

Effect of exogenous phytase on egg quality in laying hens

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An experiment was conducted to determine the effect of different levels of exogenous phytase on egg quality in laying hens fed diets with low levels of only plant original phosphorus. Twenty four Lohmann Brown hens at 31 weeks of age were housed in individual cages. In total three experiments were successively carried out with the same laying hens. In all three experiments the same diet with the same content of nutrients were always used. The control diet contained no exogenous phytase. Experimental diets differed in the level and sources of exogenous phytase. Eggs were collected daily during period of the experiment to measure egg quality. Measurement of physical parameters such as egg weight and strength of eggshell were performed daily. Shell weight and shell thickness were determined after washing and drying of shells. The shell thickness was evaluated using the micrometer. Shell proportion was calculated. The addition of phytase (150, 200, 250 and 300 FTU per kg diet) did not affect egg weight. Addition of exogenous phytase at levels 150, 200 and 250 FTU per kg had no significant effect ($P > 0.05$) on eggshell quality. Only addition of both phytases (Natuphos and Optiphos) at the level 300 FTU to the same diet had positive effect on eggshell quality ($P < 0.05$).

Keywords: laying hens, feeds, phytase, phytate, egg quality, eggshell

1. Introduction

Phosphorus (P) is one of the essential minerals for all animals. It plays a critical role in cellular metabolism, as a part of the energy currency of the cell, in cellular regulatory mechanism, and in bone (85 % of P is in bone). The challenge in diets of non-ruminant animals is the best availability of P that is present in the diet (Applegate, 2008). All animals need to absorb P mainly as phosphate (Rodehutsord, 2013). A large proportion of P in cereals, oil-seeds and grain legumes is in the form of phytate P (myo-inositol hexakisphosphate) (Marounek et al., 2008). P contained in plants in phytate form is only utilizable by monogastric animals from 20 to 30 % (Zobač et al., 1997). This unavailability is due to the very low phytase activity found in the digestive tract (Pallauf et al., 1994).

Phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) catalyses the release of P from phytate (Pandey et al., 2001). Plant ingredients of poultry diets differ greatly in native phytase activity. The phytase activity in wheat and barley is high, whereas in maize and oil-seeds the activity is very low (Eeckhout and De Paepe, 1994). Phytase has been added to poultry diets as exogenous phytase (Yao et al., 2007). Phytase can be derived from a number of sources including plants, animals and micro-organism. Recent research has shown that microbial sources are more promising for the

production of phytase on a commercial level (Pandey et al., 2001). Microbial phytases are divided into two classes on the basis of the site of initial hydrolysis on phytases: 3-phytases and 6-phytases according to position of degradation (Onyango et al., 2005). Exogenous phytase is added to diets not only to enhance phytate P utilization, but also to reduce potential environmental pollution by phytate P and also reduce dietary costs (Waldroup, 1999).

A high or low level of available P in a laying hen's diet may adversely affect the bird's performance and reduce the eggshell quality (Harms, 1982). The use of phytase in layer diets improves phytate P utilization and reduces the requirement for inorganic P. Gordon and Roland (1997) reported that hens consuming the low nonphytate P (NPP) diet with supplementary phytase performed as well as the hens fed diets containing higher levels of NPP without supplementary phytase. However, the effects of phytase in layer diets are complicated by the intimate link between Ca and P metabolism (Scott et al., 1999). Compared to broiler chicks, phytase inclusion in diets for laying hens has been the subject of less research (Selle and Ravindran, 2007).

This study was conducted to determine the effect of different levels of exogenous phytase on egg quality in laying hens fed diets with low levels of only plant original phosphorus.

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2. Material and methods

2.1 Birds and management

Twenty four Lohmann Brown laying hens, 31 weeks old, were housed in individual cages. Cages were designed for animals. These cages were made by Kovobel and were originally intended for roosters. The cages provided 1806 cm² of floor area without the nest, 43 cm of feeder and 2 nipple water dispensers. The average weight of hens was 1.7 kg at the beginning of the experiment. All hens were allowed *ad libitum* access to the feed and water.

In total three experiments were successively carried out with the same laying hens. Prior to starting the egg collection the laying hens fed the experimental diets at least five days. The egg collection period afterward lasted 5 days. A 15-h photoperiod from 06.00 to 21.00 was used throughout all experiments. Hens were weighed at the beginning and end of the period.

