P, and Ca contents of the eggshells were conducted once during the experiment. A total of 192 eggs were analysed (8 samples per treatment, 4 eggs per sample, 6 treatments; $n = 8$). The shells were dried at 105°C for 24 h, placed in a desiccator and weighed, and then the dried homogenized eggshells were ashed in a muffle furnace at 500°C for 12 h. The P and Ca contents of the dried eggshells were determined (for method see the Diet analyses part).

Statistical analysis. The data from the experiment, which consisted of a 2 × 3 full factorial design, were analysed using two-way analysis of variance (ANOVA) with the General Linear Model (GLM) procedure in the SAS software (Statistical Analysis System, Version 9.3, 2003). The main effects considered were the concentration of Ca

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(Ca), phytase supplementation (Ph) and the interaction between these two factors (Ca × Ph). 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). Performance data were analysed using cage as the experimental unit.

RESULTS

The basic feed mixtures to which Ph was added contained similar amounts of total P (4.3 and 4.2 g/kg) and NPP (1.8 g/kg), but a different concentration of Ca (35 and 42 g/kg) (Table 1). No significant differences were found in the average body weight of the hens at the beginning (24th week of age) and at the end of the experiment (36th week of age) (Table 2). A statistically significant interaction was recorded in hen-day egg production ($P = 0.029$) and egg weight ($P = 0.033$). Supplementation with Ph at 1500 FTU/kg to the diet with 35 g/kg Ca decreased hen-day egg production compared to all other dietary treatments. The lightest eggs were laid in both treatments with 1500 FTU/kg Ph in the diet and by hens fed the diet with 35 g/kg Ca without Ph addition. In addition, the increasing dose of Ph decreased egg mass production ($P = 0.001$) and increased the feed conversion ratio ($P = 0.018$).

Higher levels of Ca ($P < 0.001$) and Ph ($P < 0.001$) additions negatively influenced Haugh units, which express the albumen quality in relation to egg weight (Table 3). Higher dietary content of Ca increased shell thickness ($P = 0.024$). Shell breaking strength was positively influenced by the Ca ($P = 0.039$) and Ph ($P = 0.009$) evaluated factors. Shell percentage also increased after Ph addition to the diet ($P = 0.004$). Neither Ca nor Ph influenced ash content in egg shell.

Table 1. Composition of the basal diets (g/kg)

Item	Calcium levels in diets	
	35 g/kg	42 g/kg
Wheat	319	310
Maize	307	288
Soybean meal	250	253.9
Rapeseed oil	23	29
Dicalcium phosphate	3.6	3.5
Sodium chloride	2	2
Limestone	88	106.2
L-Lysine hydrochloride	1	1
DL-Methionine	1.4	1.4
Vitamin-mineral premix[2]	5	5
Dry matter	882	885
Crude protein	175.6	177.2
Crude fat	39.9	40.2
Crude fibre	27.4	27.6
Metabolizable energy (by calculation; MJ/kg)	10.73	10.65
Calcium	35.1	42.3
Total phosphorus	4.3	4.2
Non phytate phosphorus	1.8	1.8

[1]experimental mixed feed was supplemented with phytase (Ph) at 300 and 1500 FTU/kg in form of vitamin-mineral premix; Ph activity in three diets with 35 g/kg Ca was 146, 569, and 1491 FTU/kg and in diets with 42 g/kg Ca it was 126, 538, and 1418 FTU/kg, according to Ph addition

[2]provided per kg of mixed diet: 3.0 mg retinylacetate, 3000 IU vitamin D3, 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 ethoxyquin, 1 mg butylhydroxyanisole, 0.7 g 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

DISCUSSION

After determining that dietary Ph at 500 FTU/kg makes available less than 50% of phytate P (Camden et al. 2001), in broilers, attention has turned to higher doses of Ph, known as superdosing. In laying hens, the problem is complicated due to the high Ca requirement for egg shell formation and the negative effects of metabolic relationships of Ca : P. The decrease of hen-day egg production and egg mass production and the increase of feed conversion ratio in dietary treatments with 1500 FTU/kg at Ca concentration of 35 g/kg rather than 42 g/kg Ca demonstrated the dependence of Ph superdosing on the Ca content. Phytate can bind to cation Ca^{2+} in the small intestine, reducing the solubility of phytate and thereby reducing its accessibility by Ph. Ca binding to phytate occurs mainly in the small intestine when the pH level is above 5 (Dersjant-Li et al. 2015). Studies focused on Ph superdosing have not addressed differentiated dietary Ca concentration for laying hens. Meyer and Parsons (2011) tested Ph at 15 000 FTU/kg in lay-

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Table 2. Effect of dietary calcium and supplemental phytase on hen performance in weeks 24–36 of age

Calcium (Ca; g/kg)	35			42			SEM	Probability		
Phytase (Ph; FTU/kg)	0	300	1500	0	300	1500		Ca	Ph	Ca*Ph
BW, week 24 of age (g/hen)	1498	1473	1518	1503	1509	1458	9.9	ns	ns	ns
BW, week 36 of age (g/hen)	1664	1683	1679	1710	1699	1743	14.6	ns	ns	ns
Hen-day egg production (%)	90.5^{a}	91.5^{a}	84.1^{b}	90.9^{a}	88.6^{a}	88.6^{a}	0.40	ns	0.004	0.029
Egg weight (g)	53.9^{c}	54.9^{b}	54.1^{c}	55.0^{ab}	55.6^{a}	55.0^{ab}	0.10	0.002	< 0.001	0.033
Egg mass (g/hen/day)	46.6	47.9	43.3	48.2	46.5	45.1	0.27	ns	0.001	ns
Feed intake (g/hen/day)	115.1	112.0	112.2	114.3	110.8	108.1	0.41	ns	0.029	ns
Feed conversion ratio (g/g)	2.48	2.35	2.61	2.38	2.39	2.41	0.013	0.029	0.018	ns

