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ECOLOGICAL IMPLICATIONS OF PHYTASE ADDED TO NORMAL AND DEFICIENT AVAILABLE PHOSPHORUS DIETS OF LAYING HENS MEASURED BY N AND P EXCRETION

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Abstract

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Nitrogen and phosphorus are two main pollutants responsible for environmental eutrophication, a process of saturation of surface water with nutrients which as a result of oxygen depletion favors overgrowth of algae and grazing bacteria. An experiment was conducted to determine the effects of adding phytase to a corn-soybean meal laying hen diet over 8 weeks with different available phosphorus (AP) levels on total nitrogen (TN) and total phosphorus (TP) in excreta. In the experiment, 144 Hisex Brown hens were fed two levels of AP (0.12% and 0.46%) and two levels of phytase (0, and 600 Phytase Units-FTU)/kg). In the experiment supplemental phytase resulted in lower total excreta nitrogen (1.50 ± 0.04; 1.23 ± 0.04; 133 ± 0.07; and 1.31 ± 0.04%, for hens fed 0.12% AP–phytase, 0.12% AP + phytase, 0.46% AP - phytase, and 0.46% AP + phytase, respectively) and total excreta phosphorus (1.14 ± 0.01; 1.03 ± 0.05; 1.34 ± 0.03; and 1.37 ± 0.01, respectively). Results of this study indicate that 600 FTU added to corn soybean meal based layer diets containing 0.12% AP contributes to significantly lower levels of total excreta nitrogen and phosphorus without any adverse effects on the performance of laying hens.

Key words: phytase; excreta; laying hens; nitrogen; phosphorus

Introduction

Phosphorus is an essential and critical nutrient for the growth and production of all poultry species, and especially for those producing eggs. An insufficient supply of this mineral can cause great economic damage (Williams, 1996; Waldroup, 1999). According to the research of Boling et al. (2000), on the effects of the level of available phosphorus in the performance of poultry, older hens exhibit more quickly signs of phosphorus deficiency compared to young laying hens. It is known that the main raw materials for animal feed from a plant origin store the biggest amount of phosphorus bound in phytates, which is not accessible for nonruminants. These high levels of unavailable phosphorus in the mixture require the addition of more digestible inorganic Phosphorus (P) sources. However, high dietary levels of phosphorus result in higher P, which is excreted via faeces. When excessive amounts of poultry manure are accumulated, certain pollution concerns for groundwater and the environment in general may arise (Huff et al., 1998; Abudabos, 2012). Since poultry miss the adequate enzyme system necessary for the hydrolysis of phytate, they can use only one-third of the total phosphorus of plant origin, which requires the addition of inorganic phosphorus sources in order to compensate for the limited amount of available phosphorus. From an economic point of view, phosphorus is the third most expensive nutrient after energy and protein because the prices of traditional supplements of inorganic phosphorus are relatively high (Ahmad et al., 2000).

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Therefore, the optimal amount of P in diets for poultry should carefully be considered from both a nutritional and ecological point of view, but also from an economic standpoint (Rao et al., 1999). A poultry diet is generally made on the basis of corn-soybean meal. Since the average two-thirds of the total phosphorus in these diets are bound to phytic acid, available inorganic phosphate and phytate P is discharged through faeces (Punna and Roland, 2000).

Reducing excessive excretion of P as a result of the inability of hens to take advantage of phytate phosphorus is particularly important because of potential environmental pollution and other economic and environmental problems, which are particularly manifested in regions with intensive animal farming (Carlos and Edwards, 1998; Paik, 1999; Um and Paik., 1999). The complete diets for hens have unnecessarily high P contents (Engelmaierova et al., 2012). This fact results in increased feed costs, often decreased performance and environmental pollution by the unused P that is excreted. Excretion of phytate phosphorus contributes to pollution of surface and ground water, and this environmental problem can be prevented through the use of exogenous phytase, which improves the digestibility of phosphorus by non-ruminants (Roberson, 1999). The application of phosphorus through manure of animals in excess of the needs of plants may result in its washing by water resources (Atia and Mallarino, 2002). It is known also that phosphorus has a much lower mobility in the soil when compared with nitrogen (Koelkebeck et al., 2002), and it is obvious that there may be contamination of surface waters by these nutrients.

