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Evaluation of Phytase Nutrient Equivalency for Old Layer Hens

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Abstract: An experiment was conducted to determine the effect of phytase supplementation on old layer hens performance and evaluation of using phytase nutrient equivalency values with comparison to those fed conventional diet. In this experiment 288 Hy-line W-36 hens were used from 60 to 72 weeks of age. The treatments consisted of a control diet (C) without phytase, control diet supplemented with 300 FTU kg^{-1} phytase over the top (C+P) and the third diet contained 300 FTU kg^{-1} phytase which calculated nutrient equivalency values for phyatse (100 E). Hen day egg production for the C, C+P and 100 E group were 75.25, 77.25 and 66.0%, respectively. As egg production declined, FCR increase significantly ($p<0.05$). There were no significant differences in egg specific gravity, egg shell thickness and breaking strength, egg and toe mineralization among dietary treatments. Results indicated that using phytase nutrient equivalency did not prove energy and protein utilization but improved phosphorus utilization in old layer hens.

Key words: Phytase, nutrient equivalency, performance, layer hens

INTRODUCTION

Phytic acid, myo-inositil phosphorylated on all of its six hydroxyl groups, can ionically bind minerals and proteins in aqueous medium (Sebastian *et al*., 1997). The interactions among phytic acid, minerals and protein appear to be primarily responsible for the adverse nutritional effects of a high phytate diet. It is well documented that microbial phytase supplementation enhances the phytic acid hydrolysis and increase the availability of minerals and proteins bound to the phytic acid (Namkung and Leeson, 1999). It has been reported that phytase supplementation improved N retention in broiler chickens (Farrell *et al*., 1993) and laying hens (Van der Klis and Versteegh, 1991). In contrast, Newkirk and Classen (1995) did not find any significant effect on crude protein digestibility by either purified phytase or crude phytase supplementation to broiler diets. Little is known about the equivalency values of phytase for calcium, phosphorus and amino acids in poultry diets, especially in different ages. On the other hand, possible economic benefit of phytase as an ingredient in feed formula in different country is questionable. The present experiment was conducted to evaluation the effectiveness of commercial available phytase (Natuphos®) for improving nutrient availability and cost of feed in old layer hen's fed either Corn Soybean Meal (CSM) diet or CSM diet supplemented with phytase as over the top or CSM diet supplemented with phytase and using nutrient equivalency of enzyme in feed formulation.

MATERIALS AND METHODS

Three diets were fed to 288 Hy-line W-36 hens from 60 to 72 weeks of age. The treatments consisted of a control diet (C) without phytase, control diet supplemented with 300 FTU kg^{-1}

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phytase over the top (C+P) and the third diet contained 300 FTU kg^{-1} phytase which calculated nutrient equivalency values for phyatse (100 E). The composition of experimental diets and calculated analysis are shown in Table 1. Control diet met nutrient requirements recommended by Hy-line W-36 management guide. All diets were iso-protein and iso-energetic. Feed ingredients analyzed for crude protein, fat, crude fiber, calcium and phosphorus (AOAC, 1990). The phytase nutrient equivalency matrix values used in diets formulation was based on numerous research trials (Ravindran and Bryden, 1997; Sebastian *et al*., 1997; Korengay *et al*., 1996) and recommended by relevant company (Table 2). One Phytase Unit (FTU) is defined as the amount of enzyme activity that librates 1 mmol of inorganic phosphorus per minute from a 0.5 mM Na-phytate solution at pH 5.5 and 37°C.

Table 1: Composition and nutrient content of the experimental diets

	60-72 weeks	
	C	100 E
Ingredients	($g\ kg^{-1}$)	
Corn	670.00	692.30
Soybean meal	181.20	173.60
Soybean oil	19.30	8.70
Wheat bran	-	-
Dicalcium phosphate	11.60	5.50
Oyster shell	108.70	110.30
Common salt	3.10	3.10
DL-Methionine	0.90	0.90
L-Lysine-HCl	0.20	0.30
Vitamin premix[1]	2.50	2.50
Mineral premix[2]	2.50	2.50
Phytase premix	0.00	0.30
Calculated analysis[3]	(%)	
AME_n ($kcal\ kg^{-1}$)	2817.00	2817.00
Crude protein	13.75	13.75
Total phosphorus	0.51	0.40
Non phytate phosphorus	0.30	0.30
Calcium	4.05	4.05
Lysine	0.68	0.68
Methionine	0.32	0.32
Met+Cys	0.585	0.585

