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Effects of Reduced Calcium and Phosphorous Diets Supplemented with Phytase on Laying Performance of Hens

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Abstract: The aim of this experiment was to examine the potential for reduced environmental impact by reducing dietary calcium and phosphorus content and phytase addition of laying hen diets. A randomized complete block design with a 2×2×2 factorial arrangement of 8 dietary treatments: 2 levels of phytase (0 and 300 FTU kg^{-1}) and 2 mineral levels (Ca: 34/18 and NPP: 3.2/2.2 g kg^{-1}, respectively). A total of 240 White Leghorn (WL) layers, 25 weeks of age were used. Considering birds in 12 cages as a replicate, 5 such replicates were randomly allotted to each dietary treatment. Individual body weight of the bird was recorded at the beginning and end of the experiment. Egg production on an individual basis was recorded daily and percentage hen day egg production was calculated. The cleaned eggshells were dried for 24 h, weighed and expressed as percentage of whole egg. One bird from each experimental unit were selected at random and killed by cervical dislocation at the end of the experiment and the left tibia was removed. Dried bone samples were ashed at 680° C for 12 h for estimation of bone ash. The results of this experiment showed that reducing Ca and NPP (Non-Phytate Phosphorus) without phytase decreased BWG (Body Weight Gain), feed intake, FCE (Feed Conversion Efficiency), egg production, egg shell weight and tibia ash. However, phytase addition to low mineral diets completely corrected the adverse effects associated with low dietary Ca and NPP. It can therefore be concluded that reducing levels of Ca and NPP below current standards and phytase supplementation can reduce pollution potential from laying hen production without adversely affecting bird performance or welfare.

Key words: Ca, P, phytase enzyme, laying hens, welfare

INTRODUCTION

The major portion of P in plant feed ingredients is present in the form of phytate, which is largely unavailable to monogastric animals. The interest in the use of microbial feed enzymes such as phytase arises from the need to improve the availability of phytate-bound P and reduce the P levels in effluent from intensive livestock operations. Phytase supplementation of laying hens diets improves utilization of Non-Phytate Phosphorus (NPP) and other minerals chelated to phytic acid (Boling *et al*., 2000; Casartelli *et al*., 2005; Ceylan *et al*., 2003; Keshvarz and Austic, 2004). Numerous investigations (Gordon and Roland, 1997; Boling *et al*., 2000; Keshavarz, 2003) had shown that phytase supplementation of diets containing low Ca and NPP resulted in an increase of phytate phosphorus utilization. Based on the results of these investigations, diets with 1.5-2.0 g kg^{-1} NPP with phytase supplementation have been shown to be sufficient for satisfactory egg production performance during the laying cycle. Reduction of phosphorus in laying hens excreta is also desirable since this can cause environmental damager through eutrophication of waterways. Since, phosphorus absorption can be influenced by dietary calcium content it is necessary to maintain the ratio between these minerals for optimal utilization and thus reduce the overall dietary mineral content. However, a reduction in Ca and P below bird requirements will impair both growth and bird welfare, particular if skeletal development is compromised. It is worth to be mentioned that although many experiments have been carried out in recent years on the use of phytase in laying hens diets, but to this knowledge, in none of these experiments the effects of phytase supplementation on lower Ca content was studied and also it is worth to know to what extent the Ca content of diet could be reduced without negative impact on laying performances of hens. Therefore, the aim of this experiment was to examine the effect of reducing dietary mineral content (Ca/NPP) supplemented with phytase enzyme on aspects of performance, egg production, shell quality and tibia ash of laying hens. To achieve this, 2

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levels of phytase (0 and 300 FTU kg^{-1}) and 2 mineral levels (Ca: 34/18 and NPP: 3.2/2.2 g kg^{-1}, respectively) were compared in 2×2×2 factorial design to examine the effect on growth performance, feed and FCE, egg production, egg shell quality and tibia ash.