Phosphorus represents a limiting nutrient for algae, so its presence in poultry faeces and then in the water can stimulate flowering algae towards consuming all the oxygen in the surface layers of water (Scott et al., 1999). Enzyme supplementation of poultry feed has evolved in recent years (Chotinsky, 2015) and became powerful tool to alleviate antinutritional effects of components in feedstuffs commonly used in nonruminant nutrition. Reduction of excessive amounts of phosphorus excreted via poultry manure is possible by a combination of an effective quality control program for protein feed, choosing phosphorus supplements with high biological value, the use of exogenous phytase and real standardisation of its needs in diet (Waldroup, 1999). Van der Klis et al. (1997) noted that the addition of phytase in rations for laying hens low in available phosphorus substantially increased the absorption of phosphorus (by about 50%) compared to 22% for a diet that is served as a negative control. Gordon and Roland (1997) in their research report that the hens fed diets with 0.1% nonphytate phosphorus have increased mortality and produced eggs with less weight and of a specific weight, which is very successfully compensated when the diet was supplemented with 300 FTU per kg of feed. When phytase is added to the control diet for laying hens, it was possible to reduce the amount of used dicalcium phosphate without consequences on the performance of laying hens, but a significant reduction in phosphorus excretion by faeces was also observed (Jacob et al., 2000). Increased hydrolysis of phytate P and total retention of 15% and less faecal excretion for 34-47% was observed when the diets of laying hens were supplemented with phytase compared to those without (Leske and Coon, 1999; Keshavarz, 2000). The use of phytase in diets low in available phosphorus can reduce phosphorus excretion through excreta of hens by 41% (Um and Paik, 1999), and from 20% to 45% (Kornegay, 1999). The use of higher levels of phytase (800 FTU/ kg mixture) in broilers shows that total phosphorus retention can be 67% compared to 53% in the control group. Denbow et al. (1995), have come to a result, according to which 54% of phytic phosphorus in soybean meal was liberated by the addition of 750 FTU/kg in the diet. Budor et al. (1995) in experiments on the effect of the addition of phytase (600 FTU/ kg) in diets with 0.3, 0.2 and 0.1% of available phosphorus, found positive effects in adding phytase in diets with 0.2% of available phosphorus, resulting in 3% improved consumption, 4% less phosphorus in faeces and 20% better resorption of phosphorus. From a meta-analysis study using a data set of 168 P or P plus phytase treatments extracted from 12 published papers (Ahmadi and Rodehutscord, 2012), sugest that dietary NPP level may be decreased down to 0.18, 0.15, and 0.14%, respectively with addition of 150, 300, and 400 phytase units (FTU)/kg of feed.

The objective of this study was to evaluate the influence of phytase on decreasing the content and total excretion of nitrogen and phosphorus from the excreta of laying hens fed rations with lower available phosphorus

Materials and Methods

The trial included 144 Hisex Brown laying hens aged 22 weeks at the beginning of the experiment. The preparatory period lasted two weeks, during which uniform groups in weight and intensity of production are formed. During this period the hens were fed standard diets for this category of laying hens. Individual treatments consisted of 36 hens (three replicates of 12 hens). In this experiment, four mixtures (treatments) containing two levels of available phosphorus (0.12% and 0.46%) and two levels of Natuphos BASF® 5000 phytase (0 and 600 FTU[1]/kg) are used. Diets were formulated to contain all the necessary nutrients in accordance with the recommendations

[1] One unit of Phytase (FTU) is defined as the amount which liberates one micromole of inorganic phosphorus per minute from an excess of sodium phytate at 37°C (98.6°F) at a pH of 5.5

of NRC (1994), except total and available phosphorus (Table 1). Diets based on corn and soybean meal, known as raw materials with low content of available phosphorus were formulated using UFFDA software from the University of Georgia, USA (Pesti and Miller, 1992). During entire experiment whole excreta produced each day was collected, mixed thoroughly and circa 10% was dried at 70°C during 12 hours using forced air dryer followed by other 12 hours at room temperature. To avoid N losses during drying, two grams of fresh excreta were taken every 13th and 14th day of experimental phase to determine the content of total N. Kjeldahl method was used to do it. Determination of total phosphorus was done spectrophotometrically using ammonium vanadate-molybdate method (Gericke and Kurmies, 1952). The amount of excreted N and P was calculated from total amount of excreta produced and put in relation with the total egg mass expressed as grams of mineral (in dry matter basis) per kilogram of eggs produced. Egg mass was calculated from total number of eggs laid and their weights. Experimental data were was statistically analysed using GLM procedure of SAS (1985). One way Analysis of Variance (Anova) was used to find whether significant probability value ($P<0.05$) exists and afterwards the means were compared using Duncan's New Multiple-Range Test.

