# Effect of dietary available phosphorus and phytase on production performance of old laying hens and tibia bone quality

**Mansoori, B.*, Modirsanei, M.**

[1]*Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

**Key words:**
available phosphorus, bone quality, laying hen, phytase

**Correspondence**
Mansoori, B.
Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98(21) 61117105
Fax: +98(21) 66933222
Email: bmansoori@ut.ac.ir

Received: 1 December 2014
Accepted: 16 March 2015

## Abstract:

**BACKGROUND:** Old laying hens are more prone to dietary calcium (Ca) and phosphorus (P) deficiencies as they absorb Ca and P less efficiently than younger hens. **OBJECTIVES:** In a 2×2 factorial design, the influence of diets with two levels of available phosphorus (AP) and phytase enzyme on the laying performance, egg quality and tibia bone characteristics of laying hens at their late 2nd production phase was studied. **METHODS:** The experiment used four treatments of 20 Hy-Line W36 hens of 140 weeks old. Treatments 1 and 2 received a 3.5 g $kg^{-1}$ AP diet while Treatments 3 and 4 received a 2.5 g $kg^{-1}$ AP diet for 12 weeks. Treatments 2 and 4 received 250 FTU $kg^{-1}$ phytase in their diet. Laying performance, egg weight, shell weight and shell thickness were measured. On the last day of the experiment, all birds were weighed and blood samples were collected for serum Ca and P measurements. Ten birds from each treatment were sacrificed and both tibiae were excised, defatted, weighed and ashed for Ca and P contents, cortical thickness and bone breaking strength measurements. **RESULTS:** Hens fed with 2.5 g $kg^{-1}$ AP diet without enzyme had the lowest body weight gain, serum P, tibia cortical thickness and tibia breaking strength among the treatments ($p<0.05$). Phytase enhanced body weight gain, egg shell thickness, serum P, tibia weight/body weight and tibia ash/body weight ratios, tibia cortical thickness and breaking strength, particularly in birds receiving 2.5 g $kg^{-1}$ AP diet ($p<0.05$). **CONCLUSIONS:** Phytase may improve bone quality and strength of hens in the late 2nd laying period.

## Introduction

Most cereal grains and oilseeds contain phosphorus (P) in the form of phytate serving as the storage form of P and representing 50 to 85% of the total P (Cheryan, 1980; Singh, 2008). However, phytate P has generated nutritional as well as environmental concerns as this form of P is poorly digested by poultry (Jongbloed and Lenis, 1998; Selle et al., 2000; Adeola and Sands 2003). Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. It is reported that endogenous phytase activity is present to some extent in all sections of the digestive tract of the laying hen and consequently, in hens fed a wheat-corn-soybean diet, phytate P is partially digestible (Marounek et al., 2008; 2010). How-

ever, phytate degradation by endogenous phytase mostly occurs in the hindgut which is not utilized by the hen. Since endogenous phytase enzyme is low in the chicken gastro-intestinal tract, dietary usage of exogenous phytase with microbial origin has proven to be an effective method for enhancing the utilization of phytate P (Nys et al., 1999). Exogenous phytase enhances the digestion and absorption of phytate P by the bird and as a result, the usage of inorganic P is decreased and the amount of P excreted into the environment is reduced (Kies et al., 2001; Adeola and Sands 2003; Silversides and Hruby 2009; Junqueira et al., 2010).

Based on the cost of feed, market price of eggs and availability of replacement pullets when the flock reaches as low as 50% production, induced molting is a practical way to prolong the productive life of table laying hens and to improve the quality of produced eggs. Upon completion of the molting process, the reproductive system is rejuvenated, allowing the bird to enter into the next egg production cycle (Gordon et al., 2009). It is well documented that the addition of phytase to diets containing low levels of available P (AP) improves production parameters of laying hens such as egg production, egg mass, egg weight, egg specific gravity, eggshell quality and bone quality (Boling et al., 2000; Jalal and Scheideler, 2001; Roland et al., 2003; Keshavarz, 2000, Wu et al., 2006; Ahmadi and Rodehutscord, 2012). However, most of the documented works are on layer hens of up to 100 weeks of age. There is no documented information on the influence of phytase on the production performance and bone characteristics of older hens near the end of their second laying phase.

This study aims to evaluate the influence of adding phytase to diets containing 3.5 or 2.5 g $kg^{-1}$ AP, on the production performance of 140-152 week-old hens as well as the quality of produced eggs. Additionally, the influence of enzyme on tibia bone characteristics is also assessed.

## Materials and Methods

The experiment was carried out at the Poultry Station, Veterinary Research Institute, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. The experimental procedure was approved by the Animal Research Committee of the University of Tehran.

The experiment was designed to contain 4 treatments of 20 individually selected and identified Hy-Line W36 hens at 140 weeks old (close to the end of their second laying phase). The birds were randomly allocated in 80 raised floor wire cages (30×40×40 cm in dimension).