[1]: Provided the following per kilogram of layer diet: Vitamin A, 8800 IU; Cholecalciferol, 2500 IU; Vitamin E, 8 IU; Vitamin k3, 2 mg; Vitamin B12, 0.02 mg; Biotin, 0.015 mg; Folacin, 0.5 mg; Niacin, 30 mg; Pantothenic acid, 13 mg; Pyridoxine, 2.2 mg; Riboflavine, 5.5 mg; Thiamine 1 mg, choline, 500 mg

[2]: Provided the following per kilogram of layer diet: Copper (as cupric sulfate 5 H_2O), 6 mg; Iodine (as calciumiodate), 0.99 mg; Iron (as ferrous sulfate 7 H_2O), 50 mg; Manganese (as manganese oxide), 65 mg; Selenium (as sodium selenite), 0.2 mg; Zinc (as zinc oxide), 55 mg.

[3]: Protein, calcium and total phosphorus contents of diets were calculated on the basis of determined nutrient contents of ingredients

Table 2: Nutrient equivalency matrix values for Phytase enzyme (one kg commercial enzyme equivalent to)[1]

Nutrients	(%)
Metabolizable energy (kcal)	106000
Crude protein	450
Available phosphorus	344.9
Calcium	333
Lysine	24
Methionine	2
Met+Cys	8
Threonine	26
Tryptophan	6
Isoleucine	24

[1]: BASF D-67056 Ludwigshafen Germany

Hens selected based on very nearly the same egg production and body weight from commercial flock of 7000 birds. Birds allocated to 144 cages in 3 treatments with 4 replicate and 24 hens in each replicate, in a completely randomize design. Two hens were hosed in a 30×46 cm cage, 12 adjoining cages consisted of a replicate. Hens were exposed to a daily lighting schedule of 16L:8D. All birds were kept under uniform environmental conditions throughout the experimental period. Diets were presented in mash form and provided daily according to expected intake and hens have free access to water.

Individual body weight of the birds was recorded at the beginning and end of the experiment. Egg production on individual basis was recorded daily and percent hen day egg production was calculated. All the eggs laid were weighted daily throughout the experimental period. The three eggs were randomly chosen in each replicate from the eggs laid during the three consecutive days at every 28 days period to determine specific gravity (Densitomter, Mettler-Toledo, ISO-14001, Switerland), shell thickness and shell breaking strength (Universal Testing Machine, EZ test, 120891-04, Japan). The shell thickness was measured at three different locations (middle, broad and narrow end) using a micrometer gauge (Mitutoyo code 7027) and the mean value was calculated. Dried shells were ashed at 600°C for 4 h and shell ash analyzed for calcium and phosphorus content (AOAC, 1990). During each 28 day period, three eggs were randomly selected from each replicate for tow consecutive days to determine the Haugh unit. At the end of experimental period two birds from each replicate were selected randomly and slaughtered. The left middle toes of the birds within a pen after removed the soft tissue were pooled, then fat content of samples extracted (in a Soxtec System HT1043) by ether for 4 h and dried to a constant weight at 100°C. Toe ashed in a furnace (Heraeus Hanau) at 600°C for 4 h. The ash from toes was solubilized with nitric and perchloric acid, then phosphorus and calcium content were analyzed with spectrophotometer and atomic absorption spectrophotometer respectively.

Statistical Analysis

Data were analyzed using the General Linear Models procedure of SAS (2001) software appropriate for completely randomized design. Significant difference among individual group means was determined with Duncan's multiple range test option of the GLM procedure of SAS software.

RESULTS AND DISCUSSION

The data on mean production performance of layer hens during 60 to 72 weeks of age are shown in Table 3. Supplementation of phytase to diet and calculating nutrient equivalency values of phytase, decrease percent hen day egg production and egg mass output (g/hen/day) in hens fed 100 E diet significantly ($p<0.05$). As egg production declined, FCR increase significantly ($p<0.05$). Addition of phytase over the top of C diet slightly increase egg production, but this effect was not significant. Reduction of production performance in hens fed 100 E diet very likely due to overestimate of energy, protein and amino acid equivalency derived from enzyme. These finding are in discrepancy to the results obtained by other investigators. Keshavarz and Austic (2004) reported that when hens from 36 to 48 week of age received the 13% protein diet containing 0.2% NPP and supplemented with phytase, all the traits were comparable to those of hens fed the positive control diet. Jalal *et al*. (1999) the one of two studies with layer hens, observed a significant effect of phytase (250 to 300 U kg^{-1}) on digestibility of only four amino acids and no effect on crude protein digestibility in a CSM diet. Panda *et al*. (2005) and Dorota *et al*. (2006) reported that addition of phytase can allow the reduction of NPP content to 0.12% in the layer diet, without affecting the production performance of layer hens. One likely reason for the inconsistency in phytase effect in our study and others is that the long time monitoring performance in the latter part of production (60 to 72 weeks). Little studies are in