Results and Discussion

The content and excretion of total nitrogen through excreta

Although diets used in this experiment contained the same amount of crude protein and different levels of available phosphorus, the use of phytase affected the content of nitrogen in fresh faeces. Excreta of hens fed a diet deficient in available phosphorus in which phytase was added, contained less nitrogen in all stages of the experiment compared to other treatments, with significant differences occurring during the third and fourth phase. The average nitrogen content in fresh faeces (Table 2) was 1.50, 1.23, 1.33 and 1.31% for treatments 1, 2, 3 and 4 respectively. Adding phytase in laying hens diets low in available phosphorus (0.12%), in addition to successful maintenance of adequate body and production parameters, also demonstrated its effect on reducing the nitrogen content in fresh faeces by 18% compared to diets without phytase. This content was lower, for 7.52% and 6.1% compared with treatments with 0.46% available phosphorus without and with phytase.

Since different available phosphorus supplies affected the egg output, the amount of nitrogen excreted through faeces

Table 1
The composition and nutritional value of experimental diets

Raw materials	Diet A (%)		Diet B (%)	
	TREATMENTS			
	1	2	3	4
Ground yellow Corn	61.53	61.53	59.25	59.25
Soybean meal, 44% SP	26.64	26.64	27.08	27.08
Sunflower oil	2.00	2.00	3.00	3.00
Limestone	8.45	8.45	7.37	7.37
Dicalcium phosphate	0.00	0.00	1.92	1.92
Kitchen salt	0.30	0.30	0.3	0.3
Min-Vit premix	1.00	1.00	1.00	1.00
DL-Methionine	0.08	0.08	0.09	0.09
Natuphos^{R} Phytase	-	0.012	-	0.012
TOTAL	100.00	100.00	100.00	100.00
Calculated nutrient content of diets				
ME; MJ/kg	12.17		12.25	
Crude protein, %	17.00		17.00	
Ca, %	3.30		3.30	
Total P, %	0.35		0.70	
Available P, %	0.12		0.47	
Lysine, %	0.88		0.88	
Met + Cys, %	0.64		0.64	
Mg, %	0.12		0.12	
Zn, mg	85.21		85.13	
Cu, mg	15.31		15.36	

Table 2
The effect of AP and phytase levels on nitrogen content in fresh faeces, %

Treatment	AP[2] and Phytase level	Nitrogen content, % (Mean ± SEM[1])			
		Phase 1	Phase 2	Phase 3	Phase 4
1	0.12 AP/0FTU[3]	1.46±0.03	1.47±0.10	1.56±0.13[a]	1.51±0.07[a]
2	0.12 AP/600FTU	1.39±0.07	1.23±0.04	1.20±0.02[b]	1.09±0.09[b]
3	0.46 AP/0FTU	1.30±0.12	1.46±0.03	1.21±0.04[b]	1.37±0.11[ab]
4	0.46 AP/600FTU	1.37±0.01	1.31±0.07	1.22±0.11[b]	1.34±0.04[ab]
P value		*0.5044*	*0.1018*	*0.0563*	*0.0426*
Main effect of means					
Available Phosphorus (%)					
0.12		1.42±0.04	1.35±0.07	1.38±0.10	1.30±0.11
0.46		1.33±0.06	1.38±0.05	1.22±0.05	1.35±0.05
Added phytase (FTU/kg)					
0		1.38±0.07	1.46±0.05[a]	1.39±0.10	1.44±0.07[a]
600		1.38±0.03	1.27±0.04[b]	1.21±0.05	1.22±0.07[b]
Analysis of variance					
Source		P value			
AP		*0.2363*	*0.6233*	*0.0985*	*0.5402*
Phytase		*1.0000*	*0.0222*	*0.0829*	*0.0.271*
AP x Phytase		*0.7922*	0.3678	0.5277	0.0760

Values within the same column with different superscript differ significantly ($P < 0.05$), [1]SEM-Standard Error of Mean [2]AP-Available Phosphorus, [3]FTU-Phytase Unit

was also calculated per each kilogram of egg mass produced (Table 3). It is assumed that this is an important indicator in determining the efficiency of phytase used to decrease the excretion of this nutrient. Results of our study indicate that different levels of available phosphorus and added phytase had a certain impact on the amount of nitrogen excretion during all phases of the experiment, but the amount excreted was significantly different only in the third phase. At this stage, the highest nitrogen excretion is observed in treatment 1 (33.78 g N/kg of eggs produced). In treatments 2, 3 and 4, the amount of excreted nitrogen was not significantly different. From mean values it can also be noted that hens fed deficient AP excreted more nitrogen (34.42 g) than laying hens of treatments 2, 3 and 4, where excretion of 27.76, 26.56, and 28.81 g of nitrogen for each kilogram of eggs produced was observed. There is no significant effect of phosphorus or phytase level on the amount of nitrogen excreted (seen as main effect of means).