The average body weight of the birds was 1660±40 g and each bird within a treatment was considered as one replicate. Birds were selected at 138 weeks old according to uniformity and initial body weight and were subjected to a 14-day adaptation period.

From week 140, Treatments 1 and 2 received a corn-barley-soybean meal diet containing 40 g $kg^{-1}$ calcium (Ca) and 3.5 g $kg^{-1}$ AP. Treatments 3 and 4 received a similar diet but with 2.5 g $kg^{-1}$ AP. Treatments 2 and 4 received 250 FTU/kg of a commercially available phytase (myo-inositol-hexakisphosphate 3-phosphohydrolase, EC 3.1.3.8) in their diet as well. One FTU (phytase enzyme unit) is equivalent to the amount of enzyme that liberates a micromole of inorganic P per minute from 0.0051 mol/L sodium phytate at 37°C and at pH = 5.5 (Hall et al., 2003). Diets were formulated to contain 2700 kcal $kg^{-1}$ metabolisable energy, 148 g $kg^{-1}$ crude protein and supply other required nutrients recommended by the breeder company (Hy-Line International, West Des Moines, Iowa, 2007) using UFFDA feed formulation package (User-Friendly Feed Formulation, Done Again, Programmed by J. Hargrave, University of Georgia, 1992). The UFFDA feed formulation package was corrected for nutrient values of local varieties of corn and barley. Ingredients and calculated chemi-

cal composition of diets fed to laying hens are shown in Table 1. The birds had free access to feed and fresh water during the 12-week experimental period (140-152 weeks of age). All birds were weighed individually at the start and end of the experiment and weight gain was calculated in percent of original weight. Egg production was recorded on a weekly basis by dividing the number of eggs laid per bird per week by the number of birds multiplied by seven, and the result was expressed in percent. Egg mass output (weight of egg/hen housed/day) was calculated by multiplying the mean egg weight by the production rate and dividing by 100. Feed conversion ratio was calculated from units of feed intake per unit of egg mass produced. Broken and shell-less eggs were recorded on daily basis and evaluated as the total number of broken or defective eggs divided by the number of produced eggs, and the result expressed in percent. Egg weight, shell weight and shell thickness were measured for 7 days on weeks 4, 8 and 12 of the experiment. Each egg was weighed, broken egg shells were left to dry at room temperature for 3 days and weighed by using a balance close to 0.01g. Shell thickness was recorded as the mean of three measurements between two ends of the egg using a tripod micrometer. On the last day of the experiment, 10 birds from each treatment were randomly selected and blood samples (2 ml) were obtained from the ulnar vein. Blood tubes were centrifuged at 1200×g for 5 minutes and serum was collected in 1.5 ml Eppendorf tubes and frozen (-20°C) until measurement of serum Ca and P concentration using commercial diagnostic kits according to AOAC (http://aoac.org/). The same birds were humanely sacrificed by cervical dislocation and both tibiae were excised. Tibia width and cortical thickness were measured at the middle of left tibia using a micrometer. The cortical thickness was recorded as relative to bone width in percentage. Then the same tibia was defatted in boiling potassium hydroxide solution (2% W/V distilled water) for 10 minutes, dried at 110 °C for 24 h and ashed at 540°C for 6 h. Tibia dry matter and tibia ash weights were determined and expressed relative to live body weight in percentage. Calcium and P contents of the ashed samples were measured according to AOAC (http://aoac.org/) using volumetric (Code 935.13) and spectrophotometric methods (Code: 964.06), respectively. The Ca and P contents of tibia were calculated and expressed as a percentage of tibia weight.

Bone-breaking strength analysis was conducted on the right tibia using an Instron Materials Tester (Model 4486, Instron Corp., Canton, MA) according to the method described by Jendrall et al. (2008). Briefly, each bone was placed on 2 support points measuring 4 cm apart. A shear plate, 80 mm in length and 1 mm wide attached to a 50-kg load cell with a crosshead speed of 20 mm/min was introduced to the midpoint of the same facial plane of each bone and the breaking strength was recorded in Newton.

**Statistical Analysis:** The experiment was a complete random design with a 2×2 factorial arrangement (ANOVA) (Minitab 13.2 statistical package, Minitab Inc. State College). Fisher's LSD procedure was used to obtain confidence intervals for all pair wise differences between means. General linear model was used to determine the main effects of factors (Type of Diet, Phytase, Type of Diet×Phytase) on the response variables by using the following equation:

$$y_{ijk}=\mu+a_i+b_j+a_ixb_j + e_{ijk}$$

where $y_{ijk}$= kth observation for a hen fed diet type i, enzyme supplementation j, μ is the parametric mean, $a_i$ is the diet type (2.5 and 3.5 g $kg^{-1}$ AP), $b_j$ the enzyme supplementation (0 and 250 FTU $kg^{-1}$ phytase), $a_ixb_j$, the interaction between diet type and enzyme supplementation, respectively, and $e_{ijk}$ the error term. Correlation coefficients of tibia bone variables as influenced by factors (dietary phosphorus level and phytase) were also determined. The