MATERIALS AND METHODS

The study was lasted for 90 days and carried out at a farm in Animal Research Institute, Karaj, Iran. The experimental design was a randomized complete block design, with a 2×2×2 factorial arrangement of eight dietary treatments: 2 levels of phytase (0 and 300 FTU kg^{-1}) and 2 mineral levels (Ca: 34/18 and NPP: 3.2/2.2 g kg^{-1}, respectively). Table 1 shows the ingredients and analyzed nutrient values for each of the treatments. The diets were corn-soybean meal-based to reflect the widespread use of corn-soybean meal in Iranian laying hens diets and were formulated to provide the same level of CP and ME. A total of 240 White Leghorn (WL) layers, 25wk of age were used. Considering birds in 12 cages as a replicate, 5 such replicates were randomly allotted to each dietary treatment. Eight isonitrogenous and isocaloric diets (Table 1) were formulated to contain 2.2, 3.2 g NPP kg^{-1} and 34 and 18 g Ca kg^{-1} diets and 2 levels of microbial phytase (BASF phytase, Germany) at 0 and 300 FTU kg^{-1} diet. Each experimental diet was offered *ad libitum* for 12 weeks. A continuous 500 lux 17 h light day^{-1} was used. All the birds were maintained under uniform management conditions throughout the experimental period of 25-37 weeks of age.

Data collection: Individual body weight of the bird was recorded at the beginning and end of the experiment. Feed consumption was recorded weekly. Egg production on cage basis was recorded daily and percentage hen days egg production was calculated. Feed conversion efficiency was calculated by dividing egg production (g) to feed consumption (g). All the eggs laid during the last 3 consecutive d of each week were collected to measure the egg weight. The cleaned eggshells were dried for 24 h, weighed and expressed as percentage of whole egg. One bird from each experimental unit were selected at random and killed by cervical dislocation at the end of the experiment and the left tibia was removed, freed from soft tissue and diaphysis, defatted by soaking in 20 mL petroleum ether for 48 h and dried at 105°C. Dried bone samples were ashed at 680°C for 12 h for estimation of bone ash (AOAC, 2006).

Data analysis: Data were subjected to an Analysis of variance using the General Linear Model command in the statistical package (McKenzie and Goldman, 2005). The statistical model used for the analysis of dependent variables was:

Table 1: Ingredients and chemical analysis of the experimental diets

Chemical analysis of diets	Teatments 1	2	3	4	5	6	7	8
Phytase (FTU kg^{-1})	0.00	0.00	0.00	0.00	300.00	300.00	300.00	300.00
Mineral levels (g kg^{-1})								
Ca content	34.00	18.00	34.00	18.00	34.00	18.00	34.00	18.00
NPP content	3.20	3.20	2.20	2.20	3.20	3.20	2.20	2.20
Ingredients (g kg^{-1})								
Corn	662.50	745.40	667.80	700.00	662.50	745.40	700.00	745.40
Soybean meal	196.30	219.80	195.20	219.80	196.20	219.80	195.10	219.80
Poultry fat	29.50	0.00	27.60	0.00	29.50	0.00	27.60	0.00
Oystershell	82.00	39.70	84.60	42.70	81.60	39.70	84.60	42.70
Dicalcium phosphate (18%)	10.80	10.60	5.40	5.20	10.80	10.60	5.40	5.20
Vit./ mineral supplement[1]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
DL Methionine	0.61	0.55	0.61	0.55	0.61	0.55	0.61	0.55
Salt	2.70	7.90	2.70	8.00	2.70	7.90	2.70	8.00
Phytase enzyme (FTU kg^{-1})	0.00	0.00	0.00	0.00	300.00	300.00	300.00	300.00
Sand	6.00	6.00	6.00	8.30	6.00	6.00	6.00	8.30
Nutrients								
Analyzed value (g kg^{-1})								
Ca	35.00	19.60	34.90	19.50	35.00	19.00	35.00	19.00
Non-phytate P	3.20	3.20	2.20	2.20	3.20	3.20	2.20	2.20
Total phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
CP	145.00	145.00	145.00	145.00	145.00	145.00	145.00	145.00

[1]Provided per kilogram of vitamin mix: 1500000 IU of vitamin A from retinyl acetate, 250000 ICU of vitamin D3 from cholecalciferol, 1000 IU of vitamin E from DL-α-tocopheryl acetate, 400 mg of riboflavin, 30,864 mg of D-pantothenic acid from calcium pantothenate, 200 mg of vitamin K from menadione sodium bisulfite, 2,646 mg of folic acid, 7,716 mg of pyridoxine from pyridoxine hydrochloride, 150 mg of thiamine from thiamine mononitrate, 10 mg of Se from Na2SeO3, 176 mg of Dbiotin

$$Y_{ijklm} = \mu + C_i + Pj + E_k + B_l + C_i{*}P_j + P_j{*}E_k + Ci{*}E_k + C_i{*}P_j{*}E_k + e_{ijklm}$$

where, Y_{ijklm} was the individual observation, μ is the experimental mean, C_i is the Ca effect, P_j is the P effect, E_k is the phytase effect, $C_i{*}P_j$ is the Ca by P interaction, $P_j{*}E_k$ is the P by phytase interaction, $C_i{*}E_k$ is the Ca by phytase interaction, $C_i{*}P_j{*}E_k$ is the Ca by P by phytase interaction, B_k is the block effect and e_{ijklm} is the random error.

