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The Effects of Phytase and Different Level of Dietary Calcium and Phosphorous on Phytate Phosphorus Utilization in Laying Hens

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Abstract: This experiment was conducted to study the effects of different levels of phytase (0, 500 and 1000 FTU/kg diet), calcium (2.275 and 3.25 percent) and available phosphorus (0.175 and 0.25 percent) on phytate phosphorus utilization in laying hens. One hundreds ninety two 30-week age White Leghorn (Hy-line W-36) laying hens were randomly allocated in cages for 12 dietary treatments with arranged of 3*2*2 factorial experiment with four replicates and four hens per replicate. The experimental period lasted 90 days, when the age of hen was 42 weeks. Dietary phytase caused a significant (P<0.05) increase in feed consumption, feed conversion ratio, tibia ash weight, tibia ash percentage, tibia phosphorus, plasma phosphorus and phosphorus digestibility. However, dietary phytase caused a significantly (P<0.05) decrease in plasma alkaline phosphatase activity and excreta phosphorus percentage. Also phytase had no beneficial effect on egg shell quality traits. Available phosphorus levels had significant effect (P<0.05) on tibia ash weight and tibia ash percentage. Reduction dietary available phosphorus caused a significant (P<0.05) decrease in feed consumption. Effect of dietary calcium were significant (P<0.05) on tibia ash weight, feed consumption and plasma phosphorus. Interaction between phytase and calcium on tibia phosphorus, plasma calcium and excreta phosphorus were significant (P<0.05). Interaction between phytase and available phosphorus on tibia phosphorus were significant (P<0.05). Overall, it could be concluded that in low phosphorus diet which food consumption is low, phytase would increase food consumption as well as retention of phosphorus in bones. Also, the lower excreta of phosphorus by using phytase could decrease pollution.

Key words: Layer, phytase enzyme, calcium, phosphorus

Introduction

A number of studies have indicated that supplementing laying diets with microbial phytase results in improved performance (Van der Klis *et al*., 1996), particularly when dietary levels of non phytate P (NPP) are low (Gordon and Roland, 1997). The results of a number of research studies with laying hens have shown that a diet with 0.1-0.13% available phosphorus (AP) in the presence of 100 to 300 units phytase can results in comparable performance to the control group which were fed a normal level of 0.4-0.45% available phosphorus. It is well established that a high dietary level of calcium (Ca) reduces the activity of phytase (Ravindran *et al*., 2000). However, a portion of the phytase benefit observed in poultry fed NPP deficient diets can be attributed not only directly to P, but also to the influence of P on improved Ca utilization. Also, it has been shown that some additivity or synergistic effects exist between phytase and vitamin D_3 for increasing the availability of phytate phosphorus to the birds (Edwards, 1993). Reports of the effect of phytase enzyme in layer diets are fewer and have not fully investigated the interactions among AP, phytase and Ca. This paper reports on a laying trial in which hens were fed two levels of P, three levels of phytase enzyme and two levels of Ca in a factorial arrangement designed to investigate interactions among these factors on production parameters of layers.

Materials and Methods

Twelve diets were fed to Hy-line W-36 hens from 30 to 42 wks of age. The treatments consisted of a 3×2×2 factorial arrangement with three levels of Natuphos® phytase (0, 500, and 1000 FTU/kg diet), two levels of Ca (2.275 and 3.25%) and two levels of NPP (0.175 and 0.25%). Each treatment was randomly assigned to four replicate cages for a total of 48 cages. Each cage was an experimental unit and contained 4 hens. The experimental diets were formulated to meet National Research Council (1994) nutrient requirement of laying hens (Table 1). Records of daily egg production and weekly feed consumption were kept during the experiment. All the eggs produced during the last 3 d of every 28 d periods were saved for determination of egg weight and two eggs per cage were used to determine egg shells breaking strength, shell thickness, shell ash, and shell phosphorus. At 42 wks of age, four birds from each dietary treatment were killed and their left tibia was removed. They were solvent-extracted to remove fat and then dried and ashed. At 42 wks of age, one bird was randomly selected from each pen, and blood samples were obtained for subsequent determination of minerals (Ca and P) and alkaline phosphatase (ALP) in plasma. At 32 weeks of age, chromic oxide was added to all diets as an analytical marker for P digestibility and fed for 10 days. Representative fecal samples were collected from each cage on the last day of Cr_2O_3 feeding to determine

Table 1: Ingredients and nutrient composition of experimental diets

Ingredient (%)	0.25%AP, 3.25%Ca	0.25%AP, 2.27%Ca	0.175%AP, 3.25%Ca	0.175%AP, 2.27%CA
Corn	65.86	72.27	66.30	72.48
Soybean meal	21.37	20.13	21.28	20.09
Fat	3.1	0.50	2.92	0.50
Oyster shell	7.95	5.39	8.18	5.62
Dicalciumphosphate	0.70	0.71	0.30	0.31
Vitamin premix[1]	0.30	0.30	0.30	0.30
Mineral premix[2]	0.30	0.30	0.30	0.30
Salt	0.35	0.34	0.35	0.34
DL-Methionine	0.07	0.06	0.07	0.06
Nutrient Composition				
ME, kcal/kg	2900	2905	2900	2911
Protein (%)	15	15	15	15
Calcium (%)	3.25	2.27	3.25	2.275
Nonphytate P (%)	0.25	0.25	0.175	0.175
Sodium (%)	0.15	0.15	0.15	0.15
Arg. (%)	0.921	0.906	0.92	0.906
Lys. (%)	0.746	0.729	0.745	0.728
TSAA (%)	0.58	0.58	0.58	0.58
Try. (%)	0.197	0.192	0.197	0.192

[1]Vitamin mix supplied the following per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 500 IU; vitamin E, 10 IU; B_1, 2.2 mg; B_2, 4 mg; B_3, 8 mg; B_6, 2 mg; B_9, 0.56 mg; B_{12}, 15 mg; H_2, 0.15 mg. [2]Mineral mix supplied the following per kilogram of diet: Mn, 800 mg; Zn, 60 mg; Fe, 50 mg; Cu, 5 mg; Co, 0.1 mg; I, 1 mg; Se, 0.1 mg; Choline chloride, 200 mg.