Table 1. Feed ingredients and calculated nutritional composition of experimental diets. (*) The vitamin and mineral premix supplied per kg of diet; Vit A 8800IU, Vit D3 2500IU. Vit E 11IU, Vit B1 1.5mg, Vit B2 4.0 mg, Vit B3 (Calcium Panthotenate) 8 mg, Vit B5 (Niacin). 35 mg, Vit B6 2.5 mg, Vit B12 0.01 mg, Biotin 0.15 mg, Folic Acid 0.48 mg, Cholin Chloride 400mg. Vit K3 2.2 mg, Manganese 75 mg, Iron 75 mg, Zinc 64.8 mg, Copper 6.0 mg, Iodine 0.87 mg, Selenium. 0.2 mg.

| **Feed Ingredients (g/ kg)** | **3.5 g kg$^{-1}$ AP Diet** | **2.5 g kg$^{-1}$ AP Diet** | **Nutritional Composition** | **3.5 g kg$^{-1}$ AP Diet** | **2.5 g kg$^{-1}$ AP Diet** |
|---|---|---|---|---|---|
| Corn | 460 | 460 | Metabolizable Energy (kcal kg$^{-1}$) | 2700 | 2700 |
| Barley | 172 | 172 | Crude Protein | 148 | 148 |
| Soya Meal (44%) | 218 | 218 | Crude Fat | 52 | 52 |
| Vegetable Oil | 25 | 25 | Crude Fiber | 40 | 40 |
| Oyster Shell | 55 | 55 | Total Calcium | 40 | 40 |
| Calcium Carbonate | 46 | 50 | Total Phosphorus | 6.5 | 5.5 |
| Di-Calcium Phosphate (18% P) | 14 | 8 | Available Phosphorus | 3.5 | 2.5 |
| Vit-Min Premix* | 5 | 5 | Total Sodium | 1.8 | 1.8 |
| DL-Methionine | 1.4 | 1.4 | Methionine | 3.8 | 3.8 |
| Salt | 3.6 | 3.6 | Lysine | 7.7 | 7.7 |
| Sand | 0 | 2 | Methionine+-Cystine | 6.4 | 6.4 |
| | | | Arginine | 9.4 | 9.4 |
| | | | Threonine | 5.6 | 5.6 |

Table 2. Effect of supplementary phytase on laying performance and egg quality of laying hens fed with diets containing two levels of available phosphorus. (abc) Means with different superscripts in each row are significantly different ($p<0.05$); NS, Non Significant; (*) $p<0.05$; (***) $p< 0.001$.

| | | | | | | **Statistical Significance** | | |
|---|---|---|---|---|---|---|---|---|
| | **3.5 g kg$^{-1}$ AP Diet** | **3.5 g kg$^{-1}$ AP Diet + Phytase** | **2.5 g kg$^{-1}$ AP Diet** | **2.5 g kg$^{-1}$ AP Diet + Phytase** | **SEM** | **Diet** | **Phytase** | **Diet*-Phytase** |
| Body Weight Gain (%) | 3.5$^{a}$ | 5.1$^{b}$ | 2.9$^{a}$ | 3.4$^{a}$ | 0.37 | * | * | NS |
| Feed Intake (g/d) | 106.5 | 105.8 | 101.1 | 103.8 | 1.82 | NS | NS | NS |
| Egg Production (%) | 65.5 | 66.8 | 65.2 | 64.6 | 0.91 | NS | NS | NS |
| Egg Weight (g) | 67.6 | 68.1 | 67.7 | 68.4 | 0.31 | NS | NS | NS |
| Egg Mass (g/h/d) | 44.3 | 45.5 | 44.1 | 44.2 | 0.28 | NS | NS | NS |
| Feed Conversion Ratio | 2.40 | 2.33 | 2.29 | 2.35 | 0.055 | NS | NS | NS |
| Broken Eggs (%) | 14.4$^{a}$ | 12.4$^{a}$ | 18.8$^{b}$ | 19.9$^{b}$ | 0.74 | *** | NS | NS |
| Shell-less Eggs (%) | 1.5 | 1.1 | 1.0 | 1.2 | 0.41 | NS | NS | NS |
| Shell Weight (g) | 5.88 | 6.03 | 6.08 | 5.83 | 0.09 | NS | NS | NS |
| Shell Thickness (µm) | 347$^{a}$ | 353$^{ab}$ | 346$^{a}$ | 357$^{b}$ | 3.5 | NS | * | NS |

level of probability less than 0.05 was considered as significant.