RESULTS

Decreasing Ca and NPP had no significant effect on Body Weight Gain (BWG), feed consumption, egg weight and tibia ash. However, reducing Ca content significantly ($p<0.05$) decreased FCE, egg production and egg shell weight. There was no significant interaction between Ca content and NPP level for any of the feed intake and production performance measured except for egg production which as significantly ($p<0.050$) decreased when C a/NPP level was reduced from 34/3.2 to 18/2.2 g kg^{-1} (Table 2).

As can be shown from the Table 3 reducing Ca content or phytase supplementation had no significant effect on BWG, feed intake, egg weight. However, reducing Ca content significantly ($p<0.05$) decreased FCE, egg production and egg shell weight and phytase addition significantly ($p<0.05$) increased tibia ash. There was a significant interaction ($p<0.05$) between Ca content and phytase supplementation for egg production, egg shell weight and tibia ash and birds received diet containing 34 g kg^{-1} Ca and 300 FTU kg^{-1} phytase had higher egg production and egg shell weight than those fed the other diets. Addition of phytase to low Ca diet significantly ($p<0.05$) increased tibia ash.

As can be shown from Table 4, reducing dietary P content had no significant ($p>0.05$) on BWG, feed consumption, feed conversion efficiency, egg weight, egg production, egg shell weight and tibia ash. Supplemental phytase had no significant ($p>0.05$) on BWG, feed conversion efficiency, egg weight, egg shell weight and tibia ash. However, phytase inclusion significantly ($p<0.05$) increased feed consumption and egg production.

Table 2: Production performance of WL layers on diets with different concentrations of non-phytate phosphorus and calcium

	Ca (g kg^{-1}) (n = 12)							
	34	18	34	18				
	NPP (g kg^{-1}) (n = 12)					p-value		
Variables	3.2	3.2	2.2	2.2	Pooled SE	Ca	P	Ca×P
Body weight gain (g bird^{-1})								
Day 1 to 90	283.00	206.30	225.00	210.00	29.00	0.130	0.840	0.312
Feed consumption (g/bird/day)								
Day 1 to 90	96.20	93.50	97.30	96.20	0.55	0.196	0.191	0.320
Feed conversion efficiency (g g^{-1})								
Day 1 to 90	0.43	0.40	0.42	0.40	0.05	0.010	0.304	0.535
Egg weight (g)	56.40	56.20	55.90	56.60	0.56	0.380	0.541	0.440
Egg production (%)	73.80^{a}	66.20^{b}	73.30^{a}	67.80^{b}	0.87	0.028	0.313	0.028
Egg shell weight	12.90	11.80	12.70	12.10	0.28	0.070	0.133	0.420
Tibia ash	47.70	48.00	46.30	47.70	0.95	0.788	0.773	0.271

n: Number of cage replicates per treatment. ^{a,b}Means within a row not sharing a common superscript different significantly ($p<0.05$)

Table 3: Production performance of WL layers on diets with different concentrations of calcium with or without phytase

	Ca (g kg^{-1}) (n = 12)							
	34	18	34	18				
	Phytase (FTU kg^{-1}) (n = 12)					p-value		
Variables	0	0	300	300	Pooled SE	Ca	Phytase	Ca×phytase
Body weight gain (g bird^{-1})								
Day 1 to 90	224.00	211.30	285.00	204.00	29.00	0.130	0.870	0.240
Feed consumption (g/bird/day)								
Day 1 to 90	97.60	94.20	95.90	95.40	0.55	0.196	0.244	0.317
Feed conversion efficiency (g g^{-1})								
Day 1 to 90	0.41	0.40	0.44	0.40	0.05	0.010	0.117	0.535
Egg weight (g)	56.30	56.10	56.00	56.60	0.56	0.380	0.541	0.440
Egg production (%)	71.70^{b}	66.20^{c}	75.30^{a}	67.70^{c}	0.87	0.028	0.313	0.028
Egg shell weight	13.20^{a}	11.70^{b}	12.30^{ab}	12.10^{ab}	0.28	0.070	0.133	0.042
Tibia ash	46.40^{b}	45.60^{b}	47.60^{a}	50.10^{a}	0.95	0.788	0.050	0.048

n: Number of cage replicates per treatment. $^{a-c}$Means within a row not sharing a common superscript different significantly ($p<0.05$)

