Effects of phytase B on laying performance, eggshell quality and on phosphorus and calcium balance in laying hens fed phosphorus-deficient maize-soybean meal diets

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ABSTRACT: A study was conducted to evaluate the effects of dietary supplementation of phytase B (product of the *Aspergillus niger phyB* gene expressed in *Trichoderma reesei)* on feed intake, laying performance, eggshell quality, and on phosphorus and calcium balance in laying hens. Seventy-two, 40 weeks old Hy Line Brown hens were fed for 14 weeks the following four phosphorus-deficient (0.12% nonphytate phosphorus, NPP), maize-soybean meal-based diets: (1) calcium-deficient (2.8% Ca) control diet; (2) diet 1 + phytase B at the activity of 2.5 acid phosphatase units (AcPU/kg); (3) control diet (3.8% Ca); (4) diet 3 + phytase B at the activity of 2.5 AcPU/ kg. Each dietary treatment was fed to 18 cages of hens, 1 hen/cage kept in individual cages. Hens fed the NPP- and Ca-deficient diets consumed more feed ($P < 0.01$) and excreted less calcium ($P < 0.01$) than those receiving P-deficient diets with the standard calcium level. There were no effects of calcium level on feed utilization, egg mass, egg weight, and eggshell breaking strength. Egg production, although numerically higher in hens fed low Ca diets with no enzyme added, failed to be significantly different due to the low number of hens investigated and therefore the measurement should be considered as preliminary and supplementary. Phytase B increased mean egg weight by about 7% in layers fed the NPP- and Ca-deficient diet (Ca × phytase B interaction, $P < 0.05$), increased shell breaking strength, particularly at the standard calcium level, significantly enhanced amounts of calcium retained by layers and amounts of phosphorus retained by hens fed the Ca-deficient diets. Additionally, phytase B improved Ca retention at both dietary Ca levels and phosphorus retention in hens fed the Ca-deficient diets. Results of the study indicate that the efficacy of phytase B in NPP-deficient diets is strongly influenced by the dietary calcium level and the enzyme may modulate egg weight, eggshell quality, phosphorus and calcium retention in laying hens fed low-NPP, maize-soybean meal-based diets.

Keywords: phytase B; laying hen; laying performance; eggshell quality; retention of P and Ca

Commercial preparations of phytases used in poultry nutrition include preparations of 3-phytase A, such as Natuphos® (BASF, Lampertheim, Germany), produced by the *Aspergillus niger* strain carrying phyA genes from *Aspergillus ficuum*, which initiate phytate hydrolysis by removing the phosphate residue (iP) from position 3 of the myo-inositol ring. Another subclass of phytases – 6-phytases – like the enzyme from a recombinant *Aspergillus oryzae* strain transformed with the phytase gene of *Peniophora lycci* (Ronozyme P®, DSM-Nutritional Products, Basel, Switzerland) or enzymes from

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hosts carrying the *appA* gene from *Escherichia coli* (Phyzyme XP, Danisco, Malborough, UK; Quantum phytase, AB Enzymes GmbH, Darmstadt, Germany; OptiPhos®, JBC, Sheridan, USA), start the degradation of phytate by removing iP from position 6 or 4 on the phytic acid molecule (Hatten et al., 2001; Applegate at al., 2003; Ullah and Sedhumadhavan, 2003; Vats and Banerjee, 2004; Onyango et al., 2005). Despite many attempts to characterize and classify phytases, considerable differences in descriptions of properties and particularly in the mode of action of the same catalyst (Mullaney and Ullah, 2003; Oh et al., 2004; Vats et al., 2005; Greiner, 2006; Mullaney and Ullah, 2006) remain confusing, especially in the light of the official IUB enzyme classification. In contrast to the above-mentioned enzymes, phytase B is an enzyme with broad substrate specificity, high specific activity and low optimal pH (Oh et al., 2004; Vats et al., 2005). Phytase B, previously known as a non-specific acid phosphatase (EC 3.1.3.2), is classified nowadays as a histidine acid phytase (HAP). Although the preparation of phytase B has been available exclusively in an experimental form for some time (Finase® AP, Roal Oy, Finland), it has the full potential to be commercialized (Miettinen-Oinonen et al., 1997). Previously, it was postulated that the simultaneous application of either of phytases A along with phytase B offers an effective way to enhance phytate dephosphorylation in poultry (Żyła et al., 2000). In that study combinations of phytases A with phytase B were investigated thoroughly, but scarce information is available about the effects of phytase B fed as a sole supplemental enzyme, and nothing is known about its efficacy in the nutrition of laying hens. In broilers fed maize-soybean meal-based diets phytase B increased feed intake, BWG, toe ash and P retention, but not the retention of Ca (Żyła et al., 2004).