## Results

The effect of dietary AP level and phytase on laying performance of experimental hens and the quality of produced eggs are presented in Table 2. Treatments 3 and 4 had more broken eggs when compared with Treatments 1 and 2 ($p<0.001$). Dietary addition of phytase increased body weight gain in Treatment 2 ($p<0.05$). Dietary phytase also improved egg shell thickness in Treatment 4 ($p<0.05$). Neither dietary AP level nor supplementary phytase had an effect on other production pa-

Table 3. Effect of supplementary phytase on blood calcium and phosphorus as well as bone characteristics of laying hens fed with diets containing two levels of available phosphorus. [abc] Means with different superscripts in each row are significantly different (n=10, p<0.05); NS, Non Significant; [*] p<0.05; [**]p<0.01, [***] p< 0.001.

| | | | | | | Statistical Significance | | |
|---|---|---|---|---|---|---|---|---|
| | **g kg$^{-1}$ 3.5 AP Diet** | **3.5 g kg$^{-1}$ AP Diet + Phytase** | **2.5 g kg$^{-1}$ AP Diet** | **2.5 g kg$^{-1}$ AP Diet + Phytase** | **SEM** | **Diet** | **Phytase** | **Phytase** |
| Serum Ca (mg/dl) | 19.8 | 18.4 | 18.5 | 18.6 | 0.48 | NS | NS | NS |
| Serum P (mg/dl) | 8.2c | 7.8$^{c}$ | 5.9$^{a}$ | 6.7$^{b}$ | 0.33 | *** | * | * |
| Tibia Weight/Body weight (%) | 0.321$^{ab}$ | 0.343$^{a}$ | 0.312$^{b}$ | 0.335$^{a}$ | 0.008 | NS | * | NS |
| Tibia Ash/Body Weight (%) | 0.187$^{ab}$ | 0.199$^{a}$ | 0.177$^{b}$ | 0.196$^{a}$ | 0.005 | NS | * | NS |
| Tibia Ca/Tibia Weight (%) | 19.5 | 19.9 | 20.5 | 20.8 | 0.33 | NS | NS | NS |
| Tibia P/Tibia Weight (%) | 9.05 | 8.88 | 9.31 | 9.34 | 0.18 | NS | NS | NS |
| Cortical Thickness (%) | 14.2$^{ab}$ | 15.6$^{c}$ | 13.5$^{a}$ | 14.7$^{bc}$ | 0.43 | * | ** | * |
| Bone Breaking Strength (Newton) | 217$^{b}$ | 229$^{b}$ | 190$^{a}$ | 226$^{b}$ | 9.0 | * | * | * |

Table 4. Correlation coefficients of tibia bone variables as affected by dietary phosphorus level and phytase. Correlations are significant at [*] p<0.05; [**] p<0.01, [***] p< 0.001.

| | **Tibia Ash/Body Weight (%)** | **Cortical Thickness (%)** | **Bone Breaking Strength (Newton)** |
|---|---|---|---|
| Tibia Weight/Body weight (%) | 0.843*** | 0.365* | 0.441** |
| Tibia Ash/Body Weight (%) | | 0.419* | 0.390* |
| Cortical Thickness (%) | | | 0.554** |

rameters in the experimental birds (p>0.05). Tables 3 and 4 show the influence of dietary AP level and phytase on serum Ca and P as well as tibia bone variables and the correlations between the variables. Neither dietary AP level nor phytase affected serum Ca (p>0.05). Treatment 3 had the lowest concentration of serum P (p<0.001), the lowest weights of tibia (p<0.05) and tibia ash (p<0.05), the thinnest tibia cortex (p<0.01) as well as the weakest bone (p<0.05) among the experimental treatments. On the other hand, Treatment 4 showed a higher level of serum P compared to Treatment 3 (p<0.05). Dietary supplementation of phytase increased the relative weights of tibia and tibia ash to body weight (p<0.05) as well as cortical thickness (p<0.01) and bone breaking strength (p<0.05), particularly in birds that received the diet containing 2.5 g kg$^{-1}$ AP. An interaction was noted between dietary AP and phytase on serum P level, cortical thickness and bone breaking strength as phytase had more positive influence on birds that received diet with 2.5 gkg-1 AP. Table 4 shows a highly significant correlation (p<0.001) between tibia ash weight/body weight and tibia weight/body weight ($R^2$=0.843) and to a lesser extent (p<0.01), between bone breaking strength and tibia weight/body weight ($R^2$=0.441) as well as cortical thickness ($R^2$=0.554). Significant correlations (p<0.05) were also noted between cortical thickness and tibia weight/body weight ($R^2$=0.365) and tibia ash weight/ body weight ($R^2$=0.419) as well as bone breaking strength and tibia ash weight/ body weight ($R^2$=0.390).

