Effect of Supplementing Microbial Phytase on Performance of Broiler Breeders Fed Low Non-phytate Phosphorus Diet

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ABSTRACT : An experiment was conducted to study the production performance of broiler breeder females (25 to 40 weeks of age) fed either reference diet or low non-phytate phosphorus (NPP) diet with or without microbial phytase (500 FYT/kg) supplementation. A weighed (160 g/b/d) quantity of feed from each diet was offered daily to 40 replicates of one bird each housed in California type cage having individual feeders. Each cage was considered as a replicate. A continuous 16-h light per day was provided using incandescent bulbs. Body weight, egg production, egg weight, feed per egg mass, egg specific gravity, egg breaking strength, shell thickness, tibia ash and serum Ca and protein concentrations were not affected by reducing the NPP level from 0.30 to 0.18% in the broiler breeder diet. Supplementation of phytase (500 FYT/kg) enzyme to the diet containing 0.18% NPP had no added advantage on any of the above production parameters. The serum inorganic P was increased significantly ($p<0.05$) by either enhancing the NPP content from 0.18 to 0.30% or supplementing phytase @500 FYT/kg to the diet containing low P which were found comparable. Retention of Ca and P was positive on all the diets. P retention decreased significantly ($p<0.05$) with either increase in NPP content or phytase supplementation in the diet. Neither NPP nor phytase supplementation influenced bone mineralization in terms of tibia ash and strength. The hatchability was not influenced by either increasing the NPP content or supplementing the enzyme phytase. Similarly, the P concentration in the egg yolk and day old chick, day old and 14th day body weight and leg score was not altered by increasing the level of NPP or supplementing phytase enzyme. The mortality was within the normal limits in all the three dietary groups. Thus, it can be concluded that 0.18% NPP (288 mg NPP intake/b/d) in the broiler breeder's diet is adequate in sustaining the optimum performance from 25 to 40 wks of age. Enhancing the NPP content or supplementation of phytase (500 FYT/kg diet) to diet containing 0.18% NPP had no added advantage on performance. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1299-1304)***

Key Words : Non Phytate Phosphorus, Phytase, Broiler Breeders, Production Performance

INTRODUCTION

Phosphorous (P) is an essential mineral required in poultry for normal physiological functions and optimum production. In addition to its role as a structural constituent of skeleton and cell wall, it plays a critical role in the metabolism of carbohydrates, protein and lipids primarily in oxidative phosphorylation. Insufficient P level in the diet causes rickets in young chicks, poor shell quality and osteoporosis in laying hens (Tuijl, 1998).

The concept of non-phytate phosphorus (NPP) requirement of chickens is gaining importance because of increasing use of vegetable protein supplements in place of animal protein supplements in poultry diets. In plants, the majority of P is in the form of phytic acid (PA) and it salts with Ca, Mg, Zn and other divalent cations known as phytates (Pallauf and Rimbach, 1997). Phytate phosphorus (PP) is not available to the poultry because they do not contain sufficient amounts of intrinsic phytase required to hydrolyze the PP (Nelson, 1976; Schwarz, 1994). Therefore, poultry diets are supplemented with additional inorganic P to meet the bird's requirement. Formation of complexes of phytic acid with divalent cations and amino acids not only reduces the availability of P but also reduces the digestibility and availability of amino acids and cations to the birds (Sebastian et al., 1998). The availability of PP to chickens can be increased considerably by supplementation of microbial phytase in the diet (Rama Rao et al., 1999a). With the public concern over pollution due to P excretion considerable attention has been generated in the recent years on the use of microbial phytase in diet of broilers (Kornegay and Denbow, 1996; Sebastian et al., 1996; Zhang et al., 1999; Ravindran et al., 2000) and layer chickens (Carlos and Edwards, 1998; Um and Paik, 1999; Boling et al., 2000; Keshavarz, 2000) However, the effect of supplemental phytase on production performance of broiler breeders fed low P diets is yet to be investigated. Therefore, the present study was undertaken to determine the effect of phytase supplementation on production performance of broiler breeders in terms of egg production, shell quality, bone mineralization, retention of calcium (Ca) and P, hatchability and progeny performance.

