



EFFECTS OF SUPERDOSING PHYTASE B ON EGG PRODUCTION AND QUALITY IN LAYING HENS*

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Abstract

A trial was conducted to evaluate the addition of phytase B (PhyB) to a diet for laying hens. In the feeding trial with laying hens, three feeds were tested: a negative control diet (group I) without the addition of monocalcium phosphate, a negative control group with the addition of 0.05% (6575 AcPU/kg) phytase B (group II) and a positive control diet with recommended levels of calcium and phosphorus (group III). The experiment was carried out on 36 hens of the Bovans Brown variety, 12 birds were assigned to each test group. During the experiment, the number and weight of eggs laid by each hen, individual changes in the body mass of birds, and feed consumption were registered. From the 50th to the 54th day of the experiment, the amounts of the feed ingested and the excreta were measured in order to determine the retention of calcium and phosphorus. In order to examine the effect of diet on egg quality, 36 eggs from each study group were randomly selected for analysis. The research included measurements of physical and morphological parameters determining the quality of egg albumen, yolks and eggshells. Phytase B added to the feed for laying hens did not significantly affect feed conversion and feed consumption per egg but significantly increased the daily feed intake. Hens that consumed phytase B showed higher egg production indicators, including the number of eggs, the mean egg weight, and daily egg mass, as compared to hens from the negative control group (I). The addition of phytase B allowed obtaining similar calcium and phosphorus retention as in hens fed the positive control group diet (III) and showed reduced amounts of phosphorus in the manure. There were also significant positive effects of the addition of phytase B on the color intensity of the yolk and quality of eggshell by increasing its mass, density, and strength.

Key words: hens, eggshell quality, yolk, phytase B, phosphorus

Phytase B is a heat-tolerant enzyme that belongs to histidine acid phosphatases and was previously identified as an unspecified acid phosphatase, but at pH 2.5, it can also hydrolyze phytate efficiently (Kumar et al., 2013). Phytate phosphorus is scarce for monogastric animals, and phytic acid has anti-nutritional properties decreasing the digestibility of other nutrients (Woyengo and Nychoti, 2013). There are many publications on phytase A, but phytase B has not received such interest.

Primarily, the effectiveness of the use of phytases in the nutrition of monogastric animals was deeply studied in the feeding of broiler chickens (Denbow et al., 1998; Żyła et al., 2004; Woyengo and Wilson, 2019), but there are fewer papers describing the use of these enzymes in the diet of laying hens.

Supplementation of poultry feeds with phytase A increased the utilization of phytate phosphorus by 20 to 45% (Ravindran et al., 1995; Lei and Stahl, 2000; Maenz, 2001). However, the digestibility of total phosphorus and

phytate from feed ingredients can be affected by the diet composition, age of animals, methodology of the experiment, the level of phosphorus in the diet, and endogenous excretion of phosphorus (Lei and Porres, 2007; Musilova et al., 2017; Shen et al., 2002). The general estimation is that the addition of 300–600 units of phytase A (FTU) per kg of feed for poultry results in the release of 0.8 g of digestible phosphorus, and replaces it with 1.0 up to 1.3 g mono- and dicalcium phosphate, respectively (Ravindran et al., 1995; Esteve-Garcia et al., 2005; Lei and Porres, 2007; Musilova et al., 2017). Currently, applied exogenous phytases hydrolyze less than 35% of phytate in the feed supplied; hence there is a large space for the development of new strategies to increase the level of degradation of phytic phosphorus in poultry (Selle and Ravindran, 2007; Musilova et al., 2017). There is a widespread belief that a 0.1% reduction in non-phytate phosphorus in feed results in the 25% reduction of phosphorus in the feces of laying hens (Roland et al., 2003). On

*Project financed by the Ministry of Science and Higher Education of Poland as part of a grant for statutory activities.

the other hand, reductions in available phosphorus from 0.2 to 0.15% had no negative effect on eggshell quality but negatively affected egg production (Świątkiewicz et al., 2015).