There have been many studies conducted on laying hens fed maize-soybean meal indicating that microbial phytase A improves performance, bone mineralization, phosphorus utilization and digestion of nutrients from nonphytate phosphorus (NPP)-deficient diets (Scott et al., 1999; Wu et al., 2006; Liu et al., 2007; Zaghari et al., 2008). Multifactor experiments employing different levels of NPP and Ca clearly indicated that the Ca level and Ca/NPP ratio in a diet effectively modulate phytase efficacy in laying hens (Gordon and Rolland, 1997; Jalal and Scheideler, 2001; Hughes et al., 2009). Phytase A preparations increased feed intake, feed conversion ratio and egg mass, improved eggshell quality and reduced phosphorus excretion in laying hens fed maize-soybean meal diets comprising 0.10 to 0.15% of NPP and carefully adjusted Ca level. A few reports suggest that phytase A may reduce the impact of low dietary Ca on hen performance (Gordon and Roland, 1998) although other data indicate the opposite (Francesch et al., 2005; Liu et al., 2007). Studies where different phytase A preparations (3-phytase A; consensus 3-phytase A, 6-phytase A) were tested simultaneously did not show any significant differences among sources of the catalyst used for supplementation of feeds fed to laying hens (Jalal and Scheideler, 2001; Wu et al., 2006; Liu et al., 2007).

To our knowledge, no studies have been performed to date with phytase B as a supplemental enzyme in maize-soybean diets fed to laying hens. Therefore the purpose of the current investigations was to evaluate effects of phytase B added to nonphytate phosphorus deficient maize-soybean meal-based diets containing either 2.8 or 3.8% Ca on production characteristics, eggshell quality, and on P and Ca balance in laying hens.

MATERIAL AND METHODS

Enzyme activity measurements and units

The microbial phytase B (E.C. 3.1.3.2; Finase® AP) with the declared activity of 225 000 units/g was from AB Enzymes OY, Rajamäki, Finland. The enzyme was characterized by the producer as being derived from a genetically modified *Trichoderma reesei* strain carrying the *Aspergillus niger* acid phosphatase gene and having side activities of β-glucanase, cellulase and xylanase. In our study one unit of phytase B activity (AcPU) was equal to 1μM/min of *p*-nitrophenol liberated from 5.5mM disodium *p*-nitrophenylphosphate at 40°C, pH 4.5. Finase® AP was found to have the phytase B activity equal to 13.15 AcPU/g.

Animals, housing and experimental diets

An experimental protocol was approved by the Local Bioethical Committee for Experiments with Animals in Krakow, Poland. A total of seventy-two 40 weeks-old Hy Line Brown laying hens were placed in individual cages (40 × 40 cm equalling 1600 cm^2 of total floor space) on a wire-mesh floor

Table 1. Composition and nutrient content of the low NPP, basal diets with low and standard calcium content(%)

Ingredient	Calcium content	
	low	high
Maize meal	68.10	62.90
Soybean meal	24.00	25.00
Limestone	7.00	9.60
Vegetable oil	-	1.60
Sodium chloride	0.30	0.30
DL-methionine	0.10	0.10
Vitamin mineral premix[1]	0.50	0.50
Calculated composition (on 'AS IS' basis)		
Metabolizable energy (MJ/kg)	11.51	11.51
Crude protein	16.70	16.70
Ca	2.80	3.80
P total	0.36	0.35
NPP	0.12	0.12
Lysine	0.82	0.83
Methionine	0.37	0.37
Ca analyzed	2.73	3.64
P total analyzed	0.37	0.37