MATERIALS AND METHODS

Birds and management

Broiler breeder females (120), which were on quantitative feed restriction from 7th week of age to the day

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Table 1. Composition of experimental diets

Ingredient (% as fed basis)	NPP (%) in the diet		
	0.30	0.18	0.18*
Maize	64.14	63.78	63.60
Soybean meal	25.50	25.50	25.50
Deoiled rice bran	0.64	1.28	1.60
Common salt	0.20	0.20	0.20
Dicalcium phosphate	1.20	0.40	0.30
Oyster shell grit	8.02	8.54	8.50
DL-methionine	0.14	0.14	0.14
Sodium bicarbonate	0.16	0.16	0.16
Choline chloride 50%	0.10	0.10	0.10
Vitamin premix[1]	0.10	0.10	0.10
Trace mineral premix[2]	0.10	0.10	0.10
Nutrient composition			
Analysed value			
Crude protein (%)	16.83	16.90	16.91
Total phosphorus (%)	0.51	0.37	0.35
Non phytate phosphorus	0.31	0.18	0.17
Calcium (%)	3.03	3.12	2.98
Calculated value			
Metabolizable energy (kcal/kg)	2,727	2,727	2,727
Lysine (%)	0.84	0.84	0.84
Methionine (%)	0.41	0.41	0.41
Methionine+cystine (%)	0.70	0.70	0.70

* Microbial phytase (500 FYT/kg diet; 0.02%).

[1] Vitamin premix provided (unit/kg diet): Vitamin A, 15,000 IU; D, 3,000 ICU; E, 100 mg; K, 4 mg; thiamin, 2 mg; riboflavin, 15 mg; pyridoxine, 8 mg; cyanocobalamin, 0.25 mg; niacin, 60 mg; Pantothenic acid, 25 mg; Folic acid, 2.5mg; Biotin, 0.2 mg.

[2] Trace mineral premix provided (mg/kg diet): Manganese, 100; Zinc, 90; Iron, 40; Copper, 10; Iodine, 1; Selenium, 0.2.

of experimentation (24^{th} weeks of age) having uniform body weight were utilized in the present study. These birds were randomly distributed into three groups of forty each and housed in individual California type cage (15"×12"×12 ") having individual feeders and nipple waterers. Each cage was considered as a replicate (40 replicates/group). A continuous 16-h light per day was provided using incandescent bulbs. All the birds were kept under uniform managemental conditions throughout the experimental period of 25 to 40 weeks of age.

Experimental diets

The proximate constituents, Ca, TP, (AOAC, 1990) and PP (Haugh and Latzscha, 1983) in the feed ingredients were estimated and NPP contents in feed ingredients were calculated by subtracting the PP from TP. Two experimental diets were formulated based on corn-soybean-de oiled rice bran. A reference diet and a low P diet were formulated to contain 0.30 and 0.18% NPP, respectively at a constant level of Ca (3%). Dicalcium phosphate and shell grit was altered to obtain the desired levels of NPP and Ca (Table 1). Third diet was prepared with commercial source of microbial phytase (Bio-feed, phytase) supplemented to low P (0.18% NPP) diet at 500 FYT/kg diet (0.02%). A premix of phytase (20 g) and uniformly grounded maize (200 g) was prepared in 1:10 ratio and subsequently mixed in the feed through a vertical feed mixture. The broiler breeder females were reared on the experimental diets up to 44 weeks of age. A weighed (160 g/d) quantity of feed was offered daily to all the birds and the residue left, if any, at every 28 d period was weighed to calculate the average daily feed intake.

RESPONSE CRITERION

Body weight, egg production and egg weight

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 housed egg production (HHEP) was calculated. All the eggs laid were weighed daily throughout the experimental period and egg mass was calculated to obtain feed consumption per kg egg mass.