## Discussion

The study presented here showed that the diet containing 2.5 g kg$^{-1}$ AP had no influence on productive performance of the birds when compared with the diet containing 3.5 g kg$^{-1}$AP, except for weight gain which supports previous reports indicating that diets containing

2.0 to 2.3 g $kg^{-1}$ AP are sufficient to maintain hen performance when dietary Ca is within the range of 32.5 to 40.0 g $kg^{-1}$ (Summers, 1995; Scott et al., 1999; Keshavarz, 2000; Hughes et al., 2008, Kozlowski and Jeroch, 2011). Hughes et al. (2008) showed that there was no significant difference in production traits of laying hens fed with diets either containing 3.5 or 2.5 g $kg^{-1}$ AP. Ahmadi and Rodehutscord (2012) had a meta-analysis approach on 12 previously published data in order to describe a relationship between dietary levels of AP and phytase in laying hens of up to 76 weeks of age. Analyses of the model which was only based on egg production, egg mass and FCR revealed that diets containing AP as low as 2.2 g $kg^{-1}$ without supplemental phytase showed high performance with regards to the above variables in layers. However, in the presence of phytase at levels up to 400 FTU/kg of feed, dietary AP levels may be reduced to 1.4 g $kg^{-1}$. Diets containing 1.5 g $kg^{-1}$ or lower AP without phytase are insufficient for maintaining egg production and bone quality (Boling et al., 2000; Keshavarz, 2000; Bar et al., 2002). Francesch et al. (2005) evaluated the efficacy of phytase on performance, egg quality, tibia ash content and P excretion in laying hens fed on either corn- or a barley-based diet low in AP (1.3 or 1.1 g $kg^{-1}$). Rate of lay, daily egg mass output, feed consumption, tibia ash percentage and weight gain were reduced in hens fed low AP diets. The adverse effects of low AP were more severe in hens on corn based diet than in those on barley based diet. Adding microbial phytase improved these parameters comparable with the hens fed AP adequate diets (3.2 g $kg^{-1}$). Addition of phytase increased total P absorption at the ileal level from 0.25 to 0.51 in the corn diet and from 0.34 to 0.58 in the barley diet.

Hughes et al. (2008) showed that when the amount of dietary AP was reduced to 1.5 g $kg^{-1}$, the birds had lower total hen housed egg production and body weight and higher incidence of soft-shelled, cracked and broken eggs. Addition of phytase to the diet improved these production characteristics. Additionally, phytase increased bone ash content of laying hens fed with low AP diets. Lei et al. (2011) evaluated the effects of total removal of dietary inorganic P and reduced energy and protein without and with phytase supplementation on the performance, egg quality and bone composition of 56-week old laying hens for 20 weeks. They showed that feed intake, egg production, body weight and tibia ash were decreased with P removal, but restored by phytase inclusion. The authors concluded that P removal from the diets reduced performance and tibia quality, but phytase addition improved performance and tibia integrity. It seems that phytase has more pronounced effect on older birds as enzyme supplementation at the level of 600 to1000 FTU $kg^{-1}$ of feed in the second and third four months of production as well as in the second production cycle improves laying performance and egg quality (Silva et al., 2008; Hassanien and Sanaa, 2011).

Worth mentioning is that all reported studies on dietary P requirements of laying hens and the influence of dietary phytase on production performance, egg quality and bone characteristics are carried out on hens of up to 100 weeks old, however this study presented here evaluated the influence of phytase on laying performance, egg quality and tibia bone characteristics of hens aged 140-152 weeks old, near the end of their second laying cycle.

Intestinal absorption of dietary Ca is most important in substantial daily deposition of shell Ca. However, older hens absorb Ca more slowly and less efficiently than younger hens (Bar et al., 2002). The lack of adequate intestinal Ca absorption to support egg shell formation forces bone resorption which is accompanied by increased P excretion (Clunies and Leeson, 1994; Bar et al., 2002). Although, molted hens have lower needs for AP in comparison with first cycle hens, they seem to be

more sensitive to low dietary P as the older hens may exhibit P deficiency symptoms sooner than younger hens (Pelicia et al., 2009). Besides, there are dietary interactions between Ca and P in high egg producing layers as significant performance depression and high mortality rates are seen when low P content is combined with high Ca in the diet (Hartel, 1990). Thus, dietary AP content as well as the age of the bird have significant influences on the quality and strength of skeletal bones in laying hens. Boling et al. (2000) reported that P deficiency signs in older hens (70 weeks) occurred within 3 weeks of consuming a diet with 1.0 g $kg^{-1}$ AP compared with 8 weeks in younger hens (20 weeks). The authors suggested that older hens may exhibit P deficiency symptoms sooner than younger hens.

In the study presented here, phytase had no influence on production parameters of laying hens offered diets containing either levels of AP. However, addition of phytase increased serum P concentration and improved the tibia bone characteristics including bone weight, bone ash weight, cortical thickness and bone strength in birds that received diet containing 2.5 g $kg^{-1}$ AP to a greater extent and in birds that received 3.5 g $kg^{-1}$ to a lesser extent. These results are in accordance with other reports indicating that those birds offered diets marginal or low in AP show higher responses to the addition of dietary phytase when compared to the birds fed on adequate AP diets supplemented with enzyme (Keshavarz, 2000; Liebert et al., 2005; Liu et al., 2007; Lei et al., 2011). High correlation coefficients seen in the tibia bone variables shown in Table 4 indicate that regardless of the AP content of the diets, phytase enzyme increases the bioavailability of organic P by releasing phosphate groups from dietary phytate and hence, enhances bone quality in experimental hens. An increase in serum concentration of P in laying hens by phytase has been shown by others as well (Das et al., 2004; Hassanien and Sanaa, 2011). Clark et al. (2009) measured the incidence of bone fracture in end-of-lay high-producing, noncommercial laying hens by using radiographic technique. They reported that the overall incidence of hens with at least 1 bone fracture was 6.6 and 15.7% in 47and 65-week-old hens, respectively.