2.2 Experimental diets

In all three experiments the same diet with the same content of nutrients were always used. The diets were formulated to contain the same metabolizable energy (ME) 11.8 MJ/kg, crude protein 17.6%, Ca 3.5%, lysine 0.9%, methionine 0.42%, Met + Cys 0.71% and NPP 0.115%. The content of total phosphorus was 0.415%. Composition of the diets and content of nutrient are shown in Table

1. The control diet contained no exogenous phytase and it was the same in all experiments. Experimental diets differed in the level and sources of exogenous phytase.

2.3 Experiments

2.3.1 Experiment 1

Laying hens were divided into two groups. The experimental diet contained phytase Natuphos at the level 150 FTU/kg diet (Natuphos 150 FTU). The experiment was arranged according to latin square. The experiment lasted 20 days. After first period (10–5 days preparatory period and 5 days egg collection period) the diets were switch between groups of laying hens followed by next preparatory and collection periods.

2.3.2 Experiment 2

Laying hens were divided into three groups. There were also used two experimental diets with two different phytases at the level 300 FTU/kg diet (Natuphos 300 FTU and Optiphos 300 FTU). The experiment was arranged according to latin square. The experiment lasted 30 days with the same arrangement as in the exp. 1.

2.3.3 Experiment 3

Laying hens were divided into three groups. There were used two experimental diets contained 200 and 250 FTU of Natuphos (Natuphos 200 FTU and Natuphos 250 FTU). The experiment was arranged according to

Tables 1 Ingredients of diets and nutrient composition in %

Component	Content in the diet in %
Wheat	29.20
Maize	38.90
Soybean meal	1.50
Rapeseed oil	21.00
Limestone	4.23
Grit	4.24
Salt	0.20
Lysin	0.10
Methionin	0.15
Aminovitan	0.50
Total	100.00
ME in MJ/kg	11.80
Crude protein	17.60
Lysin	0.90
Methionin	0.42
Met + cys	0.71
Ca	3.50

Table 2 Scheme of experiments

Experiment	The number of hens in group	Compound feed		
		Group 1	Group 2	Group 3
1	12	control diet	Natuphos 150 FTU	-
2	8	control diet	Natuphos 300 FTU	Optiphos 300 FTU
3	8	control diet	Natuphos 200 FTU	Natuphos 250 FTU

latin square. The experiment lasted 30 days with the same arrangement as in the exp. 1.

Scheme of the experiments is shown in Table 2.

2.3 Egg analysis

Eggs were collected daily during period of the experiment (always 5 days after preparatory period) to measure egg quality. Measurement of physical parameters such as egg weight and strength of eggshell were performed daily. The shell breaking strength was determined on the vertical axis using Egg Force Reader. Shell weight and shell thickness were determined after washing and drying of shells. The shell thickness was evaluated using the micrometer. Shell proportion was calculated. Totally 1920 eggs were analyzed for all three experiments.

2.4 Statistical analysis

Egg quality parameters were expressed using mean and standard error mean (SEM). Data obtained from this experiment were analyzed using the single factor analysis of variation. Data were followed by LSD test at a significance level $P < 0.05$ using the software package Unistat 5.1 (UNISTAT Ltd, ENGLAND).

3. Results and discussion

The quality of eggshell is shown in tables 3, 4 and 5. These parameters indicate the deposition phosphorus into the eggshell, thus the retention of phosphorus from the feed through the intestinal wall.

The results of Experiment 1 are presented in Table 3. There were two diets, control diet and diet with phytase Natuphos 150 FTU. The supplement of phytase Natuphos at the level 150 FTU had no significant effect on eggshell quality ($P > 0.05$). Except egg weight the characteristics of eggshell was almost the same in both groups. Egg weight was slightly higher in Natuphos 150 FTU (about 0.4 g).

Results of Experiment 2 are summarized in Table 4. There were no significant differences ($P > 0.05$) between treatments in egg weight. Compared with control, diet with phytase Natuphos 300 FTU resulted in significantly higher strength of shell, shell thickness, shell weight and shell proportion ($P < 0.05$). Significant difference was also noted in higher strength of shell, shell thickness and shell proportion ($P < 0.05$) in group of hens fed diet with phytase Optiphos 300 FTU compared with control.