Egg shell quality

Twelve eggs were randomly chosen in each treatment from the eggs laid during the last three consecutive days at every twenty-eight days period to determine the specific gravity (Densitometer, Mettler-Toledo, ISO-14001, Switzerland), shell weight, shell thickness and shell breaking strength (Universal Testing Machine, EZ test, 120891-04, Japan). The cleaned egg - shells were dried for twenty-four hours, were weighed and expressed as % of whole egg. 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.

Serum bio-chemical studies

At the end of each twenty- eight days period, 2 ml of blood samples were collected from brachial vein from five birds in each treatment. Subsequently serum was separated and the levels of Ca (AOAC, 1990), P (Fiske and Subba Row, 1925) and Protein (Doumas et al., 1971) in the serum were estimated.

Metabolism trial

To determine the retention of Ca and P in breeders fed different NPP levels, a three days metabolic trial on three birds from each group involving total collection of faeces was conducted at 40 weeks of age. The daily feed intake and faeces voided was recorded. Representative samples of feed offered, residue left and total faeces voided were analyzed for dry matter, Ca and TP concentrations (AOAC, 1990).

Bone mineralization

At the end of the experimental period, three birds from

Table 2. Effect of phytase supplementation on production performance of broiler breeders during 25 to 40 weeks of age

Attributes	NPP %		
	0.30	0.18	0.18*
Initial body weight (kg)	2.89±0.04	2.962±0.04	2.918±0.04
Final body weight (kg)	3.49±0.06	3.557±0.05	3.519±0.04
Hen housed egg production (%)	61.45±2.06	64.69±1.89	64.55±1.62
Egg weight (g)	55.49±0.67	55.75±0.47	56.19±0.60
Feed per egg mass	5.13±0.33	4.66±0.17	4.53±0.17
Egg specific gravity	1.072±0.001	1.075±0.001	1.075±0.001
Egg shell breaking strength (Newton)	23.63±0.77	24.06±0.79	23.67±0.67
Egg shell weight (%)	8.71^b±0.11	9.26^a±0.12	8.59^b±0.16
Egg shell thickness (mm)	0.398±0.006	0.397±0.005	0.414±0.006
Tibia ash (%)	62.80±0.49	60.77±1.19	61.38±1.21
Tibia breaking strength (Newton)	94.97±5.35	89.87±4.55	97.27±6.23
Ca (mg/dL serum)	15.40±0.58	13.87±0.44	14.25±0.64
P (mg/dL serum)	6.66^a±0.08	6.09^b±0.06	6.61^a±0.07
Protein (mg/dL serum)	5.16±0.23	5.59±0.33	5.09±0.15
Ca retention (mg/g intake)	319±65	181±29	205±35
P retention (mg/g intake)	330^b±10	561^a±31	299^b±45

* Microbial phytase (500 FYT/kg diet).
[a, b] Means with different superscripts in a row differ significantly (p<0.05).

each treatment were selected at random and sacrificed by cervical dislocation. Both the tibia were freed from soft tissue and diaphysis, then defatted by soaking in petroleum ether for 48 h and dried at 100°C for 12 h. The right and left tibia were used for determination of bone ash and bone strength, respectively. Dried bone samples were ashed at 600±30°C for 12 h for estimation of bone ash (AOAC, 1990). Breaking strength on the left tibia was determined by universal testing machine (EZ test, 120891-04,Shimatzu-Japan).

Yolk phosphorus

During each 28 d period, three eggs were randomly selected from each treatment for three consecutive days to determine the P content in egg yolk (AOAC, 1990).

Hatchability and performance of progeny

Hatchability of eggs laid by broiler breeders fed different levels of NPP were evaluated at 280 days of age. All the hens in each treatment were inseminated with pooled semen from broiler breeder males of the same hatch (age). Eggs were collected through the 3rd to 8th day following insemination and incubated to determine the fertility and hatchability. The chicks hatched were wing banded and reared to 14 days of age in stainless steel battery brooders under uniform managemental condition to determine the survivability, body weight gain and leg abnormality (Watson et al., 1970), if any. The leg score given were 1- for completely normal leg, 2- slight amount of swelling of tibio-metatarsal joint, 3- marked degree of swelling of the joint, 4- swelling with slight amount of slipping of the Achilles tendon and 5- for swelling combined with marked degree of slipping of the tendon from its condyles.