[1]premix provided per 1 kg of diet: vitamin A, 10 000 IU; vitamin D_3, 3000 IU; vitamin E, 50 IU; vitamin K_3, 2 mg; vitamin B_1, 1 mg; vitamin B_2, 4 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 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

under controlled climate conditions with minimal temperature maintained at 20°C, lighting period 14L:10D and light intensity of 10 lux. At 40 weeks of age, hens were randomly assigned to one of the four dietary treatments with 18 cage replications of 1 hen and fed experimental diets until week 54. Feed and water were provided *ad libitum*. The ingredient and nutrient contents of basal maize-soybean meal-based diets are shown in Table 1. Experimental diets were formulated to meet or exceed nutrient recommendations (NRC, 1994) with the exception of P (0.12% NPP; 0.36% total phosphorus) and Ca (2.8 or 3.8%). One of the two diets at each level of calcium was supplemented with phytase B at 2.5 AcPU/kg. The enzyme preparation was premixed with a small quantity of feed and added to the remaining part of the diet during final mixing. Diets were isocaloric (2750 kcal/kg) and isonitrogenous (16.70% CP). The 2 × 2 factorial experimental design was used, with two levels of calcium and two levels (supplemented versus not supplemented) of phytase B.

Data collection

The number and weight of eggs were registered daily during the entire experimental period and egg production was expressed as percentage of hen-day EP, feed intake was recorded monthly whereas feed utilization was calculated for the entire experimental period and expressed as g of egg mass per g of feed mass consumed. Egg mass was calculated as the daily mass of eggs: egg production × egg weight/100 and egg weights were calculated as the mean weight of a single egg in each treatment. Eggs collected on day 95 (18 eggs from each treatment) were analysed for shell breaking strength using an Instron Testing Machine, Model 5542 (Instron Ltd., High Wycombe, UK), equipped with a 500 N load cell. The eggs were compressed at a constant crosshead speed of 10 mm/min and the breaking strength was determined at the time of the eggshell fracture. Eggs collected on day 96 (18 eggs from each treatment) were analysed for shell thickness

and shell density with semi-automated egg quality equipment [QCM+, Technical Services and Supplies (TSS), York, UK] as described by Świątkiewicz et al. (2010). Shell thickness was measured in the equator area of the egg using an electronic micrometer (QCT device, TSS, York, UK). Eggshell density (the dried shell weight per unit of shell area, mg/cm^2) was calculated by the Eggware software (TSS, York, UK). During the 52nd and 53rd week the collection of total excreta from each cage was performed. Excreta were stored in plastic bags at –20°C. After thawing, the excreta were dried in an oven at 50°C to constant weight, weighed and ground to pass a 1-mm sieve. Duplicate samples of feed and excreta were digested by a wet-ash procedure, which was validated by including standard reference material 1572 (Citrus leaves) from the National Institute of Standards and Technology, Gaithersburg, USA. Phosphorus concentration was determined colorimetrically by the molybdo-vanadate method (AOAC, 1995). Calcium concentration in feed and manure samples was analysed by flame atomic absorption spectrophotometry.

Statistical analyses

Data were analysed by the General Linear Models procedure of Statgraphics Plus for Windows statistical software (1996). Analysis of variance was based on two-way ANOVA using the 2 × 2 factorial design. Mean differences were determined using Duncan's test. Additionally, the equality of variances was assessed by Levene's test and subsequent one-way ANOVA was performed to estimate differences among means where interactions of the experimental factors were significant. Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