The content and excretion of total phosphorus through excreta

Results of the total phosphorus in dry faeces (Table 4) indicate a significant relationship with the amount of available phosphorus and phytase in a diet. Starting from the second phase, a noticeable tendency of reduction of the amount of total phosphorus in the faeces depending on the level of this mineral in the diet was observed. This can be better illustrated from the values obtained by analysis of excreta of hens fed treatment 2, where from 1.23% P (second phase) a reduction to 1.04% of phosphorus in the fourth phase was observed. In treatments 3 and 4 (with sufficient quantities of AP), increased phosphorus excretion as the experiment progressed was observed, since from 1.44% and 1.45% in the second phase, P content at the end of the experiment reached 1:53% or 1:55%. The average phosphorus content in dry faeces of laying hens fed a deficient AP diet was significantly lower compared with hens fed a treatment with sufficient amounts of AP (1.08% and 1.36% respectively). Use of 600 FTU phytase per kg of diet reduced the phosphorus content from 1.24% to 1.20%. The content of total phosphorus in dry faeces of laying hens fed with 0.12% available phosphorus was 9.65% lower compared with the diet without phytase. This reduction is more expressed (23.13%) if compared to a standard commercial layers diet with 0.46% AP. Results of total phosphorus content in dry faeces, and results on the number of eggs produced and their weight, were used to calculate the amount of phosphorus excreted per kilogram of eggs produced.

Based on these results (Table 5) it is possible to see the effect of different available phosphorus levels, and in particular the addition of phytase in the reduction of the amount of phosphorus excreted via excreta. Use of phytase in diets

Table 3
The effect of AP and phytase levels on nitrogen excretion, g/kg egg

Treatment	AP[2] and Phytase level	Gram N per kg of egg (Mean ± SEM[1])			
		Phase 1	Phase 2	Phase 3	Phase 4
1	0.12 AP/0FTU[3]	41.45±8.38	23.89±1.15	33.78±1.87[a]	38.54±8.61
2	0.12 AP/600FTU	33.60±2.37	24.42±0.66	23.43±0.82[b]	19.28±1.14
3	0.46 AP/0FTU	43.92±1.95	26.67±1.69	22.09±0.23[b]	23.89±0.38
4	0.46 AP/600FTU	43.85±7.50	24.63±0.94	23.49±1.91[b]	23.26±1.81
P value		*0.5788*	*0.4107*	*0.0012*	*0.0647*
Main effect of means					
Available Phosphorus (%)					
0.12		42.69±3.89	24.18±0.60	28.61±2.49[a]	28.915.80
0.46		38.73±4.20	25.65±0.98	22.79±0.91[b]	23.570.84
Added phytase (FTU/kg)					
0		37.53±4.27	25.28±1.10	27.94±2.75[a]	31.215.06[a]
600		43.89±3.47	24.52±0.52	23.46±0.93[b]	21.271.31[b]
Analysis of variance					
Source		P value			
AP		*0.5161*	*0.2379*	*0.0032*	*0.2640*
Phytase		*0.3070*	*0.5357*	*0.0127*	*0.0555*
AP x Phytase		*0.5241*	*0.3959*	*0.0030*	*0.0691*

Values within the same column with different superscript differ significantly ($P < 0.05$),
[1]SEM-Standard Error of Mean, [2]AP-Available Phosphorus, [3]FTU-Phytase Unit