Total phosphorus in chicks

Three chicks were randomly chosen from each treatment from the chicks hatched on day one and were killed with chloroform. Subsequently they were dried in the hot air oven at 100°C, ground and P content of individual chick was determined (Fiske and Subba row, 1925).

Statistical analysis

Data were subjected to statistical analysis employing one-way analysis of variance (Snedecor and Cochran, 1989). The mean of different treatments were compared with Duncan multiple range tests (Duncan, 1955). Significance was considered at p<0.05 levels.

RESULTS AND DISCUSSION

The data on mean production performance of broiler breeders during 25 to 40 weeks of age are presented in Table 2. The body weight, egg production (Figure 1), egg weight, feed per egg mass, egg specific gravity, egg breaking strength, shell thickness, tibia ash and serum Ca and protein concentrations were not affected by reducing the NPP level from 0.30 to 0.18% in the broiler breeder diet diet. Supplementation of phytase (500 FYT/kg) enzyme to the diet containing 0.18% NPP had no added advantage on any of the above production parameters. The serum inorganic P was increased significantly (p<0.05) by either enhancing the NPP content from 0.18 to 0.30% or supplementing phytase @ 500 FYT/kg to the diet containing low P which were found comparable. Retention of Ca and P was positive on all the diets. P retention

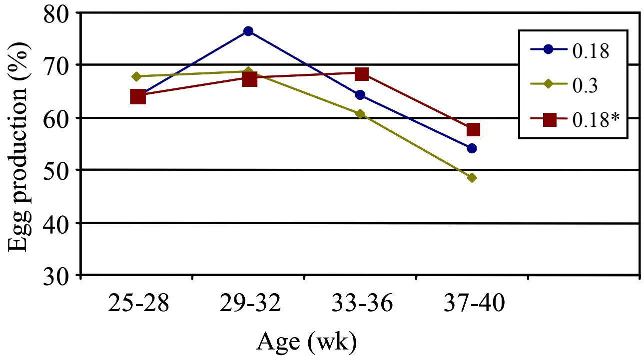

Figure 1. Egg production curve during 25 to 40 weeks in broiler breeders.

decreased significantly (p<0.05) with either increase in NPP content or phytase supplementation in the diet. Neither NPP nor phytase supplementation influenced bone mineralization in terms of tibia ash and strength.

The hatchability was not influenced by either increasing the NPP content or supplementing the enzyme phytase (Table 3). Similarly, the P concentration in the egg yolk and day old chick, day old and 14th day body weight and leg score was not altered by increasing the level of NPP or supplementing phytase enzyme. The mortality was with in the normal limits in all the three dietary groups.

Despite the growing use of phytase enzyme to reduce dietary P supplementation and the environmental load, research on use of phytase in broiler breeders is not available. The NRC (1994) suggested a level of 0.35% NPP in the diet of broiler breeder for optimum performance, at a daily intake of 400 to 450 kcal ME. Thus keeping the dietary concentration of ME to 2,700 kcal/kg and assuming a daily intake of 160 g with 0.35% NPP in the diet, birds will be provided with 560 mg NPP every day. However in the present study, 0.18% NPP (288 mg NPP intake/b/d) in the diet was adequate in sustaining the breeders performance from 25 to 40 wks of age. Only two references are available in the literature on the requirements of P for broiler breeder females. Wilson et al. (1980) observed similar egg production on NPP from 0.16 to 1.21% in diet (NPP intake 163 to 860 mg/b/d) in Cobb color sexed breeders on floor. Similarly Wilson and Harm (1984) did not find any difference on the egg production, egg weight and egg specific gravity of broiler breeder females fed 0.22% NPP to 0.27% NPP (NPP intake of 308 to 378 mg/b/d) in the diet. Body weight was variable. The lowest level of TP in diet (0.42%) met the P requirements for the broiler breeders. Assuming 0.30% P from cereal and protein sources (0.10% NPP) and 0.12% from inorganic sources, a NPP content of 0.22% (NPP intake 319 mg/b/d) was adequate to meet the requirements of phosphorus. The result of the present study is as per with the findings of O'Rourke et al. (1954) who suggested a NPP requirement of 0.18% for White Leghorn layer breeders.