The experimental design employed in the study was based on the assumption that phytase B, similarly like phytase A, may allow 0.12% NPP diets, eliminate added iP and reduce P excretion in laying hens (Panda et al., 2005). Feed utilization, calculated as the mean across all dietary treatments, for the 14 weeks experimental period was 0.504 g of egg mass per g of feed consumed; egg production averaged out at 87.4%; mean egg weight was 64.5 g, and egg mass amounted to 56.3 g per hen with the average daily feed consumption of 113 g/hen (Table 2). In spite of the fact that the present experiment comprised exclusively P-deficient diets (0.12% NPP), the results were in agreement with laying hen performance reported by various researchers (Gordon and Roland, 1997; Lim et al., 2003; Liebert et al., 2005; Hughes et al., 2008; Skřivan et al., 2010). Hens fed the low phosphorus (0.12% NPP) and low calcium (2.8% Ca) diets consumed more feed ($P < 0.05$) than those fed 0.12% NPP and 3.8% Ca diets. This observation confirms similar findings of Gordon and Roland (1998), who used 2.5, 2.8, and 3.1% Ca, P-deficient diets in a 6-week study with laying hens. Generally, NPP and Ca levels in diets seem to be the important factors affecting egg production, egg mass and egg weight as well as eggshell quality in laying hens (Boling et. al., 2000; Snow et al., 2004; Wu et al., 2006). Lim et al. (2003) reported that the low dietary Ca level (3% Ca) was associated with increased feed intake and reduced eggshell quality. Similar conclusions may be drawn from the study of Liu et al. (2007), who used 3.18 and 3.30% Ca diets to study the efficacy of 3- and 6-phytases A in laying hens. In our study, reduced feed intakes in hens that consumed NPP-deficient and Ca-adequate diets had no influence on egg production or eggshell quality whereas reduced shell thickness in hens consuming Ca-deficient diet was not significant ($P = 0.0727$). Egg production, although numerically higher in hens fed diets low in Ca with no enzyme added, failed to be significantly different due to the low number of hens investigated and therefore the measurement should be considered as preliminary and supplementary. Phytase B increased egg weights in hens fed the low calcium diet (Ca × phytase B interaction, $P < 0.05$) and improved eggshell breaking strength at both levels of calcium ($P < 0.05$). The supplemental enzyme, however, had no influence on feed utilization, egg production, egg mass, and on eggshell density. The effect of phytase B on the thickness of egg shells was approaching significance ($P = 0.0752$). Limited data are available on the efficacy of phytases A in Ca-deficient diets. Sohail and Roland (2000) reported an increase in egg specific gravity in hens fed 3.1% Ca, 0.3% NPP diets and Lim et al. (2003) observed similar improvements in egg quality as a single significant effect of phytase A addition to Ca-deficient, low NPP diets. Liu et al. (2007) reported an increase in feed intake, egg production, egg mass and eggshell thickness in hens fed low-

Table 2. Effect of phytase B and calcium level on feed intake (FI), feed utilization (FU), egg mass (EM), egg weight (EW), egg production (EP), shell breaking strength (SBS), shell thickness (STH), and shell density (SD) of laying hens fed maize-soybean meal-based diet

Calcium (%)	Phytase B	FI (g/hen/day)	FU[1] (g/g)	EM[2] (g/hen)	EW (g)	EP[3] (%)	SBS (N)	STH (μm)	SD[4] (mg/cm^2)
2.8	0	117	0.510	57.7	62.1^{a}	93.1	35.0	368.1	79.4
2.8	1	116	0.491	56.7	66.7^{b}	84.9	36.4	371.7	77.5
3.8	0	109	0.506	55.2	64.6^{ab}	85.4	34.1	371.7	78.8
3.8	1	109	0.508	55.6	64.5^{ab}	86.1	37.8	400.4	85.1
2.8		116^{B}	0.500	57.1	64.4	89.0	35.7	369.9	78.4
3.8		109^{A}	0.507	55.4	64.5	85.8	36.0	386.0	82.0
	0	113	0.508	56.4	63.3	89.2	34.6^{A}	369.9	79.1
	1	113	0.499	56.1	65.6	85.5	37.1^{B}	386.0	81.3
Mean		113	0.504	56.3	64.5	87.4	35.8	378.0	80.2
SEM		1.29	0.015	1.48	0.75	2.28	0.57	8.39	2.20
Probability									
Treatment		0.027	0.820	0.632	0.017	0.108	0.035	0.066	0.090
Phytase B		0.705	0.598	0.833	0.017	0.143	0.027	0.075	0.326
Calcium		0.004	0.680	0.253	0.832	0.195	0.834	0.073	0.116
Phytase B × calcium		0.569	0.527	0.637	0.014	0.088	0.289	0.157	0.072

Values are means of 18 replicates of 1 hen kept in individual cages

^{a,b,A,B}means within a column with no common superscript differ significantly ($P < 0.05$)