Productivity growth in poultry can be achieved by applying the so-called super-dosages of phytase (above 2500 FTU/kg), and the results obtained could not be accounted only for an increase in the amount of available phosphorus (Kim et al., 2017; Woyengo and Wilson, 2019). Cowieson and Wilcock (2011) reported that the effects of the super-dosages of phytases could be justified in three ways: by improving the proportion of phosphorus to calcium during the degradation of phytate (most calcium is released in the first stage of hydrolysis), by reduced anti-nutritional effects of phytate (lower *myo*-inositol phosphates are more soluble) and also by the generation of free *myo*-inositol.

The preparations of phytases A currently used are not able to perform full hydrolysis of the phytate molecule, and their products include penta-, tetra-, tri-, di- and monophosphates of *myo*-inositol and orthophosphate. Using a mixture of phytase A with nonspecific acid phosphatase (also called phytase B) is theoretically possible to carry out complete hydrolysis of the phytate molecule. Phosphate ions are released in this way, as is free inositol (Duliński et al., 2016). Although the use of acid phosphatase in poultry nutrition has been studied before (Żyła et al., 2004, 2012), the potential of using high-dose phytase B in the feeding of laying hens was not determined to date. The application of this enzyme may release a larger amount of phosphate from phytic acid, which gives the opportunity to reduce or completely eliminate inorganic phosphate as a feed additive and thus reduce the amounts of phosphorus in the manure that goes to the environment.

The aim of this study was to estimate the effects of superdosing phytase B in the feed for Bovans Brown laying hens. In the experiment, the effects of the addition of phytase B on the performance of laying and egg quality were investigated.

Material and methods

Animals and diets

A recombinant phytase B (EC. 3.1.3.2; Finase AP) was kindly provided by AB Enzymes OY (Rajamäki, Finland), and the specific activity was 13,150 AcPU/g of the product (1 AcPU – Acid Phosphatase Unit – is defined as the amount of enzyme that liberates 1 μ M/min of p-nitrophenylphosphate at pH 4.5 and 40°C).

The experiment was performed on 36 hens of the Bovans Brown variety. The birds were purchased at a poultry farm Jawiszowice-Zasole, at the age of 18 weeks. From their arrival to the start of the experiment, the hens were fed a standard feed that covered all their needs for nutrients, as indicated in Poultry Nutrition Standards (Smulikowska and Rutkowski, 2005). The experiment

started when the hens were 45 weeks of age and continued for 12 weeks. The birds were placed in metal cages measuring 40 cm $\times$ 40 cm, equipped with a mesh bottom, and had free (*ad libitum*) access to water and feed. The chicken coop temperature was kept at 17–19°C. The light program assumed 14 hours of daylight and 10 hours of night daily.

The composition and nutritional value of the feeds used in the experiment are shown in Table 1. The experiment was performed in three dietary treatment groups with 4 replications of 3 layers. The negative control (group I) comprised hens that did not receive feed enzymes and calcium phosphate (Table 1). Group II was the experimental group, in which birds were fed a mixture of the same composition as the negative control but with the addition of 0.05% phytase B at a dose of 6,575 AcPU/kg feed. Group III was a positive control, which received a standard amount of calcium phosphate (1.25%) in feed according to Polish Nutrient Requirements of Poultry (Smulikowska and Rutkowski, 2005). During the time of the study, feed compounds were stored in a refrigerator at a temperature of 8°C.

Determination of the production parameters

During the experiment, the number and weight of eggs laid by each hen were registered daily. At the beginning (45 weeks) and at the end of the experiment (57 weeks), individual changes in the body mass of birds were monitored, and feed consumption was controlled. Based on these data, the following production parameters were calculated: egg production, the total mass of eggs and mean weight of an egg, the daily mass of eggs per laying hen, egg laying rate in %, the daily feed intake by the hen, feed conversion (feed consumed per 1 g of produced egg mass) and feed consumption (g) per one egg. The egg laying rate (productivity) was calculated as a ratio of the eggs laid by a single hen during the whole period of research to the number of days of the experiment and expressed as a percentage.

