Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Apr - Jun 2005 / v.7 / n.2 / 93 - 98

Effect of phytase in laying hen diets with different phosphorus sources

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Keywords

Commercial laying hens, eggshell quality, phosphorus sources, phytase enzyme.

Arrived: december / 2004
Approved: march / 2005

ABSTRACT

An experiment was conducted to evaluate the effects of the enzyme phytase in diets formulated with different phosphorus sources on performance, eggshell quality and excretion of commercial laying hens. Two hundred and eighty-eight commercial Hyssex Brown laying hens were evaluated during two production phases, which included eight twenty-eight-day cycles, using a completely randomized design in a 3x2 factorial with six replicates of eight birds per treatment. Three phosphorus sources (calcium and sodium phosphate, micro-granulated dicalcium phosphate and triple super phosphate) and two phytase levels (0 or 1000 FTU/kg diet) were tested in the composition of the diets. After the post-peak period, triple super phosphate decreased bird performance and eggshell quality. It was possible to reduce the levels of phosphorus supplementation when phytase was added to the diet. Besides, phytase supplementation reduced phosphorus, calcium and nitrogen excretions, but affected mean egg weight at production peak.

INTRODUCTION

Bird diets are generally formulated based on corn and soybean meal. All vegetal sources have available phosphorus around 33%, except for rice bran, which has 20% of available phosphorus (Rostagno *et al.*, 2000). The 66% of phosphorus unavailability in almost all vegetal ingredients is a consequence of inositol binding, which forms a molecule of phytic acid or inositol hexaphosphate, a reactive anion responsible for the formation of chelates with divalent cations such as calcium, magnesium, manganese, zinc, copper and iron. Since such organic minerals are very important, this reaction has been proposed as a major antinutritional factor that reduces the availability of these minerals in monogastric animals (Cousins, 1999, Yi *et al.*, 1996).

Due to the low content of available phosphorus in plants and the low phytase activity in birds, diets must be supplemented with an inorganic phosphorus source. Nevertheless, similar to phytic phosphorus, a great proportion of inorganic phosphorus is excreted by birds (Fireman & Fireman, 1998). The use of inorganic phosphorus in diets has many disadvantages, as the increase of nutrition costs and, consequently, production costs. Besides, river, lakes, fens and underground water contamination are environmental problems caused by the higher phosphorus elimination in the excretions. Such problem is even greater especially when bird manure is used as fertilizer, since the amount of phosphorus added to soil exceeds plant requirements.

According to Syers *et al.* (1973), phosphorus (P) and nitrogen (N) are considered limiting elements for aquatic plant growth, because their excess causes an increase of the eutrophication process and, consequently, reduction of the water quality. In Brazil, Perdomo (1996) reported high indexes of water pollution in some cities in the South of Santa Catarina State, where many large swine and bird farms are located.

Inorganic phosphorus sources are non-renewable, and this may pose problems to the future. Therefore, the efficiency of vegetal phosphorus utilization must be increased to prevent the gradual reduction in the available sources.

Phytase supplementation of laying hen diets improves the utilization of available phosphorus and other minerals chelated to phytic acid (Boling *et al.*, 2000; Jalal & Scheideler, 2001; Borrmann *et al.*, 2001; Ceylan *et al.*, 2003 and Keshavarz & Austic, 2004). The quantification of phosphorus excretions has been studied in birds fed diets with reduced levels of phosphorus and supplemented with phytase and some reports show a reduction of approximately 45% in phosphorus excretion without affecting performance parameters.

The main mineral source of phosphorus is dicalcium phosphate, which is used worldwide in diet supplementation for livestock. In conventional, corn-soybean meal based diets for birds, sources of inorganic phosphorus must be used to fulfill maintenance and production requirements. This kind of supplementation is performed using inorganic phosphates with high biological availability, as monocalcium and dicalcium phosphates, but the costs are high. Some inorganic phosphorus sources are cheaper, such as natural rock or single super phosphate, but they have low biological availability and undesirable results have been reported in animal nutrition assays, probably because of the high fluorine levels present in these sources.