[1]g egg per g of feed

[2]daily mass of eggs = hen-day egg production × egg weight/100

[3]hen-day egg production = 100 × number of eggs laid/number of hens × days

[4]dried shell weight/egg shell surface area

Ca diets supplemented with 3- and 6-phytases A. Mean egg weights in those studies were increased by *Aspergillus niger* 3-phytase A, but not by two different preparations of 6-phytases A derived from *Escherichia coli*. Liebert et al. (2005), who tested the effects of a consensus phytase A expressed in *Hansenula polymorpha*, observed no influence of the supplemental enzyme on feed intake but rather on the feed conversion ratio that was significantly improved in laying hens fed low NPP, Ca-deficient maize-soybean meal-based diets. There seems to be a general consent that the productivity of laying hens fed maize soybean meal-based diets, containing 0.10 to 0.15% of NPP and supplemented with phytase A, is similar to that of hens fed diets with a normal NPP concentration (Gordon and Roland, 1997; Liu et al., 2007; Hughes et al., 2008). Wu et al. (2006) reported that both 3- and 6-phytases A prevented the decline in feed intake of hens fed P-deficient diets. Similar conclusions may be drawn from the data provided by Sohail and Roland (2000) and Augspurger et al. (2007). Decreased feed intakes in hens fed diets with 3.8% of calcium and inability of phytase B to overcome this effect observed in our study suggest that in contrast to phytases A, phytase B does not stimulate feed intake in hens fed low NPP, adequate-Ca diets. Sohail and Roland (2000) observed no effect of phytase A on egg weight and egg production in hens fed 0.3% NPP diets while Augspurger et al. (2007), who studied the effects of *Escherichia coli* 6-phytase A on the performance of laying hens fed the 3.8% Ca, 0.10% NPP diets, reported significant increases in egg weight associated with the presence of the supplemental enzyme. It seems therefore that both the inability of phytase B to enhance performance parameters of hens fed low NPP and adequate-Ca diets and its ability to increase egg weight in hens fed low NPP and Ca-deficient diets are unique features of the enzyme.

Table 3. Effect of phytase B and calcium level on phosphorus and calcium excretion and retention in laying hens fed maize-soybean meal-based diet

Calcium (%)	Phytase B	Phosphorus			Calcium		
		excreted	retained	retention (%)	excreted	retained	retention (%)
		(mg/hen/day)			(mg/hen/day)		
2.8	0	334^{bc}	93^{a}	26.4^{a}	1097	1700	61.2
2.8	1	229^{a}	225^{b}	45.7^{b}	610	2545	80.7
3.8	0	294^{b}	98^{a}	24.5^{a}	2539	1704	41.7
3.8	1	358^{c}	114^{a}	18.9^{a}	2402	2505	51.3
2.8		281	179	36.0	853^{A}	2122	71.0^{B}
3.8		326	114	23.0	2470^{B}	2105	46.5^{A}
	0	314	103	25.5	1818	1702^{A}	51.5$^{\alpha}$
	1	294	190	33.5	1506	2525^{B}	66.0$^{\beta}$
Mean		304	147	29.5	1662	2114	58.7
SEM		18.9	30	5.2	165	140	2.63
Probability							
Treatment		0.002	0.000	0.000	0.000	0.001	0.000
Phytase B		0.292	0.001	0.037	0.083	0.000	0.000
Calcium		0.035	0.007	0.002	0.000	0.900	0.000
Phytase B × calcium		0.008	0.001	0.000	0.309	0.875	0.086

Values are means of 18 replicates of 1 hen kept in individual cages

$^{a\text{-}d; A\text{-}B; \alpha\text{-}\beta}$means within a column with no common superscript differ significantly ($P < 0.05$)

The best eggshell quality was reported for either high or low levels of both Ca and NPP (Scott et al., 1999). In the study presented here, phytase B clearly enhanced shell breaking strength at both levels of Ca ($P < 0.05$) and tended to increase the shell thickness ($P < 0.1$; Table 2). In hens fed the adequate level of calcium, however, both effects were much more pronounced. Therefore, the efficacy of phytase B resembles the effectiveness of phytase A reported by Jalal and Scheideler (2001) and Hughes et al. (2008), who observed improved eggshell quality in hens fed diets supplemented with Natuphos and Quantum phytase, respectively.

There were significant calcium with phytase B interactions in relation to the amounts of phosphorus excreted, P retained and to the percentage of P retention in laying hens (Table 3). Different levels of calcium in the experimental diets affected the amounts of P excreted, P retained and Ca excreted as well as the percentage of P and Ca retention ($P < 0.05$) but did not influence the amounts of Ca retained by experimental birds. Phytase B significantly increased the amounts of P and Ca retained by hens and improved the percentage of P and Ca retention ($P < 0.05$). Significant calcium with phytase B interactions observed in this study reflect the well-known phenomenon that in laying hens a proper Ca to available P ratio is the key factor influencing the efficacies of supplemental phytases and the digestibility of minerals (Sohail and Roland, 2000; Lim et al., 2003; Wu et al., 2006; Liu et al., 2007; Hughes et al., 2009). Lim et al. (2003) observed no effects of different Ca levels or 6-phytase A on the g/hen/day retention of P and Ca in hens fed 0.15% NPP. In a similar study, Liu et al. (2007) observed an improved P ileal digestibility due to the supplementation 3- and 6-phytases A and a higher Ca digestibility in hens fed 6-phytase A. Increased amounts of P excreted by hens fed Ca-adequate diets that had been supplemented with phytase B were the most unexpected result of the study presented here. Liebert et al. (2005) did not find any effects of a consensus phytase A on P excretion and P utilization in NPP and Ca-deficient diets. Wu et al. (2006)

