Effects of Dietary Non-phytate Phosphorus Levels on Egg Production, Shell Quality and Nutrient Retention in White Leghorn Layers

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ABSTRACT : An experiment was conducted (28 to 44 weeks) to study the laying performance, shell quality, and nutrient retention of White Leghorn layers fed different levels of non-phytate phosphorus (NPP). Six levels of NPP (0.15, 0.18, 0.21, 0.24, 0.27 and 0.30%) at a constant calcium (Ca) level (3.5%) in maize-soya-deoiled rice bran based diets were formulated, and each experimental diet was offered *ad libitum* for 16 weeks to five replicates with five birds in each replicate. The body weight of WL layers fed diet containing 0.15% NPP was significantly ($p<0.05$) lower than those fed diet with 0.30% NPP, at 44 weeks of age. However, the hen day egg production, egg weight, daily feed intake and feed consumed per dozen eggs were not influenced by the variation in the NPP levels in the diet. The bone ash content was significantly ($p<0.05$) higher in the birds fed 0.30% NPP as compared with those fed diets up to 0.24% NPP. Bone ash content was intermediate in the birds fed diet containing 0.27% NPP. The tibia strength followed the same trend as that of bone ash. Dietary NPP content had no influence on serum Ca and protein concentration and activity of alkaline phosphatase. However, serum inorganic P concentration increased linearly with NPP content in the diet. The concentration of P was significantly ($p<0.05$) higher in the birds fed 0.27% NPP or higher as compared with those fed 0.15% NPP. Levels of dietary NPP had no influence on egg quality parameters like shell wt, shell thickness, shell strength and specific gravity. The retention of nutrients such as DM, N and Ca were comparable among the WL layers fed different levels of NPP. However, the retention of P decreased linearly with increase in the level of NPP in the diet. The retention of P in the birds fed diets up to 0.24% NPP in the diet was comparable, however further increasing the content of NPP (either 0.27% or 0.30%) reduced the retention of P. Based on the results of the present study, 0.15% NPP (180 mg/b/d) in the diets of WL layers is adequate for optimum production performance during 28 to 44 weeks of age, however, WL layers require 0.27% NPP (324 mg /b/d) in the diet for optimum production with better bone mineralization. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1171-1175)***

Key Words : Non Phytate Phosphorus, Egg Production, Shell Quality, Nutrient Retention, White Leghorn Layers

INTRODUCTION

Phosphorous (P) is an essential and critical mineral in laying hens' diet. It plays an important role in metabolism, being a part of many organic compounds involved in metabolic processes such as phosphates used for energy and synthesis of DNA and RNA (Scott et al., 1982). Dietary levels of P that are too high or too low adversely affects not only egg production but also egg shell quality (Owing et al., 1977). Deficiency of P in the diet is associated with cage layer fatigue and mortality during heat stress (Garlich et al., 1985). P does not exist in 'pure' phytic acid (PA) form, but always as a salt with divalent cations (phytate). About two thirds of total P (TP) in vegetable feed ingredients is in phytate form (NRC, 1994) and is not available to poultry due to lack of or insufficient amount of phytase in their digestive system (Paik, 2004). Therefore, non-phytate phosphorus (NPP) gives precise estimates of the P requirements for poultry (Rama Rao et al., 1999b).

Much of the egg production and shell quality research has emphasized the role of calcium, since approximately 98 % of the shell is composed of calcium carbonate. Dietary P has become a focus of attention lately and as a result the nutritional strategy has shifted to include optimising the P requirement in relation to production. Research reports indicated that P requirement of the hen vary depending on the composition of the diet (Pointillart et al., 1987) and quantity of other nutrients such as Ca (Hartel. 1990). vitamin D_3 (Kesharvarz, 1996) and phytase (Gordon and Ronald. 1997), hen age (Sell, 1979), and climatic environment (Sell et al., 1987). Although, NRC (1994) recommended 250 mg NPP /hen/d, many workers reported that the NPP requirement of laying hens may be even lower than the above recommendation (Van der Klis et al., 1996; Gordon and Ronald. 1997; Parsons. 1999).

Despite considerable research on P requirement of laying hens, some uncertainty exists about dietary P levels that are nutritionally adequate and economical. Because of the high cost of P sources and the public concern over the contribution of poultry excreta to environmental pollution, the P requirement of laying hens need to be reinvestigated. Therefore, the present investigation was carried out to study the effects of dietary NPP levels on egg production, shell quality and retention of nutrients in WL layers.