Another limiting factor of phosphorus digestibility in birds is the particle size, because the larger the particle size, the higher is the mineral availability. Therefore, grinding level is an important variable for inorganic phosphorus utilization by animals (Potter, 1988). However, Burnell *et al.* (1990) noted to the controversial results of studies that assessed the effects of phosphate sources and particle size on solubility and, consequently, on available phosphorus and calcium. These authors also evaluated five different particle sizes (2.0, 1.25, 0.5, 0.18 and 0.05 mm of mesh screen) and suggested that larger particles were retained longer in the proventricle, partially blocking food passage. Consequently, the mineral supplement would stay longer within the acid environment of the higher digestive tract and could be dissolved faster than if proceeding to the lower digestive tract.

The aim of this study was to evaluate the effect of phytase in laying hen diets with different sources of phosphorus on performance, eggshell quality and excretion quality.

MATERIAL AND METHODS

A trial was carried out at Experimental Poultry Farm of Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, São Paulo, Brazil. Two hundred and eighty-eight commercial Hyssex Brown laying hens were housed in cages (two per cage) in 36 experimental parcels with eight birds each. The birds were evaluated during eight periods of 28 days and divided into two phases: peak egg production (32 to 48 weeks of age) and post-peak production (48 to 64 weeks of age).

A completely randomized experimental design in a 3x2 factorial was used with 6 replicates per treatment. There were three phosphorus sources (calcium and sodium phosphate, micro granulated dicalcium phosphate or triple super phosphate) and two phytase levels (0 or 1000 FTU/kg diet). It is considered that 100% of the phosphorus of corn and soybean meal becomes available after phytase action; it was thus possible to reduce total phosphorus levels (Pt) from 0.50% to 0.30% after phytase was added.

Calcium, phosphorus, fluorine and sodium contents were analyzed in phosphorus sources, as well as the particle size (Table 1). Experimental diets based on corn and soybean meal were formulated in order to contain the same levels of protein (18% CP), energy (2800 kcal ME/kg) and calcium (3.64%), according to Rostagno *et al.* (2000) (Table 2).

After the experimental period, forty-eight birds were randomly chosen for sampling during a period of eight days. The first four days were the adaptation period, and excretion sampling was carried out in the next four days, according to a completely randomized design with four repetitions of two birds distributed in 24 parcels. The birds housed in metabolism cages were given water *ad libitum* and controlled amounts of diets during this experimental period. The experimental diets used during this assay were similar to the diets used during the performance trial (Table 1).

After the adaptation period, excretion samplings were performed using 1% ferric oxide in the diet as marker of the onset and end of sampling period. Trays covered with plastic were placed under the experimental cages for collecting excretions twice a day. Immediately after sampling, excretions were frozen until the end of the period. Before analyses,

Table 1- Analysis from the phosphorus sources.

Traits	Calcium and sodium phosphate	Micro granulated dicalcium phosphate	Triple super phosphate
Calcium (%)	20.29	24.09	15.10
Phosphorus (%)	18.36	18.08	20.31
Fluorine (%)	0.12	0.11	1.94
Sodium (%)	6.82	-	-
Particle size (mm)	0.04	0.15	0.04

Table 2 – Composition and nutritional levels of the experimental diets.