reported that hens fed low NPP and Ca-adequate diets did not excrete less phosphorus when they consumed feeds supplemented with Natuphos 3-phytase A or Phyzyme 6-phytase A. A similar conclusion was drawn by Hughes et al. (2009), who studied phytate phosphorus digestibility in feeds supplemented with Quantum 6-phytase A. An experimental consensus 3-phytase A from *Aspergillus niger* was efficacious in promoting P absorption but not Ca absorption in laying hens fed maize- or barley-based feeds (Francesch et al., 2005). In our study, the supplementation of phytase B to Ca-deficient diets reduced the amounts of P excreted, greatly increased the amounts of P retained and, in consequence, highly improved P retention (46% vs. 27%). Similarly, the greater improvements in the retention of Ca (81% vs. 61%) resulted from phytase B addition to Ca-deficient feeds and were caused by the elevated amounts of Ca retained by hens. It should be emphasized that phytase B, although increased the amounts of Ca retained by hens at both dietary calcium levels, was more effective in reducing Ca excretion and in promoting the retention of that nutrient in laying hens fed Ca-deficient diets.

Therefore it seems that the strain of *Trichoderma reesei* carrying the *Aspergillus niger* phytase B genes produces an interesting and unique enzyme protein that, in contrast to phytases A, may be efficacious in low-NPP and Ca-deficient maize-soybean meal-based feeds fed to laying hens. However, further studies with a higher number of hens are necessary to elucidate the effects of long-term feeding of phytase B on laying hen performance and the efficacy of the enzyme in NPP-adequate diets.

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REFERENCES

AOAC, Association of Official Analytical Chemists. (1995): 4. Animal feeds. Phosphorus. 965.17 Photometric method. In: Official Methods of Analysis. Washinghton, DC., USA, p. 27.

Applegate T.J., Webel D.M., Lei X.G. (2003): Efficacy of a phytase derived from *Escherichia coli* and expressed in yeast on phosphorus utilization and bone mineralization in turkey poults. Poultry Science, 82, 1726–1732.

Augspurger N.R., Webel D.M., Baker D.H. (2007): An *Escherichia coli* phytase expressed in yeast effectively replaces inorganic phosphorus for finishing pigs and laying hens. Journal of Animal Science, 85, 1192–1198.

Boling S.D., Douglas M.W., Johnson M.L., Wang X., Parsons C.M., Koelkebeck K.W., Zimmerman R.A. (2000): The effects of dietary available phosphorus level and phytase on performance of young and older laying hens. Poultry Science, 79, 224–230.

Francesch M., Broz J., Brufau J. (2005): Effects of 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.

Gordon R.W., Roland D.A. (1997): Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. Poultry Science, 76, 1172–1177.

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

Greiner R. (2006): Phytate-degrading enzymes: Regulation of synthesis in microorganisms and plants. In: Turner B.L., Richardson A.T., Mullaney E.J. (eds): Inositol Phosphates Linking Agriculture and the Enviroment. CABI Publishing, Wallingford, 78–92.

Hatten L.F., Ingram D.R., Pittman S.T. (2001): Effect of phytase on production parameters and nutrient availability in broilers and laying hens: A review. Journal of Applied Poultry Research, 10, 274–278.

Hughes A.L., Dahiya J.P., Wyatt C.L., Classen H.L. (2008): The efficacy of quantum phytase in a forty-week production trial using white leghorn laying hens fed corn-soybean meal-based diets. Poultry Science, 87, 1156–1161.

Hughes A.L., Dahiya J.P., Wyatt C.L., Classen H.L. (2009): Effect of quantum phytase on nutrient digestibility and bone ash in white leghorn laying hens fed corn-soybean meal-based diets. Poultry Science, 88, 1191–1198.