Table 3 Results of Experiment 1

Parameters ± SEM	Group					
	Control diet			Natuphos 150 FTU		
Egg weight in g	59.9 ^a	±	0.432	60.3 ^a	±	0.406
Strength of shell in N	40.2 ^a	±	0.303	40.3 ^a	±	0.288
Shell thickness in mm	0.39 ^a	±	0.003	0.39 ^a	±	0.003
Shell weight in g	5.79 ^a	±	0.063	5.8 ^a	±	0.048
Shell proportion in %	9.61 ^a	±	0.069	9.65 ^a	±	0.061

Table 4 Results of Experiment 2

Parameters ± SEM	Group								
	Control diet			Natuphos 300 FTU			Optiphos 300 FTU		
Egg weight in g	61.2 ^a	±	0.399	61.3 ^a	±	0.416	60.4 ^a	±	0.483
Strength of shell in N	39.8 ^a	±	0.338	40.6 ^b	±	0.206	40.8 ^b	±	0.270
Shell thickness in mm	0.39 ^a	±	0.003	0.40 ^b	±	0.003	0.40 ^b	±	0.003
Shell weight in g	5.97 ^a	±	0.051	6.15 ^b	±	0.050	6.08 ^{ab}	±	0.049
Shell proportion in %	9.77 ^a	±	0.088	10.1 ^b	±	0.067	10.1 ^b	±	0.090

^{a,b} indicate statistical significant difference between groups ($P < 0.05$) for the same characteristics

Table 5 Results of Experiment 3

Parameters ± SEM	Group								
	Control diet			Natuphos 200 FTU			Natuphos 250 FTU		
Egg weight in g	64.1 ^a	±	0.402	64.7 ^a	±	0.467	63.7 ^a	±	0.481
Strength of shell in N	38.4 ^a	±	0.386	38.1 ^a	±	0.453	37.3 ^a	±	0.423
Shell thickness in mm	0.39 ^a	±	0.002	0.39 ^a	±	0.003	0.39 ^a	±	0.003
Shell weight in g	6.04 ^{ab}	±	0.052	6.19 ^b	±	0.050	6.03 ^a	±	0.056
Shell proportion in %	9.43 ^a	±	0.064	9.59 ^a	±	0.062	9.48 ^a	±	0.065

^{a, b} indicate statistical significant difference between groups ($P < 0.05$) for the same characteristics

Phytase level 300 FTU positively affect quality of eggshell. There was no significant difference between the effect of Natuphos and Optiphos.

The effects of phytase level 200 FTU and 250 FTU are summarized in Table 5. Result showed that only diet with phytase Natuphos 200 FTU improved shell weight ($P < 0.05$) compared with diet with phytase Natuphos 250 FTU and no significant differences were found in egg weight, strength of shell, shell thickness and shell proportion among three treatments.

The recommendation for laying hen diets is 0.25 % available phosphorus (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). Inadequate dietary P results in low feed intake and poor performance (Owings et al., 1977). In the presented experiments, despite low levels of NPP in diets, the performance and eggshell quality was not negatively affected probably mainly because of short term periods of using the diets.

Gordon and Roland (1997) reported that feeding 0.1 % NPP diet decreased egg production compared to 0.2 to 0.5 NPP diet but 0.1 % NPP diet supplemented with 300 FTU of phytase per kg diet completely corrected the adverse effect. Summers (1995) reported that a low dietary AP without supplemental phytase decreased performance characteristic, such as egg weight. Conversely, an increase egg weight was found after phytase addition (Cabuk et al., 2004; Englmaierová et al., 2012) but it was not confirmed by these experiments. The addition of phytase (150, 200, 250 and 300 FTU kg diet) did not affect egg weight. The same results were published by Berry et al. (2003) who did not find any significant effect of phytase on the egg weight.

Um and Paik (1999) reported no significantly different of eggshell strength in hens fed with control diet without phytase and diet with phytase 500 FTU kg diet.

This study also has shown that eggshell quality (strength, thickness, weight and proportion of eggshell) of hens fed low NPP (0.115 %) diets with 150, 200 and 250 FTU of exogenous phytase/kg diet were not

significantly different from those of the control without exogenous phytase. But the addition of both phytases (Natuphos and Optiphos) to the diets with a low dietary content of NPP positively affected the eggshell quality but only at the level 300 FTU kg diet.

4. Conclusions

Addition of exogenous phytase at levels 150, 200 and 250 FTU/kg to the diets with 0.115% NPP and with the total content of phosphorus 0.415% had no significant effect on eggshell quality (weight, thickness, strength, and proportion). Only addition of both phytases (Natuphos and Optiphos) at the level 300 FTU to the same diet had positive effect on eggshell quality ($P < 0.05$).

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