Determination of calcium and phosphorus retention

From day 50 to day 54 of the experiment, the amounts of the feed ingested and the excreta which were stored after freezing were measured. After obtaining the total collection of excreta from each experimental group, the samples were weighed and then dried to air-dry weight. The dried excreta were weighed again and then homogenized. In the dried excreta, calcium and phosphorus were determined for each of the experimental groups. In order to determine the amount of calcium and phosphorus absorbed by the laying hens, on the basis of the results obtained (Ca and P consumed, excreted and retained), retention rates were calculated, i.e., the ratios of elements retained in the body to the ones consumed, expressed as a percentage. The values of calcium and phosphorus retained were calculated as the difference between the amount of the element ingested and the amount

excreted. Calculations were performed separately for each bird.

Table 1. The composition and nutritional level of experimental laying hens' diets

Ingredient	Group I (%)	Group II (%)	Group III (%)
Corn	62.90	62.86	61.75
Soybean	25.35	25.34	25.70
Rapeseed oil	1.25	1.25	1.00
Limestone	9.60	9.60	9.40
Monocalcium phosphate	–	–	1.25
Sodium chloride	0.30	0.30	0.30
DL-methionine (99%)	0.10	0.10	0.10
Vitamin-mineral premix ¹	0.50	0.50	0.50
Acid phosphatase (phytase B)	–	0.05	–
Total ingredients (%)	100	100	100
Calculated composition (on an as-is basis)			
Crude protein ²	16.7	16.7	17.0
Energy (MJ/kg)	11.51	11.51	11.55
Lys ²	0.83	0.83	0.88
Met ²	0.375	0.375	0.350
Ca ²	3.80	3.80	3.70
P (digestible) ²	0.124	0.124	0.375
Ca analyzed	3.2	3.7	4.5
P total analyzed	0.411	0.388	0.678

¹The premix provided, per kg of diet: vitamin A, 10,000 IU; vitamin D₃, 3,000 IU; vitamin E, 50 IU; vitamin K₃, 2 mg; vitamin B₁, 1 mg; vitamin B₂, 4 mg; vitamin B₆, 1.5 mg; vitamin B₁₂, 0.01 mg; Ca-pantothenate, 8 mg; niacin, 25 mg; folic acid, 0.5 mg; choline chloride, 250 mg; manganese, 100 mg; zinc, 50 mg; iron, 50 mg; copper, 8 mg; iodine, 0.8 mg; selenium, 0.2 mg; cobalt, 0.2 mg.

²Calculated according to an analysis of the chemical composition of raw feedstuffs (AOAC, 1990).

The amount of calcium in the excreta was determined using the flame atomic absorption spectrophotometer AA-240FS, after microwave preparation of samples (MarsXpress), according to the method presented in AOAC (1990), method 968.08. The amount of phosphorus in the excreta was determined by spectrophotometry UV-VIS with ammonium-vanadium molybdate reagents after samples had reached 500°C, according to the procedure based on the methodology included in the AOAC (1990), method 965.17.

Determination of physical characteristics and quality of eggshell

In order to investigate the effects of the diet on the quality of the eggs at 57 weeks of age from each of the laying hens, eggs (36 eggs from each treatment) were randomly selected for analysis. The study was performed within 48 hours after laying. The study included meas-

urements of physical and morphological parameters, and variables that determine the quality of eggshells.

The assessment covered: weight, shape index, the amount of protein, Haugh units, and weight and color of egg yolks. The egg shape index is understood as the ratio of the width to the length of the egg and is expressed as a percentage. A Haugh unit (HU) is calculated using the value of dense white height (H) and egg weight (W) according to the formula given by Williams (Williams, 1992):

$$HU = 100 \log (H + 7.7 - 1.7 W^{0.37}).$$

The HU value informs about the quality of eggs. The higher the value of the Haugh unit, the higher the quality of an egg. The yolk color was determined visually using a 15-point Roche scale. The lowest values represent egg yolks that are almost colorless, and the highest correspond to darker yolks. The assessment also covered the variables that determine eggshell quality: color, weight, thickness, and mechanical strength. The color of the shell was specified by the percentage of white. The darker the shell, the lower the percentage of white. Shell density and thickness were measured using a semi-automated egg quality devices system QCM+ (Technical Services and Supplies (TSS), York, UK). Eggshell density was calculated as the ratio of dried shell weight per unit of shell surface (mg/cm²). The QCT device – an electronic micrometer (TSS, York, UK) was used to measure shell thickness near the equator of the egg. The strength of the shell was measured using an Instron Testing Machine, model 5542 (Instron Ltd., High Wycombe, England), equipped with a 500-Newton load cell. During this test, eggs were compressed with a constant speed of 10 mm/min, and the strength was determined when the eggshell cracked.