It is shown that the tibia bone breaking strength is at maximum in hens fed 3.0 to 4.0 g $kg^{-1}$ AP (Sohail and Roland, 2002) and dietary AP at lower levels may affect bone quality of the birds (van der Klis et al., 1997; Panda et al., 2005). However, phytase increases bone quality of laying hens (Hughes et al., 2008; Junqueira et al., 2010; Lei et al., 2011). van der Klis et al. (1997) showed that tibia ash content of laying hens fed on phytase-supplemented diets was not different than hens fed with mono-calcium phosphate supplemented diets. However, both groups showed higher tibia ash contents than hens fed on a basal diet containing 1.3 g $kg^{-1}$ AP. Panda et al. (2005) evaluated the influence of four diets containing 1.2, 1.8, 2.4 and 3.0 g $kg^{-1}$ AP with the two lowest AP (1.2 and 1.8) added with phytase at 500 FTU per kg diet on tibia bone ash content of White Leghorn layers at 32 - 48 weeks of age. They documented that adding enzyme to the 1.8 g $kg^{-1}$ AP diet enhanced tibia ash content.

**Conclusions:** Findings of this study suggest that diets containing AP at 2.5 g $kg^{-1}$ may be sufficient to support the production performance of laying hens near the end of their $2^{nd}$ laying cycle. However, due to lower bone integrity of these hens when compared to hens receiving diets with higher AP content, these birds are more prone to skeletal disorders such as osteoporosis and broken bones. Osteoporosis causes bone breakage in hens which not only leads to a decrease in production performance but also has major consequences on the health of the bird particularly during the depopulation of the flock and it is an animal welfare concern. Additionally, broken bones have a negative impact on food safety due to the possible presence of

bone parts and splinters in the meat product of spent hen. The addition of phytase enzyme to diets with 2.5 g $kg^{-1}$ AP may benefit both hens and human by reducing the incidence of osteoporosis in old laying hens and minimizing the presence of bone parts in meat product.

## Acknowledgments

Authors wish to thank Miss Jila Honarzad and Mr. Ali Ahmadi for their invaluable help during the growth experiment and lab work.

## References

1. Adeola, O., Sands, J.S. (2003) Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J Anim Sci. 81: 78-85.
2. Ahmadi, H., Rodehutscord, M. (2012) A meta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. Poult Sci. 912: 2072–2078.
3. Bar, A., Razaphkovsky, V., Vax, E. (2002) Re-evaluation of calcium and phosphorus requirements in aged laying hens. Br Poult Sci. 43: 261–269
4. Boling, S.D., Douglas, M.W., Johnson, M.L., Wang, X., Parsons, C.M., Koelkebeck, K.W., Zimmerman, R.A. (2000) The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult Sci. 79: 224-230.
5. Ceylan, N., Scheideler, S.E., Stilborn, H.L. (2003) High available phosphorus corn and phytase in layer diets. Poult Sci. 82: 789-795.
6. Cheryan, M. (1980) Phytic acid interactions in food systems. CRC Crit. Rev Food Sci Nutr. 13: 297–335.
7. Clark, W.D., Cox, W.R., Silversides, F.G. (2008) Bone fracture incidence in end-of-lay high-producing, noncommercial laying hens identified using radiographs. Poult Sci. 87: 1964-1970.
8. Das, S.N., Kumar, V., Chatterjee, S., Singh, J., Agrawala, S.K. (2004) Effect of MagaCal and commercial phytase supplementation on blood and bone mineral content of layer hens. Phytomedica. 5: 41-45.
9. Francesch, M., Broz, J., Brufau, J. (2005) Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on corn- or barley-based diets. Br Poult Sci. 46: 340-348.
10. Gordon, R., Bryant, M.M., Roland, D.A. (2009) Performance and profitability of second-cycle laying hens as influenced by body weight and body weight reduction during molt. J Appl Poult Res. 18: 223-231.
11. Hall, L.E., Shirley R.B., Bakalli R.I., Aggrey S.E., Pesti G.M., Edwards H.M.Jr. (2003) Power of two methods for the estimation of bone ash of broilers. Poult Sci. 82: 414-418.
12. Hartel, H. (1990) Evaluation of the dietary interaction of calcium and phosphorus in the high producing laying hen. Br Poult Sci. 31: 473-494.
13. Hassanien, H.H.M., Sanaa, H.M.E. (2011) Comparison difference levels of phytase enzyme supplementation on laying hen performance, egg quality and some blood parameters. Asian J Poult Sci. 5: 77-85.
14. Hughes, A.L., Dahiya, J.P., Wyatt, C.L., Classen, H.L. (2008) The efficacy of quantum phytase in a forty-week production trial using white leghorn laying hens fed corn-soybean meal-based diets. Poult Sci. 87: 1156-1161.
15. Hughes, A.L., Dahiya, J.P., Wyatt, C.L., Classen, H.L. (2009) Effect of Quantum phytase on nutrient digestibility and bone ash in White Leghorn laying hens fed maize-soybean meal-based diets. Poult Sci. 88: 1191-1198.
16. Jendral M.J., Korver D.R., Church J.S., Feddes J.J.R. (2008) Bone mineral density and breaking strength of white leghorns housed in conventional, modified, and commercially available colony battery cages . Poult Sci. 87: 828-837.
17. Jongbloed, A.W., Lenis, N.P. (1998) Envi-