	Diets					
	Calcium and sodium phosphate		Micro granulated dicalcium phosphate		Triple super phosphate	
Ingredient	Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme
Corn	58.52	58.89	58.41	58.89	58.14	58.87
Soybean meal	28.30	28.30	28.30	28.30	28.36	28.30
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50
Calcium and sodium phosphate	1.41	0.13	-	-	-	-
Dicalcium phosphate	-	-	1.44	0.14	-	-
Triple super phosphate	-	-	-	-	1.23	0.12
Limestone	8.46	9.13	8.30	9.10	8.72	9.14
Salt	0.28	0.50	0.52	0.52	0.52	0.52
Vitamin mineral premix [A]	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.03	0.03	0.03	0.03	0.03	0.03
Phytase enzyme	-	0.02	-	0.02	-	0.02
Total	100	100	100	100	100	100
Calculated composition						
Metabolizable energy (kcal/kg)	2,800	2,800	2,800	2,800	2,800	2,800
Sodium (%)	0.23	0.23	0.23	0.23	0.23	0.23
Available phosphorus(%)	0.36	0.12	0.36	0.12	0.36	0.12
Calcium (%)	3.64	3.64	3.64	3.64	3.64	3.64
Crude protein (%)	18.0	18.0	18.0	18.0	18.0	18.0
Methionine (%)	0.39	0.39	0.39	0.39	0.39	0.39
Methionine + Cystine (%)	0.68	0.68	0.68	0.68	0.68	0.68
Lysine (%)0.93	0.93	0.93	0.93	0.93	0.93	
Analyzed composition						
Crude protein (%)	18.0	17.7	18.2	17.9	18.0	17.9
Nitrogen (%)	2.88	2.84	2.91	2.87	2.88	2.87
Calcium (%)	3.70	3.37	3.66	3.22	3.57	3.32
Total phosphorus (%)	0.55	0.32	0.51	0.31	0.52	0.33

A - * Supplied per kilogram of diet: Vitamin A - 8,000 UI; Vitamin D3 - 2,200 UI; Vitamin E - 50 mg; Vitamin K3 - 3 mg; Vitamin B1 - 1.5 mg; Vitamin B2 - 4 mg; Vitamin B6 - 0.12 mg, Vitamin B12 - 15 mcg; Folic acid - 0.6 mg; Pantothenic acid - 10 mg; Niacin - 30 mg; Biotin - 0.1 mg; Choline chloride - 300 mg; Iron - 50 mg; Copper - 10 mg; Zinc - 70 mg; Manganese - 100 mg; Iodine - 1 mg; Selenium - 0.3 mg; ethoxyquin - 50 mg.

the samples were thawed, homogenized, weighed and pre-dried at 55°C for 72 hours in forced air oven. Dried samples were ground and phosphorus, calcium and nitrogen were determined. Diets and excretion samples were analyzed according to AOAC (1995).

Data were statistically analyzed using SAS software (SAS, 1996) and means were compared by the Tukey's test at 5% of significance level.

RESULTS AND DISCUSSION

Performance and eggshell quality

Performance and eggshell quality during the periods from 32 to 48 and from 48 to 64 weeks of age are shown in Tables 3 and 4, respectively.

At the beginning and peak of laying period (Table 3) there were no significant effects of phosphorus source on performance parameters and eggshell quality. In regard to phytase addition, birds fed diets with no enzyme and therefore with higher levels of total phosphorus laid heavier eggs ($p<0.05$) in comparison to birds fed phytase-supplemented diets with low levels of available phosphorus. In this first trial, it was also seen that the treatment with phytase improved eggshell quality (specific gravity and shell percentage). Eggshell quality improved with the decrease in egg size.

Different effects were found during the post-peak period (Table 4). There were no significant effects of phytase addition on performance and eggshell quality. However, phosphorus sources affected some variables. Triple super phosphate caused lighter eggs ($p<0.05$) compared to the other sources. Hens fed calcium and sodium phosphate laid eggs with higher shell

Table 3 - Means of performance and egg quality parameters from 32 to 48 weeks of age: feed intake (FI), egg production (EP), egg weight (EW), feed conversion (FC), shell percentage (SP), egg specific gravity (ESG) and shell thickness (ST).