Statistical analysis

Statistical calculations were performed using Statgraphics Centurion 18 for Windows. All data were analyzed by the one-way analysis of variance (ANOVA). The significance of differences between groups for all traits was verified using Duncan's multiple-range tests. The differences between treatments were considered statistically significant when $P < 0.05$.

Results

The effects of phytase B on the production parameters of laying hens

Effects of the addition of phytase B to low phosphorus and calcium feed on the production parameters of laying hens are shown in Table 2. Significant differences were found in the effects of dietary treatment on the total number of eggs laid during the studied period, the total mass of eggs, the daily egg mass, the egg production, and the daily feed intake.

Table 2. The effects of phytase B on the production parameters of laying hens fed low calcium and low phosphorus feed

Parameters	Experimental groups			SEM	p
	I control –	II phytase B	III control +		
Number of eggs (pieces)	211 a	236 b	249 b	4.8362	0.0011
Total egg mass (g)	13050.1 a	14920.5 b	16191.6 b	426.61	0.0018
Mean egg weight (g)	61.86 a	64.73 b	65.36 b	0.8494	0.0378
Daily egg mass (g/hen/day)	51.18 a	58.51 b	63.50 b	1.6725	0.0018
Productivity (laying rate) (%)	82.75 a	92.55 b	97.65 b	1.8965	0.0011
Daily feed intake (g/day/pieces)	120.22 a	127.56 b	131.36 b	1.6183	0.0027
Feed efficiency index (g eggs/g feed)	0.45	0.46	0.48	0.0146	0.3193
Feed conversion ratio (g feed/g eggs)	2.23	2.19	2.07	0.0710	0.2961
Feed consumption/egg (g)	141.19	141.47	135.24	3.1711	0.3366

Within a single line mean values of the same parameter, which differ significantly, are described with various letters (Duncan test, $\alpha = 0.05$). SEM – standard error, p – the probability.

Table 3. The effects of phytase B on the balance of calcium and phosphorus in laying hens fed low calcium and low phosphorus feed with the addition of phytase B

Parameters	Experimental groups			SEM	p
	I control –	II phytase B	III control +		
Ca ingestion (mg/day/pieces)	3423.27 a	4791.81 b	6305.44 c	239.23	0.0000
Ca excreted (mg/day/pieces)	2188.29	2248.68	2443.84	196.18	0.6331
Ca retained (mg/day/pieces)	1234.96 a	2543.12 b	3861.59 c	200.92	0.0000
Ca retention (%)	<u>34.42 a</u>	<u>53.25 b</u>	<u>61.73 b</u>	4.0238	0.0001
P ingested (mg/day/pieces)	420.18 a	508.68 b	978.43 c	30.493	0.0000
P excreted (mg/day/pieces)	306.31 a	326.88 a	630.74 b	22.968	0.0000
P retained (mg/day/pieces)	113.88 a	181.81 b	347.69 c	19.206	0.0000
P retention (%)	<u>26.44 a</u>	<u>35.26 b</u>	<u>35.57 b</u>	1.9856	0.0033

Within a single line mean values of the same parameter, which differ significantly, are described with various letters (Duncan test, $\alpha=0.05$). SEM – standard error of mean, p – the probability.

Table 4. Effects of phytase B on the physical and morphological composition of eggs

Feature	Experimental groups			SEM	p
	I control –	II phytase B	III control +		
Shape index $\bar{x}$ (%)	78.33	76.49	77.36	0,5422	0.0526
Albumen height $\bar{x}$ (mm)	5.95	6.20	6.75	0,2538	0.0685
Haugh units $\bar{x}$	73.95	75.59	79.30	1,6810	0.0644
Yolk weight $\bar{x}$ (g)	17.39	18.70	27.90	6,4938	0.4383
Yolk color $\bar{x}$ (pt)	4.27 ^a	4.75 ^b	4.06 ^a	0,1133	0.0001

Within a single line mean values of the same parameter, which differ significantly, are described with various letters (Duncan test, $\alpha=0.05$). $\bar{x}$ – mean values, SEM – standard error of mean, p – the probability.