ronmental concerns about animal manure. J Anim Sci. 76: 2641-2648.
18. Junqueira, O.M., da Silva Filardi, R., Ligeiro, E.C., Casartelli, E.M., Sgavioli, S., Assuena, V., Duarte, K.F., de Laurentiz, A.C. (2010) Technical and economical evaluation of the nutritional matrix of phytase enzyme diets with sunflower meal for laying hens. Rev Bras Zootecn. 39: 2200-2206.
19. Keshavarz, K. (2000) Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poult Sci. 79: 748–763.
20. Kies, A.K., Van Hemert, K.H.F. and Sauer, W. C. (2001) Effect of phytase on protein and amino acid digestibility and energy utilization. World Poult Sci J. 57: 109-125.
21. Kozlowski, K., Jeroch, H. (2011) Efficacy of different levels of Escherichia coli phytase in hens fed maize-soyabean meal based diets with a decreased non-phytate phosphorus content. J Anim Feed Sci. 20: 224-235.
22. Lei, Q.B., Shi, L.X., Zhang, K.Y., Ding, X.M., Bai, S.P., Liu, Y.G. (2011) Effect of reduced energy, protein and entire substitution of inorganic phosphorus by phytase on performance and bone mineralization of laying hens. Br Poult Sci. 52: 202-213.
23. Liebert, F., Htoo, J.K., Sünder, A. (2005) Performance and nutrient utilization of laying hens fed low-phosphorus corn-soybean and wheat-soybean diets supplemented with microbial phytase. Poult Sci. 84: 1576-1583.
24. Liu, N., Liu, G.H., Li, F.D., Sands, J.S., Zhang, S., Zheng, A.J., Ru, Y.J. (2007) Efficacy of phytases on egg production and nutrient digestibility in layers fed reduced phosphorus diets. Poult Sci. 86: 2337-2342.
25. Marounek, M., Skrivan, M., Dlouhá, G., Brenová, N. (2008) Availability of phytate phosphorus and endogenous phytase activity in the digestive tract of laying hens 20 and 47 weeks old. Anim Feed Sci Technol. 146: 353-359.
26. Marounek, M., Skrivan, M., Rosero, O., Rop, O. (2010) Intestinal and total tract phytate digestibility and phytase activity in the digestive tract of hens fed a wheat-maize-soyabean diet. J Anim Feed Sci. 19: 430-439.
27. Nys, Y., FrnPPin, D., Pointillart, A. (1999) Occurrence of phytase in plants, animals and microorganisms. in Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp, Mount Olive, NJ, USA.
28. Panda, A.K., Rao, S.V.R., Raju, M.V.L.N., Bhanja, S.K. (2005) Effect of microbial phytase on production performance of White Leghorn layers fed on a diet low in non-phytate phosphorus. Br Poult Sci. 46: 464-469.
29. Pelicia, K., Garcia, E.A., Faitarone, A.B.G., Silva, A.P., Berto, D.A., Molino, A.B., Vercese, F. (2009) Calcium and available phosphorus levels for laying hens in second production cycle. Rev Bras Cienc Avic Braz J Poult Sci. 11: 39-49.
30. Scott, T.A., Kampen, R., Silvesides, F.G. (1999) The effect of phosphorus, phytase enzyme, and calcium on the performance of layers fed corn-based diets. Poult Sci. 78: 1742–1749.
31. Selle, P.H., Ravindran, V., Caldwell, R.V., Bryden, W.L. (2000) Phytate and phytase: Consequences for protein utilization. Nutr Res Rev. 13: 255-278.
32. Silva, J.H.V., Araujo, J.A., Goulart, C.C., Costa, F.G.P., Sakomura, N.K., Martins, T.D.D. (2008) Calcium:available phosphorus ratio and phytase levels for semi heavy laying hens in the first and second posture cycle. Rev Bras Zootecn. 37: 2166-2172.
33. Silversides, F.G., Hruby, M. (2009) Feed formulation using phytase in laying hen diets. J App Poult Res. 18: 15-22.
34. Singh, P.K. (2008) Significance of phytic acid and supplemental phytase in chicken nutrition: A review. World's Poult Sci J. 64: 553-580.
35. Sohail, S.S., Roland, D.A. (2002) Influence of dietary phosphorus on performance of Hy-Line W36 hens. Poult Sci. 81: 75-83.
36. Summers, J.D. (1995) Reduced dietary phosphorus levels for layers. Poult Sci. 74: 1977–1983.