(g/bird/day)	FI (%)	EP (g)	EW (kg/kg)	FC (%)	SP (g/cm³)	ESG (mm)	ST Phosphate
source (P)							
Calcium and sodium phosphate	107.58	95.36	62.64	1.793	9.60	1.0909	0.384
Micro granulated dicalcium phosphate	105.24	95.00	61.81	1.793	9.57	1.0909	0.377
Triple super phosphate	105.51	93.29	62.28	1.810	9.54	1.0904	0.377
Phytase (Ph)							
0 FTU/kg diet	106.45	94.48	62.79 ^{a}	1.796	9.48 ^{b}	1.0904 ^{b}	0.379
1000 FTU/kg diet	105.76	94.62	61.70 ^{b}	1.801	9.66 ^{a}	1.0911 ^{a}	0.379
Interaction (P x Ph)	NS	NS	NS	NS	NS	NS	NS
CV (%)	2.84	2.73	2.48	4.24	1.87	0.09	2.57

a,b - Means with different superscripts within a column are significantly different (p<0.05).NS = non-significant (p>0.05).

Table 4 - Means of performance and egg quality parameters: feed intake (FI), egg production (EP), egg weight (EW), feed conversion (FC), shell percentage (SP), egg specific gravity (ESG) and shell thickness (ST), from 48 to 64 weeks of age.

(g/bird/day)	FI (%)	EP (g)	EW (kg/kg)	FC (%)	SP (g/cm³)	ESG (mm)	ST Phosphate
source (P)							
Calcium and sodium phosphate	105.66	89.31 ^{a}	63.83	1.876	9.54 ^{a}	1.0862	0.386 ^{a}
Micro granulated dicalcium phosphate	104.73	89.22 ^{a}	63.52	1.866	9.49 ^{ab}	1.0890	0.378 ^{ab}
Triple super phosphate	105.44	86.57 ^{b}	63.57	1.946	9.29 ^{b}	1.0886	0.375 ^{b}
Phytase (Ph)							
0 FTU/kg diet	105.12	88.54	64.13	1.874	9.38	1.0880	0.381
1000 FTU/kg diet	105.43	88.19	63.15	1.918	9.50	1.0878	0.378
Interaction (P x Ph)	NS	NS	NS	NS	NS	NS	NS
CV (%)	3.70	3.57	2.39	4.91	2.69	0.47	1.26

a,b - Means with different superscripts within a column are significantly different (p<0.05).NS = non-significant (p>0.05).

percentage and shell thickness than hens fed triple super phosphate. Hens fed micro granulated dicalcium phosphate had results similar to the other treatments.

The lower performance and the poorest quality of eggs from hens fed triple super phosphate might be related to the fluorine levels in this source, once fluorine in excess should be considered a toxic mineral with accumulative effect (Veloso, 1991).

Fluorine contents in diets with triple super phosphate and non-supplemented with phytase was 0.024%, whereas levels were 0.002% when phytase was supplemented. Levels higher than 0.057% should be considered toxic to hens. In our study, toxic effects of fluorine were observed only at the second phase of the study, indicating a putative accumulative effect responsible for the reduction of egg production and eggshell quality.

In terms of phytase utilization, it was evident that the addition of this enzyme permitted to reduce the level of available phosphorus (0.12%) whereas performance was similar to that observed in hens fed diets with higher levels of available phosphorus (0.36%), except for mean egg weight during the laying peak phase, which was reduced.

The reduction in the available phosphorus requirements and consequently in the inorganic phosphorus added to the diets of laying hen is largely reported. Gordon & Roland (1997) evaluated the performance and eggshell quality in laying hens fed corn-soybean diets with different levels of available phosphorus (0.1 to 0.5%) and two levels of phytase (0 and 300 U/kg). There was a reduction in performance and eggshell quality in hens fed diets with 0.1% of phosphorus. However, these undesirable effects were reversed when 300 U of phytase was added per kilogram of diet. It was also reported that there were no benefits of phytase addition when phosphorus level ranged from 0.2 to 0.5%. Um & Paik (1999) reported that the addition of 500 U phytase per kilogram in corn-soybean meal based diet allowed the reduction of available phosphorus and inorganic phosphorus for laying hens.