In the group of birds fed the negative control diet (I), the lowest number of eggs was collected (211). The total mass of eggs collected during the 12-week experiment was also the lowest in the negative control group (13.05 kg). The addition of phytase B to the feed (group II) resulted in a significant difference in the number of eggs

laid by hens (236), as well as in the total weight value of 14.92 kg as compared with group I (negative control). The greatest number of eggs laid (249) and the highest total mass (16.19 kg) were observed in the positive control (III group). There were no statistically significant differences between experimental group II and positive

control (group III). The highest values of productivity were observed in the positive control group (III), while the lowest were in the negative control group (I). The addition of phytase B increased egg production by almost 10%. The higher value of daily intake of feed was characteristic of hens receiving feed with added phytase B (group II) and in the positive control group (group III).

The effects of phytase B on calcium and phosphorus retention in laying hens

The highest amount of ingested calcium was observed in group III, lower in group II and the lowest in group I (Table 3). Differences among the values of the calcium retained presented a similar trend to the values of calcium ingestion. The addition of phytase B in group II significantly increased calcium retention by 18.83%. Although the retention of calcium in group II was lower by 8.48% than in the positive control group (III), this difference was not significant.

Amounts of phosphorus excreted in the negative control group and in the phytase group were similar (306.3 and 326.9 mg/day/hen), whereas hens fed positive control diet excreted almost twice as much (630.7 mg/day/hen).

Laying hens fed the negative control diet (I) retained only 26.4% of the phosphorus ingested. Significantly higher phosphorus retention was recorded in birds fed the diet containing phytase (II) as well as in those fed the positive control diet (III) and these values did not differ significantly from each other.

The effects of phytase B on egg quality

For the physical egg parameters, only the differences in the color of egg yolks were significant (Table 4). The lowest score (4.06) on the Roche scale was obtained by egg yolks from hens fed the positive control diet, whereas the highest (4.75) by eggs from the group fed the diet with phytase B. Usually this feature depends largely on the number of carotenoids in the feed given to hens.

In Table 5, the analysis of eggshell quality parameters is presented. The highest eggshell density (93.5 mg/cm²) was recorded for eggs laid by hens fed phytase (II). Eggshells from hens fed feed with phytase (II) and the positive control

group (III) had similar strengths (34.18 and 33.42 N), which was a significant difference in comparison to eggs laid by hens fed with the negative control (I) diet (25.96 N).

Discussion

Effects of phytase B on the production parameters of laying hens

Żyła et al. (2005, 2012) were the only to conduct studies on the application of phytase B in laying hens. In their experiments conducted on hens fed the corn-soybean feed with low content of calcium and total phosphorus (2.8% and 0.36% respectively), phytase B caused a significant increase in mean egg weight, which is consistent with the results obtained in this work. The addition of phytase B alone to the corn-soybean diet increased feed intakes, egg mass, and daily egg production (Żyła et al., 2012). Żyła et al. (2005) showed that the supplementation of feed for laying hens with phytase B increased egg production in the group receiving also 6-phytase A and did not cause changes in the case of 3-phytase A. Similar results, but only with respect to phytase A, were reported by other researchers. The number of eggs, egg weight, laying rate, and egg mass presented here are consistent with the findings of Kozłowski and Jeroch (2011), who examined the influence of *Escherichia coli* 6-phytase (Optiphos) on the performance parameters of Lohmann Brown hens fed corn-soybean. Also, Narasimha et al. (2013) reported that hens fed corn-soybean feed containing 3.6% calcium and 0.33% available phosphorus with 675 U of phytase A showed higher egg weight (53.53 g) and egg mass (36.45 g) than in the negative control diet (52.98 and 35.92 g, respectively). In their research, productivity, feed intake, and feed conversion ratio were higher in the group receiving phytase A than in the negative control diet. Van der Klis (1997), using a corn-soybean feed, with the addition of sunflower seeds (22 g/kg), with a calcium content of 3% and 0.32% of total phosphorus, showed that White Leghorn hens fed the negative control feed (without the addition of phytase or monophosphate calcium) had a significantly lower laying rate than those receiving the supplement of phytase A (Natuphos) or calcium monophosphate.