37. van der Klis, J.D., Versteegh, H.A.J., Simons P.C.M., Keis, A.K. (1997) The efficacy of phytase in corn-soybean meal-based diets for laying hens. Poult Sci. 76: 1535–1542.
38. Wu. G., Liu, Z., M. Bryant, M., Roland, Sr. D.A. (2006) Comparison of natuphos and phyzyme as phytase sources for commercial layers fed corn-soy diet. Poult Sci. 85: 64–69.

مجله طب دامی ایران، ۱۳۹۴، دوره ۹، شماره ۲، ۱۳۴-۱۲۵

# تأثیر فسفر در دسترس و فیتاز جیره غذایی بر عملکرد تولیدی مرغان تخم‌گذار پیر و کیفیت استخوان درشت نی

**بهزاد منصوری* مهرداد مدیر صانعی**

**گروه بهداشت و تغذیه دام طیور، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران**

(دریافت مقاله: ۱۰ آذر ماه ۱۳۹۳، پذیرش نهایی: ۲۵ اسفند ماه ۱۳۹۳)

## چکیده

**زمینه مطالعه:** مرغان تخم‌گذار مسن نسبت به کمبود کلسیم و فسفر به‌علت کاهش قدرت جذبی روده و کاهش سرعت جذب کلسیم و فسفر نسبت به مرغان جوان حساس‌ترند. **هدف:** در طرح فاکتوریل ۲×۲، تأثیر جیره‌های با دو سطح فسفر در دسترس و به همراه آنزیم فیتاز بر عملکرد تخمگذاری، کیفیت تخم‌مرغ و خصوصیات درشت‌نی مرغان تخم‌گذار در اواخر دوره دوم تخمگذاری مطالعه شد. **روش‌کار:** این آزمایش دارای چهار تیمار بیست مرغ تخم‌گذار نژاد Hy-Line W۳۶ با سن ۱۴۰ هفته بود. تیمارهای ۱ و ۲ جیره حاوی ۳/۵ g/kg فسفر در دسترس و تیمارهای ۳ و ۴ جیره حاوی ۲/۵ g/kg فسفر در دسترس بود. تیمارهای ۲ و ۴ به همراه جیره خود آنزیم فیتاز به مقدار ۲۵۰ واحد آنزیمی به ازای هر کیلوگرم خوراک دریافت کردند. عملکرد تخم‌گذاری، وزن تخم‌مرغ، وزن و ضخامت پوسته اندازه‌گیری‌شده در آخرین روز آزمایش تمام پرندگان وزن شد. نمونه خون از ورید بالی جهت تعیین مقدار کلسیم و فسفر سرم تهیه شد. سپس، از هر تیمار ده پرنده کشته و استخوان درشت‌نی هر دو پا جدا، چربی‌گیری و وزن شد. به‌منظور اندازه‌گیری میزان خاکستر، کلسیم و فسفر، ضخامت کورتکس و میزان استحکام استخوان استفاده شد. **نتایج:** مرغانی که جیره حاوی ۲/۵ g/kg فسفر در دسترس دریافت کردند وزن‌گیری کمتر، فسفر سرمی پایین‌تر، ضخامت کورتکس درشت‌نی کمتر و استحکام کمتری در برابر شکست استخوان داشتند. در عین حال تخم‌مرغ شکسته بیشتری تولید کردند. فیتاز باعث بهبود وزن‌گیری، ضخامت پوسته تخم‌مرغ، افزایش فسفر سرم، وزن نسبی درشت‌نی و خاکستر درشت‌نی به وزن بدن، ضخامت کورتکس و استحکام استخوان در برابر شکست به‌خصوص در مرغانی که دارای جیره ۲/۵ g/kg فسفر در دسترس شد. **نتیجه‌گیری‌نهایی:** آنزیم فیتاز باعث ارتقای کیفیت و استحکام استخوان در مرغان تخم‌گذار در اواخر دوره دوم تولید شد.

**واژه‌های کلیدی:** فسفر در دسترس، کیفیت استخوان، مرغ تخمگذار، فیتاز

---

*) نویسنده مسؤول: تلفن: ۶۱۱۱۷۱۰۵ (۲۱)۹۸+ نمابر: ۶۶۹۳۳۲۲۲ (۲۱)۹۸+ Email: bmansoori@ut.ac.ir