Enhancing the NPP content to either 0.30% or adding 500 FYT phytase to 0.18% did not prove to have any additional advantage. Similarly, Wilson et al. (1980) did not find any significant difference in egg production and egg shell quality in broiler breeders fed 163 to 863 mg NPP per day. In the present study also the daily intake of NPP in broiler fed basal diet was 280 mg NPP per bird. Probably this level of NPP was enough to maintain the optimum performance up to 40 wks of age. Since the net intake of NPP in breeders diet met its P requirement, supplementation of phytase could not prove additionally advantageous. Similarly, Rama Rao et al. (1999b) and Scott et al. (1999) reported that 0.2% NPP in the diet was adequate for optimum layer performance. Supplementation of phytase (250 or 500 FYT/kg) to diets containing adequate (0.2%) NPP did not have any additional advantage (Rama Rao et al., 1999b; Scott et al., 1999). However improvement in P utilization (Boling et. al., 2000) and egg production and shell quality (Rama Rao et al., 1999b; Narahari andJayaprasad, 2001) was reported when phytase (300-500 FTU/kg) was supplemented to diet containing sub optimal levels of NPP (0.1%) in WL layers.

The hatchability was not influenced by either increasing the NPP content or supplementing the enzyme phytase indicating similar requirement of NPP for both egg production and hatchability. Earlier reports (Wilson et al., 1980; Wilson and Harms, 1984) also indicated similar NPP requirement for both egg production and hatchability. Contrary to this, though, Waldrop et al. (1967) reported

Table 3. Hatchability and performance of progeny of broiler breeders on diets with phytase supplementation

Attribute	NPP %		
	0.30	0.18	0.18*
Hatchability (%)	79.33±3.85	80.67±2.87	74.40±3.86
Egg yolk P (mg/100 g)	728±17	744±19	749±21
Chick P (%)	0.67±0.02	0.67±0.02	0.66±0.02
Body weight at day one (g)	42.65±0.34	43.35±0.32	43.65±0.18
Body weight at 14th day (g)	261±2.71	245±5.82	249±2.94
Leg score	1.09±0.03	1.05±0.02	1.07±0.02
Mortality %	1.75	2.76	2.38

* Microbial phytase (500 FYT/kg diet).

higher P requirement for hatchability (0.19%) compared to maximum egg production (0.16%), the levels recommended for hatchability in their study was almost similar to the NPP level present in the basal diet. Since the deposition of P in egg yolk did not differ due to variation either in the NPP content or phytase supplementation, the hatchability, P content in day old chicks and leg abnormality score at 14 day of age were similar. Similar findings were also reported by Harms et al. (1964) when gradient levels of P was supplemented in breeder diet.

Broiler breeder hens are usually fed on a controlled basis to maintain body weight within the breeder guidelines and feed intake is adjusted according to the energy content of the diet. Diet containing ME 2,700 kcal/kg with 0.18% NPP and offering 160 g feed daily did not need any phytase supplementation to have any additional advantage on phosphorus utilization. Hence further research is needed to elicit the benefit of supplemental phytase in broiler breeders fed sub-optimal levels of NPP (<0.18%).

Based on the result, it can be concluded that on an intake of 288 mg NPP/b/d is adequate for broiler breeders (25-40 weeks of age) for optimum egg production, hatchability and progeny performance. Supplementation of microbial phytase (500 FYT/kg diet) in diets containing 0.18% NPP did not prove beneficial in broiler breeders.

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