Table 5. Effects of phytase B on eggshell quality parameters

Feature	Experimental groups			SEM	p
	I control –	II phytase B	III control +		
Color $\bar{x}$ (% of white)	25.67	24.72	24.64	0,6888	0.4956
Weight $\bar{x}$ (g)	6.98 a	7.49 b	7.11 a	0,1228	0.0101
Thickness $\bar{x}$ (μ m)	396.64	404.61	401.81	5,9144	0.6173
Density $\bar{x}$ (mg/cm ²)	88.01 a	93.46 b	90.96 ab	1,3262	0.0145
Strength $\bar{x}$ (N)	25.96 a	34.18 b	33.42 b	1,6749	0.0009

Within a single line mean values of the same parameter, which differ significantly, are described with various letters (Duncan test, $\alpha=0.05$). $\bar{x}$ – mean values, SEM – standard error of mean, p – the probability.

The efficiency of phytase A depends, therefore, on the level of available phosphorus, which is confirmed by numerous authors describing the dominant role of non-phytin phosphorus (available phosphorus) deficiency as the cause of the deterioration of production parameters. For example, Gordon and Roland (1997) studied the effects of the addition of 3-phytase A Natuphos at a dose of 300 U/kg of feed on the production parameters in hens fed with diet with a different available phosphorus content. The positive effects of the addition of phytase A were only observed for the feed containing the lowest level of available phosphorus – 0.1% (0.33% total phosphorus). During their experiment, high hen mortality of about 12.5% in the negative control group was observed, while in the other groups receiving more phosphorus or phytase, mortality was at 2.4–2.5%.

The same authors also showed that hens receiving the lowest level of phosphorus without the addition of phytase demonstrated a 29.6% decrease in egg laying. This effect was overcome by the addition of phytase, similarly as in the case of the egg mass. At higher levels of phosphorus in the feed, there was no positive effect of Natuphos added at a dose of 300 U/kg on the production parameters. Punna and Roland (1999) showed that the response of hens to feed containing different levels of phosphorus or phytase depends on how these birds were fed during the growth and development period. The decrease in available phosphorus from 0.4 to 0.2% had no effect on egg production and average egg weight, but a decrease to 0.1% resulted in a reduction in laying rate and increased mortality (up to 55%), but the addition of 300 FTU phytase prevented the formation of these defects (mortality dropped to 5%).

Roland et al. (2003) state that a good laying rate can be obtained without the addition of inorganic phosphate to the feed with a total phosphorus content of 0.34% only of plant origin if a diet of barley, wheat, and oats is used as a source of energy. It is known that these grains contain a higher level of endogenous phytase (Eeckhout and De Paepe, 1994). As used in this study, a corn-soybean feed has a low activity of endogenous phytase, and probably a better effect of the use of phytase B (alone) in the feeding of laying hens can be achieved with feeds containing components with a higher activity of endogenous phytase (barley, wheat), which will cooperate in the hydrolysis of phytic acid.

In studies by Rao et al. (1999), supplementation with phytase A (250 PU/kg) of feed containing 0.1% non-phytin phosphorus increased the laying rate from 63% in the negative control to 91% in the group receiving phytase A, reaching the level of the reference feed (91%) containing 0.2% available phosphorus. Probably the reduction of available phosphorus from 0.124% to 0.1% in the experiment carried out with the use of phytase B would provide unequivocal results on production parameters, such as in studies by Rao et al. (1999).

Carlos and Edwards (1998) observed no effect of phytase A on the mean egg weight, as did Rao et al.

(1999) and Hughes et al. (2008). On the other hand, Um and Paik (1999) and Keshavarz (2000) obtained a greater mean egg weight.