Jalal M.A., Scheideler S.E. (2001): Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poultry Science, 80, 1463–1471.

Liebert F., Htoo J.K., Sünder 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., Paik I.K. (2003): Effects of phytase supplementation on the performance, egg quality, and

phosphorus excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorus. Poultry Science, 82, 92–99.

Liu N., Liu G.H., Li F.D, Sands J.S., Zhang S., Zheng A.J., Ru Y.J. (2007): Efficacy of phytases on egg production and nutrient digestibility in layers fed reduced phosphorus diets. Poultry Science, 86, 2337–2342.

Miettinen-Oinonen A., Torkkeli T., Paloheimo M., Nevalainen H. (1997): Overexpression of the *Aspergillus niger* pH 2.5 optimum acid phosphatase gene in a heterologous host *Trichoderma reesei*. Journal of Biotechnology, 58, 237–243.

Mullaney E.J., Ullah A.H.J. (2003): The term phytase comprises several different classes of enzymes. Biochemical and Biophysical Research Communication, 312, 179–184.

Mullaney E.J., Ullah A.H.J. (2006): Phytases: Atributes, catalytic mechanisms and applications. In: Turner B. L., Richardson A.T., Mullaney E.J. (eds): Inositol phosphates: Linking Agriculture and the Enviroment. CABI Publishing, Wallingford, 97–110.

NRC (1994): Nutrient Requirements of Poultry. 9th ed. National Academic Press, Washington, DC, USA.

Oh B.C., Choi W.C., Park S., Kim Y.O., Oh T.K. (2004): Biochemical properties and substrate specificities of alkaline and histidine acid phytases. Applied Microbiology and Biotechnology, 63, 362–372.

Onyango E.M., Bedford M.R., Adeola O. (2005): Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. Poultry Science, 84, 248–255.

Panda A.K., Rama Rao S.V., Raju M.V.L.N., Bhanja S.K. (2005): Effect of microbial phytase on production performance of white leghorn layers fed on a diet low non-phytate phosphorus. British Poultry Science, 46, 464–469.

Scott T.A., Kampen R., Silversides F.G. (1999): The effect of phosphorus, phytase enzyme, and calcium on the performance of layers fed corn-based diets. Poultry Science, 78, 1742–1749.

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

Snow J.L., Douglas M.W., Koelkbeck K.W., Batal A.B., Persia M.E., Biggs P.E., Parsons C.M. (2004): Minimum phosphorus requirement of one-cycle and two-cycle (molted) hens. Poultry Science, 83, 917–924.

Statgraphics Plus for Windows (1996): Advanced regression. Manugistics, Inc. Rockville, USA.

Świątkiewicz, S., Koreleski, J., Arczewska, A. (2010): Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. Czech Journal of Animal Science, 55, 294–306.

Ullah A.H.J., Sedhumadhavan K. (2003): *PhyA* gene product of *Aspergillus ficuum* and *Peniophora lycii* produces dissimilar phytases. Biochemical and Biophysical Research Communication, 303, 463–468.

Vats P., Banerjee U.C. (2004): Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): an overview. Enzyme and Microbial Technology, 35, 3–14.

Vats P., Bhattacharyya M., Banerjee U.C. (2005): Use of phytases (myo-inositolhexakisphosphate phosphohydrolases) for combating environmental pollution: A biological approach. Critical Reviews in Environmental Science and Technology, 35, 469–486.

Wu G., Liu Z., Bryant M.M., Roland D.A. (2006): Comparison of Natuphos and Phyzyme as phytase sources for commercial layers fed corn-soy diet. Poultry Science, 85, 64–69.

Zaghari M., Gaykani R, Shivazad M., Taherkhani R. (2008): Evaluation of phytase nutrient equivalency for old layer hens. Asian Journal of Poultry Science, 2, 24–29.

Żyła K., Koreleski J., Świątkiewicz S., Wikiera A., Kujawski M., Piironen J., Ledoux D.R. (2000): Effects of phosphorolytic and cell wall-degrading enzymes on the performance of growing broilers fed wheat-based diets containing different calcium levels. Poultry Science, 79, 66–76.

Żyła K., Wikiera A., Mika M., Stodolak B., Koreleski J., Świątkiewicz S. (2004): Towards complete dephosphorylation and total conversion of phytates in poultry feeds. Poultry Science, 83, 1175–1186.

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