MATERIALS AND METHODS

Stocks, diet and husbandry

White Leghorn (WL) layers of 28 weeks of age were

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Table 1. Composition of experimental diets

Ingredient	NPP (%) in the diet					
(% as fed basis)	0.15	0.18	0.21	0.24	0.27	0.30
Maize	65.24	65.24	65.24	65.24	65.24	65.24
Soybean meal	22.02	22.02	22.02	22.02	22.02	22.02
Deoiled rice bran	2.47	2.46	2.43	2.40	2.35	2.31
Oyster shell grit	9.34	9.20	9.08	8.96	8.86	8.75
Dicalcium phosphate	0.25	0.40	0.55	0.70	0.85	1.00
DL-methionine	0.08	0.08	0.08	0.08	0.08	0.08
Common salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Nutrient composition						
Analyzed value						
Crude protein (%)	16.58	16.57	16.55	16.54	16.52	16.52
Calcium (%)	3.49	3.48	3.48	3.46	3.46	3.45
Non-phytate phosphorus (%)	0.15	0.18	0.21	0.24	0.27	0.30
Calculated value						
Metabolizable energy (kcal/kg)	2,762	2,760	2,758	2,757	2,756	2,755
Lysine (%)	0.84	0.84	0.84	0.84	0.83	0.83
Methionine (%)	0.35	0.35	0.35	0.35	0.35	0.34

[1] Supplies per kg diet: Vitamin A, 8,250 IU, vitamin D_3, 1,200 ICU, Vitamin E, 12 mg; vitamin K, 2 mg. Vitamin B_1, 1.2 mg; vitamin B_2 10 mg ; vitamin B_6, 2.4 mg; vitamin B_{12}, 0.01 mg; niacin, 19 mg; pantothenic acid, 12 mg.

[2] Supplies per g diet: Mn, 50 mg; Zn, 112.5 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg.

randomly divided into 6 groups of 25 each and were housed in individual California type cages. Considering birds in five cages as a replicate, 5 such replicates were randomly allotted to each dietary treatment. Six isonitrogenous and isocaloric diets were formulated to contain 0.15, 0.18, 0.21, 0.24, 0.27 and 0.30% NPP. Each experimental diet was offered *ad libitum* for 16 weeks. A continuous 16 h light per day was provided using incandescent bulbs. All the birds were maintained under uniform managemental conditions throughout the experimental period of 28 to 44 weeks of age.

Response criterion

Body weight, egg production and egg weight : Individual body weight of the bird was recorded at the beginning and end of the experiment. Egg production on individual basis was recorded daily and percent hen day egg production (HDEP) was calculated. All the eggs laid during the last three consecutive days of every 28 day period, were collected to measure the egg weight.

Egg shell quality : Twelve eggs were randomly chosen in each treatment from the eggs laid during the last three consecutive days of each 28-day 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, 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, Japan) and mean value was calculated.

Serum biochemical studies

At the end of each 28-day period, 3 ml of blood was collected from brachial vein from 10 birds in each treatment. Subsequently serum was separated and the levels of Ca (AOAC, 1990), P (Fiske and Subba row, 1925), protein (Doumas et al., 1971) and alkaline phosphatase (ALP) activity (Bergmeyer, 1974) in the serum were estimated.

Metabolism trial

A three days metabolic trial on three birds from each group involving total collection of faeces was conducted at 44 weeks of age to determine the retention of dry matter (DM), N, Ca and P. The daily feed intake and faeces voided were recorded. Representative samples of feed offered, residue left and faeces voided were analyzed for DM, N, Ca and TP concentrations (AOAC, 1990).

Bone mineralization

Three birds from each treatment were selected at random and sacrificed by cervical dislocation at the end of experiment. Both the tibiae were freed from soft tissue and diaphysis, defatted by soaking in petroleum ether for 48 h and dried at 100°C for 12 h. The right and left tibiae 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, Shimadzu-Japan).

Table 2. Performance of WL layers fed diet with different levels of non-phytate phosphorus (NPP)

Traits	NPP (%)						
	0.15	0.18	0.21	0.24	0.27	0.3	SEM
Initial body weight (g)	1,342	1,341	1,350	1,364	1,368	1,367	9.25
Final body weight (g)	1,426[b]	1,480[ab]	1,490[ab]	1,496[ab]	1,504[ab]	1,523[a]	12.65
Hen-day egg production (%)	91.76	91.88	92.05	92.02	92.19	92.17	0.24
Egg wt (g)	51.16	51.31	51.35	51.52	51.48	52.22	0.28
Feed intake (g/b/d)	120.13	120.12	119.81	119.04	119.84	119.14	0.42
Feed (kg)/12 eggs	1.572	1.568	1.561	1.552	1.559	1.553	0.14
Bone ash (%)	44.43[b]	44.74[b]	44.33[b]	44.86[b]	46.89[ab]	48.20[a]	0.45
Tibia strength (Newton)	64.2[b]	63.66[b]	64.93[b]	63.73[b]	67.81[ab]	68.46[a]	0.39

[a, b] Means with different superscripts in a row differ significantly ($p<0.05$).