A positive effect of phytase A on feed intake was described by Van der Klis et al. (1997), Rao et al. (1999), Musapuor et al. (2006) for laying hens of the White Leghorn variety and Roland et al. (2003) for laying hens of the Bovans variety. In a study by Jalal and Scheideler (2001), low phosphorus feed had a negative effect on feed intake since phosphorus deficiency causes a lack of appetite and anorexia, and the addition of 6-phytase increased feed intake due to the release of phosphorus. Van der Klis et al. (1997) did not observe significant differences in the values of the feed conversion ratio (kg feed/kg eggs). Similarly, Um and Paik (1999) showed that the feed conversion ratio did not differ significantly among the groups receiving phytase or not.

Gordon and Roland (1998), Keshavarz (2000), Boling et al. (2000), Jalal and Scheideler (2001) and Hughes et al. (2008) demonstrated that the application of phytase A to the feed with reduced non-phytin phosphorus content resulted in an increase in the feed conversion ratio (kg feed/kg eggs).

Effects of phytase B on calcium and phosphorus retention

The research has shown that phytase B introduced into the feed at a concentration of 0.05% positively affects the retention of phosphorus and calcium. This indicates that an additive to the feed of reduced available phosphorus and calcium preparation of phytase B effectively compensated for deficiencies of these elements. A similar increase in total phosphorus retention was described by Leske and Coon (1999), but after the application of phytase A of 300 FTU/kg of feed containing 0.1% non-phytin phosphorus. On the other hand, Van der Klis et al. (1997), using phytase A (Natuphos), showed increased uptake of phosphorus from 22% to more than 50% for hens receiving phytase in the feed (500 FTU/kg).

Narasimha et al. (2013) showed that phosphorus retention in hens receiving phytase was higher (35.39%) than in hens fed the negative control basal diet (30.58%) and even the positive control standard diet (32.52%). Similar results were obtained in this study with the use of phytase B. Musapuor et al. (2006) also observed a decrease in phosphorus levels in manure from hens fed a diet containing Natuphos phytase.

Boling et al. (2000) showed that the concentration of phosphorus in the feces of hens receiving phytase A with the feed containing 0.1% non-phytin phosphorus was 50% lower compared to chickens receiving 0.45% of the available phosphorus. This is compatible with the results obtained in this work.

Therefore, it can be presumed that in our work a concentration of phytase B (0.05%) in feed can replace 2.51 g of inorganic phosphate per kilogram of feed because it is such an amount of non-phytin phosphate that the feed from the positive control differs from the feed with

phytase B. Dephosphorylation with phytase B also ensured an increase in the amount of calcium released from phytic complexes, which resulted in significantly higher calcium retention than in the negative control.

Effects of phytase B on morphological composition and quality of eggshells

Albumen quality of eggs decreases with an increase in protein and amino acids content in the feed, and increases due to supplementation of feed for laying hens with lysine, ascorbic acid, and vitamin E (Roberts, 2004). In turn, the flock age affects the quality of the egg yolk (with age, the size of the yolk increases) and the carotenoid content in the feed, which are responsible for its color. Moreover, the low content of vitamin A in the feed and stress can cause the appearance of blood stains in the yolk. However, the quality of the shell is affected mainly by the calcium content in the feed and the ratio of its content to the phosphorus content because high levels of phosphorus can interfere with the absorption of calcium and degrade the quality of the shell (Roberts, 2004).