It was also possible to reduce the levels of inorganic phosphorus supplemented to laying hens when phytase was added to diets with high levels of rice bran. No matter which phytase source was used in rice bran diets for laying hens, enzyme addition allowed to remove the inorganic phosphorus source from the diets with no changes in performance or decrease in eggshell quality (Tangendjaja *et al.*, 2002).

Excretion Traits

There were no significant (p>0.05) effects of phosphorus sources on mineral intake and excretion (Table 5). On the other hand, the supplementation of phytase in diets with low levels of available phosphorus resulted in lower phosphorus, calcium and nitrogen intake and fewer excretions. A reduction in phosphorus intake was expected, once phosphorus levels in phytase-supplemented diets were reduced in 40%. Calcium and nitrogen intakes were also reduced because the calcium and nitrogen contents of these diets were lower (Table 2). The addition of phytase to the diets resulted in great losses of phosphorus (47.1%), calcium (12.4%) and nitrogen (17.4%) in the excretions.

Although these reductions were related to lower levels of available phosphorus, calcium and nitrogen in phytase-supplemented diets, it is necessary to point out that there were no adverse effects on performance or eggshell quality, except for mean egg weight during the laying peak. Therefore, phytase contributed to reduce the environmental impact of excretions, mainly the effects related to phosphorus levels. According to Barreto (1994), the increase of phosphorus contents in diets causes higher levels of phosphorus in the excreta.

The supplementation of phytase to the diet of laying hens causes the release of the phosphorus linked to phytate, and it becomes available to be metabolized by the bird. Thus, phosphorus levels in diets may be reduced and therefore decrease also in the excreta (Kornegay, 2001). Phytase addition to laying hen diets containing 0.25% of available phosphorus increased phosphorus retaining in 139% and nitrogen and calcium were retained 25% and 32% more, respectively (Nahashon *et al.*, 1994).

The effects of phytase also on nitrogen and calcium retaining and, consequently, on their excretion, evidenced that this enzyme has and effective role on releasing these minerals from phytate-protein-amino acid complex, enhancing mineral metabolization by birds.

An excellent report on phytase effects on phytate-phosphorus and phytate-protein complexes was published by Keshavarz & Austic (2004). In that trial, low phosphorus and low protein diets were supplemented with limiting amino acids and phytase and there were no effects on bird performance compared to the control diet, although nitrogen and phosphorus excretions were reduced in approximately 45% and 48%, respectively.

CONCLUSIONS

Considering the evaluated phosphorus sources, triple super phosphate had undesirable long-term effects on commercial laying hen performance and eggshell quality. The addition of 1000 FTU phytase/kg diet permitted to reduce dietary phosphorus levels and decreased the losses of phosphorus, calcium and nitrogen in the excreta. However, diets with low phosphorus levels reduced mean egg weight during the laying peak period.

The supplementation of laying hen diets with phytase might be an alternative to reduce the environmental impact of bird manures, especially when it is to be added to the soil.

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Table 5 – Effect of treatments on phosphorus, calcium and nitrogen excretion (means of ingestion and excretion/day/bird on dry matter).

	Phosphorus (mg)		Calcium (g)		Nitrogen (g)	
	Ingestion	Excretion	Ingestion	Excretion	Ingestion	Excretion
Phosphate source (P)						
Calcium and sodium phosphate	388	289	3.14	1.13	2.53	1.48
Micro granulated dicalcium phosphate	382	279	3.19	1.10	2.65	1.36
Triple super phosphate	378	279	3.09	0.96	2.58	1.40
Phytase (Ph)						
0 FTU/kg diet	496 [a]	369 [a]	3.42 [a]	1.13	2.69 [a]	1.55 [a]
1000 FTU/kg diet	269 [b]	195 [b]	2.86 [b]	0.99	2.48 [b]	1.28 [b]
Interaction (P x Ph)	NS	NS	NS	NS	NS	NS
CV (%)	6.49	12.12	6.54	18.02	6.02	9.68

a,b - Means with different superscripts within a column are significantly different ($p<0.05$). NS = non-significant ($p>0.05$).

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