Table 3. Influence of levels of dietary non-phytate phosphorus (NPP) on serum concentration of Ca, P and protein and ALP activity

Serum parameters	NPP (%)						
	0.15	0.18	0.21	0.24	0.27	0.30	SEM
Ca (mg/dl)	25.59	25.17	25.02	25.73	25.23	24.5	0.22
P (mg/dl)	3.63[b]	4.12[ab]	4.16[ab]	4.22[ab]	4.54[a]	4.64[a]	0.10
Protein (g/dl)	4.08	4.11	4.09	4.28	3.56	3.74	0.10
ALP[1] (IU/L)	56.05	53.38	56.53	57.71	57.5	57.43	1.17

[a, b] Means with different superscripts in a row differ significantly ($p<0.05$).

[1] Alkaline phosphatase (international unit/litre).

Statistical analysis

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

RESULTS AND DISCUSSION

The production performance of layers fed different levels of NPP is presented in Table 2. Body weight was not different among various treatments at the start of the experiment. However, at 44 weeks of age, the body weight of WL layers fed 0.15% NPP was significantly lower than those fed diet with 0.30% NPP. The hen day egg production, egg weight, daily feed intake and feed consumed per dozen eggs did not vary significantly due to NPP levels. However, bone ash content in the birds fed diet with NPP levels upto 0.24% was significantly lower than those fed diet containing 0.30% NPP. Birds fed diet containing 0.27% NPP had intermediate bone ash content. The tibia breaking strength (Newton) followed the same trend as that of bone ash.

Dietary NPP had a linear effect on body weight. As NPP increased in the diet body weight increased and the groups fed 0.30% NPP recorded significantly higher weight than those fed 0.15% NPP. Similar findings are also reported previously (Summers, 1995; Usayran and Balnave, 1995). Neither the egg production nor the egg weight was influenced by the variation in the NPP content on the present study, suggesting that 0.15% NPP in the diet of WL layers was adequate in sustaining both egg production and maintaining the egg weight. Concomitant to the findings of the present study, Boling et al. (2000) reported similar egg production and egg weight in laying hens fed various concentration of NPP from 0.15 to 0.45% in the diet. However, further reduction in the NPP level (0.10%) lowered the egg production. In another study with WL layers, Rama Rao et al. (1999a) also observed comparable egg production between birds fed diet with 0.15% or higher levels of NPP (0.20% or above) indicating that 0.15% NPP in the diet was sufficient for WL layers for optimum production. The daily feed consumption (g) and feed intake (kg) per dozen eggs in the present study was not influenced by the levels of NPP in the diet. On an average, hens consumed approximately 119 to 120 g feed per hen/d during the 16 wk study. This finding is consistent with the report of Boling et al. (2000). Keshavarz (2000) also observed no influence of dietary NPP levels (0.15 to 0.40%) on either feed consumption or feed conversion in WL layers during 30 to 42 weeks of age. Bone ash content and tibia breaking strength (Newton) was influenced by the levels of NPP content in the diet. Similarly, Keshavarz (2003) reported higher bone ash content in WL layers fed 0.45% NPP as compared to those fed either 0.20 or 0.25% NPP. Sohail and Ronald (2002) observed higher bone strength in WL layers by enhancing the NPP levels in the diet from 0.10 to 0.30%. The increase in bone strength at the higher NPP level may be attributed to increased bone accretion as reflected by the higher bone ash content in the same group (Ronald and Gordon, 1996). Thus, the NPP requirement for bone mineralization and tibia strength is higher (0.27%) than that for optimum egg production and shell quality (0.15%) as observed in the present study.