Table 3: Effect of phytase supplementation on performance, egg quality and egg shell ash (Ca and P) of layer hen from 60 to 72 weeks of age

	Dietary treatments			
Attributes	C	C+P	100 E	SEM
Average daily feed intake (g/hen/day)	104.99	104.55	104.31	1.140
Average hen day egg production (%)	75.25[a]	77.25[a]	66.00[b]	2.230
Average egg weight (g)	61.03	62.90	63.36	0.670
Egg mass (g/hen/day)	45.80[ab]	48.74[a]	41.97[b]	1.330
Feed conversion ratio[1]	2.31[ab]	2.15[b]	2.52[a]	0.071
Haugh unit	81.24	83.69	83.17	2.210
Egg specific gravity	1.08	1.08	1.08	0.001
Egg shell thickness (mm)	38.80	38.30	39.17	0.580
Egg shell breaking strength (N cm^{-2})	3.06	2.90	2.83	0.160
Egg shell ash (%)	86.61	87.49	89.16	1.090
Egg shell ash calcium (%)	30.97	32.82	37.27	1.540
Egg shell ash phosphorus (%)	0.25	0.24	0.25	0.006
Body weight change during 60 to 72 weeks (g)	-1.25	0.00	12.50	0.001
Toe ash (%)	93.42	91.52	93.09	0.630
Toe ash calcium (%)	27.83	24.70	27.28	1.030
Toe ash phosphorus (%)	15.94	15.83	16.61	0.680

[1]: Grams of feed per gram of egg mass, [a-b]: Means not sharing a common superscript letter(s) within a row differ significantly ($p<0.05$)

agreement with present study. Snow *et al*. (2003) concluded that phytase addition, numerically decrease the amino acid digestibilities for CSM layer diet, these researchers suggested that, phytase did not have a significant effect on digestible energy. Bhanja *et al*. (2005) reported that supplementation of phytase to diet containing 0.18% NPP had no added advantage on broiler breeder performance. Author observed that addition of enzyme in latter part of production cycle had no effect on broiler breeder egg production (unpublished data). Hence further research is needed to elicit the nutrient equivalency values of phytase for old age birds.

Despite of dietary NPP concentration decrease from 0.3 in C diet to 0.19% in 100 E diet, no significant difference was observed among dietary treatments on average daily feed intake (Table 3). Wu *et al*. (2006) reported that in hens from 21 to 33 weeks of age, as dietary NPP decrease from 0.26-0.11% in the diet without phytase, feed intake significantly decrease about 9.35%. In present study when dietary NPP was 0.19% (100 E), NPP intake of hen was 198.1 mg/hen/day, which was lower than dietary NPP requirement of hens, 250 mg/hen/day, recommended by NRC (1994). Therefore it seems that phytase supplementation prevented the decline of feed intake of hens fed the P deficient diet (100 E), release P equivalency value and improved P availability in hens 60 to 72 weeks old. As shown in Table 3, there were no significant differences in Haugh unit, egg specific gravity, egg shell thickness and breaking strength, egg shell ash, egg shell ash Ca and P content, toe ash and toe ash Ca and P content among dietary treatments. These results are in agreement with results obtained on younger hen by other researchers (Keshavarz, 2003; Roland *et al*., 2003; Wu *et al*., 2006). As discussed above, NPP intake of hens fed C, C+P and 100 E diets were 314.9, 313.6 and 198.18 mg/hen/day respectively. For hens fed 100 E diet, NPP intake was not sufficient. It meaning that the addition of phytase improved shell and bone mineralization in 60 to 72 weeks of age layer hen fed NPP deficient diet. In consistent to other studies (Bhanja *et al*., 2005; Scott *et al*., 1999) supplementation of phytase to diet containing adequate NPP (C+P diet) did not have any additional advantage.

Addition of phytase at the expense of its nutrient equivalency increase a 5.9% in feed cost per kg egg produced. In contrast to 100 E diet, supplementing C diet with phytase over the top (C+P) decrease a 6.4% in feed cost, because phytase slightly increase egg production percentage and improved FCR in C+P group.

CONCLUSIONS

Based on the results, it can be concluded that using phytase nutrient equivalency values did not prove energy and protein utilization but improved phosphorus utilization in old layer hens. These results probably occurred because any effect of phytase on protein and energy utilization in latter part of production cycle is small. Thus using the same AME and protein equivalent values for young and old layer is not valid.

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