Table 4
The effect of AP and phytase levels on Phosphorus content, %

Treatment	AP[2] and Phytase level	Phosphorus content in dry faeces, % (Mean ± SEM[1])			
		Phase 1	Phase 2	Phase 3	Phase 4
1	0.12 AP/0FTU[3]	1.09±0.10 [ab]	1.11±0.02[b]	1.22±0.09[a]	1.12±0.07[b]
2	0.12 AP/600FTU	0.87±0.06[bc]	1.23±0.04[a]	0.98±0.06[b]	1.04±0.05[b]
3	0.46 AP/0FTU	0.97±0.01[a]	1.44±0.04[a]	1.43±0.08[a]	1.53±0.01[a]
4	0.46 AP/600FTU	1.26±0.04[c]	1.45±0.06[a]	1.23±0.01[a]	1.55±0.02[a]
P value		*0.0111*	*0.0535a*	*0.0099*	*0.0001*
Main effect of means					
Available Phosphorus (%)					
0.12		0.98±0.07	1.17±0.07[b]	1.10±0.07[b]	1.08±0.04[b]
0.46		1.12±0.07	1.44±0.03[a]	1.33±0.06[a]	1.54±0.01[a]
Added phytase (FTU/kg)					
0		1.03±0.05	1.27±0.08	1.33±0.07[b]	1.33±0.10[a]
600		1.07±0.09	1.34±0.09	1.10±0.06[a]	1.30±0.12[b]
Analysis of variance					
Source		P value			
AP		*0.0623*	*0.0111*	*0.0094*	*0.0001*
Phytase		*0.5681*	*0.4548*	*0.0101*	*0.05259*
AP x Phytase		*0.0034*	*0.5250*	*0.07721*	*0.03325*

Values within the same column with different superscript differ significantly ($P < 0.05$), [1]SEM-Standard Error of Mean [2]AP-Available Phosphorus, [3]FTU-Phytase Unit

Table 5
The effect of AP and phytase levels on Phosphorus excretion, g/ kg eggs

Treatment	AP[2] and Phytase level	Gram Phosphorus per kg of egg (Mean ± SEM[1])			
		Phase 1	Phase 2	Phase 3	Phase 4
1	0.12 AP/0FTU[3]	7.70±0.80	5.98±0.74	9.28±±1.69[a]	9.77±2.62
2	0.12 AP/600FTU	6.66±0.85	5.63±0.76	4.20±0.19[b]	4.09±0.36
3	0.46 AP/0FTU	6.43±0.28	6.23±0.78	6.12±0.77[b]	6.01±0.70
4	0.46 AP/600FTU	9.88±1.31	6.65±0.26	5.76±0.35[b]	6.38±0.70
P value		*0.0869*	*0.7497*	*0.0302*	*0.1075*
Main affect					
Available Phosphorus (%)					
0.12		7.18±0.57	5.80±0.48	6.74±1.37	6.93±1.74
0.46		8.16±0.98	6.44±0.38	5.94±0.39	6.19±0.45
Added phytase (FTU/kg)					
0		7.07±0.47	6.10±0.49	7.70±1.09[a]	7.89±1.47
600		8.27±1.00	6.14±0.42	4.98±0.39[b]	5.23±0.62
Analysis of variance					
Source		P value			
AP		*0.3033*	*0.3701*	*0.4227*	*0.6154*
Phytase		*0.2135*	*0.9598*	*0.0211*	*0.0962*
AP x Phytase		*0.0354*	*0.5825*	*0.0378*	*0.0638*

Values within the same column with different superscript differ significantly ($P < 0.05$), [1]SEM-Standard Error of Mean [2]AP-Available Phosphorus,[3]FTU-Phytase Unit

with insufficient available phosphorus was more effective in reducing the total amount of phosphorus excreted per kg of egg mass produced. The trend of reduction of excreted P throughout the experiment was linear: 6.66, 5.63, 4.20 and 4.09 g P/kg of egg mass for respective phases of the experiment. A similar tendency of reduction of the amount of phosphorus excreted is also observed in hens fed treatment 4: 9.88, 6.65, 5.76 and 6.38 g P/ kg of egg mass. Sobolewska et al. (2015), reported more phosphorus in excreta for about 8% and 21% compared to the hens treated with 500 FTU and 1000 FTU phytase in feed respectively. (Yao et al., (2007) show that wheat bran phytase improved the production performance of laying hens and utilisation of total phosphorus and crude protein. Our results are consistent with results of other authors who have also reported the positive effect of added phytase on the reduction of excretion of P by 4% (Budor et al., 1995), 13% (Lim et al., 2003), 39% (Jeroch, 1994), up to 50% (Bolling et al., 2000).

Conclusions

Based on the results of this study, ecological contribution of 600 FTU/kg in a low available phosphorus diet is expressed in less nitrogen and phosphorus for 4.32% and 17.00% respectively compared with standard laying hen diet.

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