Dietary NPP content had no influence on serum Ca and

Table 4. Influence of dietary non-phytate phosphorus (NPP) levels on egg quality of WL layers

Traits	NPP (%)						
	0.15	0.18	0.21	0.24	0.27	0.3	SEM
Shell wt (%)	9.11	9.18	9.21	9.23	9.22	9.24	0.12
Shell thickness (mm)	0.38	0.38	0.39	0.39	0.39	0.39	0.02
Sp gravity	1.079	1.074	1.075	1.076	1.076	1.076	0.05
Shell strength (Newton)	22.98	23.25	23.52	23.38	23.4	23.43	0.51
Haugh unit	75.47	76.02	76.21	76.27	75.5	76.29	0.96

Table 5. Influence of dietary non-phytate phosphorus (NPP) levels on retention of different nutrients

Retention (%)	NPP (%)						
	0.15	0.18	0.21	0.24	0.27	0.3	SEM
DM	68.26	68.88	67.32	68.46	66.81	65.2	1.97
N	55.24	56.82	53.92	54.89	56.62	55.89	1.42
Ca	63.86	64.00	64.08	67.06	68.07	69.66	1.26
P	48.1[a]	45.86[a]	44.2[a]	43.96[a]	39.01[b]	37.03[b]	1.06

[a, b] Means with different superscripts in a row differ significantly ($p<0.05$).

protein concentration (Table 3). However, serum inorganic P concentration increased linearly with NPP content in the diet. The concentration of P was significantly higher in the birds fed diet with 0.27% or higher NPP as compared to those fed diet containing 0.15% NPP. The P concentration in the serum of birds fed other levels of NPP (0.18 to 0.24%) did not differ significantly with either 0.15 or 0.30% NPP dietary group. The ALP activities in the serum of WL layers did not vary significantly due to variation in NPP content in the diets. Other workers (Miles et al., 1983; Rama Rao et al., 1999b) also observed similar findings.

Levels of dietary NPP had no influence on egg quality parameters like shell wt, shell thickness, shell strength and specific gravity (Table 4). Similarly, the haugh unit score was comparable among the diets containing different levels of NPP in the diet. Though, the serum inorganic P level increased with increase in dietary NPP content, it was not reflected either in increased egg shell quality or egg production as observed by Rama Rao et al. (1999b).

The retention of nutrients such as DM, nitrogen and Ca was not affected by the levels of NPP tested in the present study. However, the retention of P decreased linearly with increase in the level of NPP in the diet (Table 5). The retention of P in the birds fed diets upto 0.24% NPP in the diet was comparable, however further increasing the content of NPP (either 0.27% or 0.30%) reduced the retention of P. Keshavarz (2000) also reported that dietary NPP levels (0.15 to 0.40%) had no influence on N retention. Birds fed higher levels of NPP had lower retention of NPP. This was expected as intake and retention of nutrients are inversely correlated (Rama Rao et al., 1999b; Keshavarz, 2003; Selle et al., 2003).

In the present experiment, the birds were in the first phase of the laying cycle and on an average, hens consumed approximately 119 to 120 g feed per hen/d during the 16-wk study. Thus considering daily intake of 120 g feed per bird, an amount of 180 mg NPP per day is adequate in maintaining the production performance. Thus, the lowest level of 0.15% NPP employed in the diet of WL layers was adequate for maintaining the egg production, feed efficiency, egg weight, shell quality (shell weight, shell thickness, shell strength and specific gravity) and optimum retention of nutrients (Ca, P, nitrogen and DM). However, the bone mineralization interms of tibia ash content and tibia breaking strength was significantly increased with increase in level of NPP from 0.15 to 0.27%. Therefore, considering the body weight change and bone mineralization a level of 0.27% NPP appears to be optimum in layer diet during their peak egg production phase (28 to 44 weeks of age). Contrary to this finding several authors (Gordon and Ronald, 1997; Rama Rao et al., 1999 a,b; Boling et al., 2000) suggested lower levels of NPP (0.15 to 0.20%) for WL layers. These authors suggested lower NPP levels mostly considering the performance interms of egg production, feed efficiency and shell quality but not the bone mineralization, which is similar to the 0.15% NPP for the same above parameters in this study. However, requirement of NPP for optimum bone mineralization is higher i.e. 0.27% as observed in the present study which is consistent with the findings of Keshavarz (2003). Based on the bone mineralization profile found in the present study, the level of 0.24% NPP or lower appear to be sub optimal even though egg production or shell quality is not affected. The sub optimal levels of NPP might have stimulated bone resorption to sustain optimum performance and shell quality and resulted in significantly lower bone ash contents in those birds. The lack of information on bone mineralization (bone ash and bone breaking strength) may limit the utilization of low NPP levels (0.15 to 0.24%) in practical layer diets.

Based on the results of the present study, 0.15% NPP (180 mg/b/d) in the diets of WL layers is adequate for optimum production performance during 28 to 44 weeks of age, however. WL layers require 0.27% NPP (324 mg /b/d) in the diet for optimum production performance with better bone mineralization.

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