Table 4: Production performance of WL layers on diets with different concentrations of non-phytate phosphorus with or without phytase

	NPP (g kg^{-1}) (n = 12)							
	3.2	2.2	3.2	2.2				
	Phytase (FTU kg^{-1}) (n = 12)					p-value		
Variables	0	0	300	300	Pooled SE	P	Phytase	P×phytase
Body weight gain (g bird^{-1})								
Day 1 to 90	193.00^{b}	241.00^{ab}	296.00^{a}	194.00^{b}	29.00	0.847	0.840	0.016
Feed consumption (g/bird/day)								
Day 1 to 90	93.40^{bc}	91.60^{c}	100.20^{a}	98.00^{ab}	0.55	0.191	0.019	0.001
Feed conversion efficiency (g g^{-1})								
Day 1 to 90	0.41	0.40	0.42	0.42	0.05	0.370	0.117	0.279
Egg weight (g)	56.10	56.40	56.50	56.10	0.56	0.380	0.390	0.414
Egg production (%)	67.60^{b}	70.30^{ab}	72.40^{a}	70.60^{ab}	0.87	0. 281	0.006	0.023
Egg shell weight	12.40	12.60	12.20	12.20	0.28	0.133	0.718	0.524
Tibia ash	46.90^{b}	45.10^{b}	48.80^{a}	49.00^{a}	0.95	0.788	0.773	0.027

n: Number of cage replicates per treatment. $^{a-c}$Means within a row not sharing a common superscript different significantly (p<0.05)

Table 5: Production performance of WL layers on diets with different concentrations of calcium and non-phytate phosphorus with or without phytase

	Teatments (n = 12)									
Production performance of layers	1	2	1	2	1	2	1	2	Pooled SE	p-value
Mineral levels (g kg^{-1})										
Ca	34.00	34.00	18.00	18.00	34.00	34.00	18.00	18.00	-	-
NPP	3.20	2.20	3.20	2.20	3.20	2.20	3.20	2.20	-	-
Phytase (FTU kg^{-1})	0.00	0.00	0.00	0.00	300.00	300.00	300.00	300.00	-	-
Variables										
Body weight gain (g bird^{-1})										
Day 1 to 90	222.50^{ab}	222.20^{ab}	163.50^{b}	159.00^{b}	343.50^{a}	228.00^{ab}	249.00^{ab}	260.00^{ab}	41.0	0.036
Feed consumption (g/bird/day)										
Day 1 to 90	94.50^{abc}	94.00^{abc}	92.70^{c}	88.80^{c}	97.80^{ab}	100.70^{a}	98.20^{ab}	99.70^{a}	0.77	0.027
Feed conversion efficiency (g g^{-1})										
Day 1 to 90	0.41^{ab}	0.41^{ab}	0.39^{a}	0.38^{a}	0.42^{ab}	0.43^{ab}	0.41^{ab}	0.45^{b}	0.07	0.027
Egg weight (g)	56.20	56.50	57.00	56.30	56.50	55.40	56.50	56.80	0.80	0.341
Egg production (%)	69.80^{cd}	73.00^{bc}	65.40^{e}	67.00^{de}	77.80^{a}	73.60^{b}.	67.10^{de}	68.30^{de}	1.23	0.025
Egg shell weight	13.30^{a}	13.00^{a}	11.40^{b}	12.10^{ab}	12.30^{ab}	12.40^{ab}	12.10^{ab}	12.10^{ab}	0.40	0.040
Tibia ash	47.40^{abc}	45.40^{bc}	46.50^{abc}	44.80^{c}	47.90^{abc}	47.30^{abc}	49.50^{ab}	50.70^{a}	1.35	0.015

n: Number of cage replicates per treatment. $^{a-c}$Means within a row not sharing a common superscript different significantly (p<0.05)

There was significant interaction (p<0.05) between P content and phytase supplementation and birds fed diet containing low NPP (2.2 g kg^{-1}) supplemented with phytase had higher egg production and tibia ash compared with those received the other diets.