Narasimha et al. (2013) noted heavier shells (5.72 g) in the group receiving phytase than in the negative control basal diet (5.5 g) and even the positive control standard diet (5.37 g). In studies by Żyła et al. (2005, 2012), the introduction of phytase B resulted in an increase in the weight, thickness, and density of the shell. Similar results were obtained by Punna and Roland (1999) in studies with the use of phytase A. The positive influence of phytase A on shell thickness was also observed by Kozłowski and Jeroch (2011), while Gordon and Roland (1998) showed that supplementation with phytase A feed containing low levels of available phosphorus (0.1%) resulted in an increase in the quality of the shell. Such an effect was not observed at a higher level of phosphorus when the levels of calcium (about 4%) satisfied the requirements. Englmaierová et al. (2014) recorded lower eggshell breaking strength and lower shell thickness from hens fed diets containing 150 FTU/kg of Natuphos phytase than hens without exogenous phytases. An increase in eggshell quality associated with the use of phytase cannot be attributed solely to the release of phosphorus from the phytate and can be partly explained by the release of calcium from phytate complexes. High levels of calcium prevent deficits of this element which is required to disclose the effects of phosphorus released from phytate to the use of calcium and shell quality. High levels of dietary calcium (above 3.6–3.9%) usually do not have a positive influence on eggshell quality and even could lead to decreasing shell thickness (Świątkiewicz et al., 2015). Rao et al. (2014) demonstrated that White Leghorn layers require lower concentrations of calcium (minimum 3.32 g/bird/day) when the diets are supplemented with phytase A (500 FYT/kg) compared to those fed without phytase (minimum 3.58 g/bird/day); moreover, higher concentrations of calcium in the diet (40 g/kg and more) significantly reduced layer performance sug-

gesting the inhibitory effect on utilization of phosphorus by phytase.

In a study by Gordon and Roland (1998), the introduction of phytase A to feed containing 2.8% calcium and 0.1% non-phytin phosphorus resulted in an increase in the weight of shells from 4.89 to 5.17 g. Rao et al. (1999) showed that phytase A supplementation to hens receiving a feed containing 0.1% and 0.15% non-phytin phosphorus resulted in a significant increase in the weight of the shells, even higher than in the positive control. Jalal and Scheideler (2001) showed, however, that the addition of phytase A to feed with different content of available phosphorus did not affect the strength of the shell. In turn, in a study by Hughes et al. (2008), there was a significant decline in the quality of the shell at lower levels of available phosphorus, to 0.15%.

In the study of Narasimha et al. (2013), Haugh units, albumen index, and yolk index were not significantly different among research groups. Jalal and Scheideler (2001) did not observe significant effects of adding phytase (Finn Feeds and Natuphos) on the Haugh unit and wet yolk percentage (in relation to albumen), although the percentage of albumen and the dry yolk was greater in the group fed with feed containing 0.1% phosphorus and receiving phytase A. Phytase A influence on yolk color was reported before by Englmaierová et al. (2014) and Kozłowski and Jeroch (2011).

Indeed, more colored yolks in eggs from hens treated with phytase compared to eggs from control groups may indicate the influence of phytase B on the metabolism of fats and fat-soluble compounds. Karadas et al. (2010) showed that superdosing 12,500 FTU of phytase A per kg of feed resulted in the livers of broilers with significantly increased levels of vitamins fat-soluble E and A and coenzyme Q₁₀. It is also known that in the liver, there are synthesized lipids for the nascent yolk. Phytase B ensured an increased supply of *myo*-inositol, which could also increase the synthesis of glycerophospholipids, which include, among others, phosphatidylinositol. Furthermore, it is known that *myo*-inositol phosphates act as signaling molecules and thus are involved in many biological processes. However, Gao et al. (2013) observed lightening in yolk color in the group fed with a high level of phytase A transgenic corn (5,000 FTU/kg) compared to those fed conventional corn, but explained this by lower levels of lutein (6.43 mg/kg vs. 11.69 mg/kg).

Conclusions

Phytase B added at a concentration of 0.05% to the feed with low levels of calcium and inorganic phosphorus increased the production parameters of laying hens and reaching values similar to those of animals fed with feed containing standard levels of calcium and inorganic phosphorus. Moreover, phytase B supplementation effectively compensated for the deficiencies of these elements, enabling the animals to increase feed intake and achieve phosphorus and calcium retention at levels not

different from hens receiving feed containing a standard dose of these elements. Hens receiving phytase B in a superdose (6,575 AcPU/kg of feed) excreted less phosphorus than hens receiving the reference feed, and the eggs produced by them were characterized by a greater strength and density of the shell and more intensive coloring of the yolk.

The general conclusions can be drawn that the use of a superdose of phytase B in the feeding of laying hens has the potential to increase the efficiency of egg production through savings in the purchase of inorganic phosphate and calcium, thereby increasing income, and also provides benefits in the form of environmental protection and improved egg quality.

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Received: 20 VI 2023

Accepted: 4 IX 2024