BW = body weight, ns = not significant

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

ing hens, but stable concentrations of Ca (38 g/kg) and NPP (1.05 g/kg) were included in the diet. Ten times higher dietary supplementation of Ph, in comparison to the amount used in our experiment, did not influence egg production, egg weight, feed intake or ash percentage in the tibia. In addition, Kim et al. (2017) applied diets with unchanged Ca (39.1 g/kg) and NPP (2.6 g/kg) contents. These authors tested dietary supplements of 1000, 2000, and 3000 FTU/kg. In contrast to the experiment presented here, they recorded significantly higher egg production at 1000 and 2000 FTU/kg of Ph, but there were no differences in egg mass production, feed intake, feed conversion ratio, egg shell strength or Haugh units.

A warning on Ph superdosing in laying hens is strongly supported by the European Food Safety Authority (EFSA). In 2013, the EFSA released a scientific opinion on the safety and efficacy of Quantum® Blue (6-phytase) as a feed additive for laying hens and laying poultry species (EFSA 2013). The scientific opinion was based on 3 experiments with Lohmann Brown and Hy-Line Brown hens, both of which were fed graduated Ph supplements from 150 to 1200 FTU/kg. A higher egg laying rate and egg mass production occurred with the 150 FTU/kg feed dose than with the negative control. Both performance characteristics did not increase further with supplementation of 300 or 1200 FTU/kg. No concentration of Ca in diets was reported for any of the 3 experiments. The Scientific Panel on additives and products or substances used in animal feed (FEEDAP Panel), which belongs to the EFSA, concluded that the additive has the potential to be effective in laying hens at a dose of 150 FTU/kg feed. This conclusion was confirmed by Englmaierova et al. (2014), who reported that using dietary contents 2.1 g/kg

Table 3. Effect of dietary calcium and supplemental phytase on egg quality

Calcium (Ca; g/kg)	35			42			SEM	Probability		
Phytase (Ph; FTU/kg)	0	300	1500	0	300	1500		Ca	Ph	Ca*Ph
Haugh units	88.5	86.8	85.7	85.9	83.1	84.9	0.26	< 0.001	< 0.001	ns
Albumen percentage (%)	66.0	65.7	65.5	65.8	65.6	65.4	0.09	ns	ns	ns
Yolk percentage (%)	23.8	24.0	24.0	23.9	24.0	24.1	0.08	ns	ns	ns
Shell thickness (μm)	341.4	343.3	345.7	343.5	352.8	348.0	1.04	0.024	ns	ns
Shell breaking strength (N)	42.5	42.6	44.8	43.1	45.01	44.8	0.26	0.039	0.009	ns
Shell percentage (%)	10.2	10.3	10.5	10.3	10.4	10.5	0.03	ns	0.004	ns
Egg shell ash (%; week 34 of age)	95.1	95.3	95.2	95.5	95.1	94.8	0.08	ns	ns	ns
Shell Ca content (g/kg ash)	395.3	394.5	396.4	391.1	390.2	391.9	0.79	0.003	ns	ns
Shell phosphorus content (g/kg ash)	1.195	1.204	1.206	1.187	1.193	1.273	0.011	ns	ns	ns

ns = not significant

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NPP and 41 g/kg Ca with the addition of 150 FTU/kg 3-phytase Natuphos® resulted in higher egg production and higher Ca and P concentrations in egg shells compared to the positive control (3 g/kg NPP). Olukosi et al. (2013) determined that a high dose of Ph in the diet releases more Ca from phytate and that this greater release is reflected in a higher concentration of Ca in bones. Our research did not include the analysis of Ca in bones, but Ph at 1500 FTU/kg increased the Ca content in the egg shells. On the other hand, changes in the shell thickness and shell strength, in favour of dietary Ca at 42 g/kg, are attributed to the well-known relationship between lower egg production and higher egg shell quality. The concentrations of Ca and NPP as well as the level of Ph superdosing in the experiment described here and in the literature differ. However, in all cases, the effect on performance was zero or negative.

In broilers, where the dietary content of Ca is at least three-and-a-half times lower than that in laying hens, Ph superdoses are effective (Shirley and Edwards 2003; Cowieson et al. 2011; Monobhavan et al. 2016). The cause of the differences between the two categories of poultry is Ca. Sebastian et al. (1996) reported that dietary Ca concentration had a significant effect on the response to Ph and that growth performance and mineral utilization in broilers were more optimized from diets supplemented with 6 g/kg Ca and Ph than from those supplemented with 10 g/kg Ca. These results suggest that a poultry diet with a high Ca concentration may be detrimental to performance.

CONCLUSION

Based on the results of this work, the authors conclude that at dietary Ca concentrations of 35 g/kg, a dietary superdose of Ph at 1500 FTU/kg is a factor that can reduce the performance of laying hens. In general, the main problem in diets for laying hens is the high content of Ca (4 times higher than in broiler diets) which causes the negative effect of high doses of Ph.

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