Table 2: Influence of phytase, P and Ca levels on production traits and bone ash measurements

Diet	Egg weight (g)	Egg production (%)	Feed intake (g/h/d)	Feed conversion ratio (g:g)	Bone ash (%)	Bone phos. (%)
Phytase(FTU/kg)						
0	57.02	83.40	95.28^{c}	2.01^{b}	61.55^{b}	7.87^{b}
500	57.32	83.72	96.61^{b}	2.02^{ab}	62.35^{ab}	8.82^{a}
1000	57.37	83.51	99.46^{a}	2.08^{a}	62.54^{a}	8.60^{a}
Calcium (%)						
2.27	57.20	82.61	94.88^{b}	2.10	62.19	8.45
3.25	57.27	82.48	99.35^{a}	2.11	62.10	8.41
Phosphorus (%)						
0.175	57.09	83.40	96.32^{b}	2.03	61.75^{b}	8.45
0.25	57.39	83.69	97.91^{a}	2.05	62.54^{a}	8.41
Pooled SEM	4.32	16.18	2.08	0.015	3.23	1.71

^{abc}means with in columns with no common superscript differ ($p<0.05$).

digestibility of P at 42 wks. Diets and excreta were analyzed for P (AOAC, 1995) and chromium (Fenton and Fenton, 1979). Analysis of variance was performed on the data using the General Linear Models of SAS® software (SAS Institute, 1995).

Results and Discussion

Eggshell quality measurements were not consistently affected by the dietary treatments. Other researchers have reported mixed results of phytase supplementation on eggshell quality measurements (Gordon and Roland, 1998). Addition of 1000 FTU/kg phytase to the diet significantly increased feed intake and feed conversion of laying hens (Table 2). There were no significant effects of enzyme supplementation on egg weight and egg production. The inclusion of phytase to the diets possibly increased feed intake by liberating the phytate phosphorus. These finding are in agreement with those of Keshavarz (2000). Supplementing diets with 1000 FTU/kg phytase resulted in an increased bone ash weight and percentage and bone mineral content (P). At the 0.175% NPP level, hens with phytase had higher bone phosphorus percent than when diets were not supplemented with phytase (Table 2). The improvement in bone ash and bone phosphorus content associated with phytase supplementation could be attributed to phytate P liberation. There was a highly significant positive influence of phytase supplementation on P digestibility at 42 wks of age as expected (Table 3). Other study (Um and Paik, 1999) found that

Table 3: Influence of phytase, P and Ca levels on production traits and bone ash measurements

Diet	Plasma Ca (mg/100ml)	Plasma P (mg/100ml)	Alkalin Phosphatase (U/L)	Excreta ash (%)	Excreta phosphorus (%)	Phosphorus digestibility (%)
Phytase(FTU/kg)						
0	17.34	5.55[b]	632.19[a]	37.63	1.13[a]	34.59[a]
500	18.16	6.88[a]	506.81[b]	39.09	0.97[b]	42.88[b]
1000	17.30	6.63[a]	544.50[b]	37.69	0.94[b]	48.12[c]
Calcium (%)						
2.27	17.67	6.06[b]	544.58	37.58	0.87[b]	42.24
3.25	17.53	6.57[a]	577.75	38.68	1.15[a]	41.49
Phosphorus (%)						
0.175	17.76	6.35	600.75[a]	37.14	1.05	40.36
0.25	17.44	6.29	521.58[b]	39.13	0.97	43.04
Pooled SEM	2.89	1.02	-	-	0.043	-

[abc]Means with in columns with no common superscript differ ($p<0.05$).

supplementation phytase increased P retention by increasing the liberation of bound phytate P. As expected, phytase supplementation to diets increased ($p<0.05$) plasma P level, and decreased plasma ALP activity (Table 3). The increase in plasma P and decrease in ALP activity associated with the diets supplemented with phytase might be reflected the down regulation of this enzyme resulting from the increased availability of phosphorus. Laying hen performance, bone ash measurements, plasma Ca and ALP level and phosphorus digestibility were not affected by dietary Ca level (Table 2, 3). Hens fed low 2.275% Ca had significantly lower feed intake, plasma P level and excreta phosphorus percent than hen fed diets with high level of Ca (Table 2, 3). Although the extent of Ca withdrawal practiced in this study is not at this point recommended commercially, but hens consuming the 2.275% Ca diet performed as well as hen fed diets containing higher level of Ca. In our current study, the use of a NPP regimen of 0.175% which was used for the age periods of 30-42 wks of age was adequate to support all the production traits, in spite of negative effect on feed intake and bone ash measurements at 0.175% NPP. Apparently, under condition of 0.175% AP diets, the P could be utilized for production and was not deposited in the bones. From our results it is concluded that supplemental phytase has beneficial effects on the performance of laying hen. It is recommended that laying hen diets be formulated to provide 0.175% NPP, 2.275% Ca with supplemental phytase to hen early in the production cycle. Microbial phytase supplementation with low-Ca, low-P diet can decrease the level of phytate P excretion in the manure and limit soil and water contamination.

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