There were significant interactive (p<0.05) effects of dietary treatments on BWG, feed consumption, feed conversion efficiency, egg production, egg shell weight and tibia ash except for egg weight and supplementation of diets containing low Ca and NPP with phytase increased BWG, feed intake, FCE, egg production, egg shell weight and tibia ash compared with the other diets. As can be seen from Table 5 egg production was higher in diet containing 34 (g kg^{-1}) Ca, 3.2 (g kg^{-1}) NPP supplemented with phytase which can be attributed to higher feed intake for this treatment (Table 5).

DISCUSSION

Reducing Ca content from 34 to 18 g kg^{-1} had no significant effect on body weight gain, feed intake, egg weight and tibia ash, suggesting that even with reduced Ca level, there was a sufficient level of this mineral to sustain growth. However, Ca reducing significantly decreased FCE, egg production and egg shell weight. These results are in agreement with those of Roland *et al.* (1985), who reported that egg specific gravity was increased in hens fed high Ca and low NPP, while Keshavarz (2000) reported increased egg specific gravity in low NPP diet. The National Research Council (1994) reports Ca requirements of 3.25% for hens eating 100 g day^{-1}, although industry norms are likely higher than this. Low levels of dietary Ca have been associated with increased feed consumption, reduced shell quality and reduced egg production (Roland *et al.*, 1985, 1996).

Reducing Ca levels and phytase supplementation significantly increased egg production and tibia ash. Present results are agree with those of Scott *et al.* (1999), who reported that supplementation of low Ca diet with phytase increased egg production.

In current experiment, reducing NPP and supplementing diets with phytase increased feed intake, egg production and tibia ash. However, it had no significant effect on BWG, FCE, egg weight and egg shell weight. Panda *et al*. (2005) also reported that reducing NPP and phytase supplementation increased feed consumption, egg production and tibia ash. Egg weight was not influenced by variation in the NPP content of the diets and addition of phytase to either the 2.2 or 3.2 g kg^{-1} NPP diet failed to increase the egg weight. Thus, the lowest NPP (2.2 g kg^{-1}) content was sufficient to support the normal egg weight, even in the absence of supplemental phytase. Similar findings have also been reported in the literature by Boling *et al*. (2000) and Keshavarz (2000). Tibia ash was significantly lower in the birds fed on the 2.2 g kg^{-1} NPP diet as compared to 3.2 NPP levels; however, addition of phytase to the 2.2 g kg^{-1} NPP diet alleviated the adverse effect on tibia ash.

The aim of current experiment was to examine the effect of reducing dietary mineral content (Ca/NPP) supplemented with phytase enzyme on aspects of performance, egg production, shell quality and tibia ash of laying hens. In the current experiment, reduced (Ca/NPP) diets containing low levels of Ca and P without phytase had lower BWG, egg production, FCE, egg shell weight and tibia ash compared with those supplemented with phytase enzyme. These finding suggest that reducing mineral content of diets without phytase supplementation impaired laying hen performance and addition of phytase enzyme overcame the adverse effect reduced mineral diets. Present finding are similar to the results of Lim *et al*. (2003), who reported that supplementation of phytase at a level of 300 U kg^{-1} diet of laying hens can improve egg production, decrease broken and soft egg production rate and P excretion. Keshavarz (2003) reported that supplementation diets containing NPP (0.25-0.20-0.15%) with phytase (300 FTU kg^{-1}) can secure performance of the commercial strains and also had the potential to reduce P excretion by over 50%. Gordon and Roland (1998) indicated that phytase supplementation (300 U kg^{-1} diet) completely overcame the adverse effects associated with low dietary P (0.1 NPP) and significantly reduced the impact of low dietary Ca (2.5, 2.8 and 3.1%) on laying hen performance.

Base on the data of this experiment reducing Ca and NPP without phytase decreased BWG, feed intake, FE, egg production, egg shell weight and tibia ash. However, phytase addition to low mineral diets completely corrected the adverse effects associated with low dietary Ca and NPP. It can therefore be concluded that reducing levels of Ca and NPP below current standards and phytase supplementation can reduce pollution potential from laying hen production without adversely affecting bird performance or welfare.

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