

# Use of Microbial Phytase for Decrease of Pollutant Due to Environmental Poultry Excreta Phosphorus

A. MUSAPUOR, M. AFSHARMANESH AND H. MORADI SHAHRBABA<sup>1†</sup>

*Department of Animal Science, Faculty of Agriculture Shahid Bahonar University of Kerman, Kerman Iran*

*†Department of Animal Science, Faculty of Agriculture, University of Tehran, Karaj, Iran*

<sup>1</sup>Corresponding author's e-mail: [hmoradis@ut.ac.ir](mailto:hmoradis@ut.ac.ir)

## ABSTRACT

This experiment was conducted to study the effects of different levels of microbial phytase (0, 500 & 1000 FTU/ kg diet), calcium (2.275 & 3.25%) and available phosphorus (0.175 & 0.25%) on phytate phosphorus utilization in laying hens. One hundred ninety two 30-week aged White Leghorn (Hy-line W-36) laying hens were randomly allocated in cages for 12 dietary treatments with arranged of 3\*2\*2 factorial experiment with four replicates and four hens per replicate. The experimental period lasted 90 days, when the age of hen was 42 weeks. Dietary phytase caused a significant ( $P < 0.05$ ) improvement in feed intake, feed conversion ratio, tibia ash weight, tibia ash percentage, tibia phosphorus, plasma phosphorus and phosphorus digestibility. Available phosphorus levels had significant effect ( $P < 0.05$ ) on tibia ash weight and tibia ash percentage. Reduction dietary available phosphorus caused a significant ( $P < 0.05$ ) decrease in feed consumption. Effect of dietary calcium were significant ( $P < 0.05$ ), on tibia ash weight and feed consumption. Interaction between phytase and available phosphorus on tibia phosphorus were significant ( $P < 0.05$ ). Overall, it could be concluded that in low phosphorus diet where feed consumption is low, phytase would increase feed consumption as well as retention of phosphorus in tibia bone. Also, the lower excreta of phosphorus by using phytase could decrease environmental pollution.

**Key Words:** layer; Microbial phytase; Phosphorus; Pollution

## INTRODUCTION

Phosphorus from poultry litter has become an environmental issue and it is a limiting nutrient in algal growth, as feed phosphorus not retained by the poultry can ultimately enter and contaminate water supplies and stimulate its algal blooms that deplete dissolved oxygen in surface water (Sharpley *et al.*, 1993; Liu *et al.*, 1998). Mineral, plant and animal are the sources for preparing the phosphorus of laying hen diet. About two-thirds of the total phosphorus contained in feed ingredient of plant origin occurs as phytates and availability of phytate phosphorus is low in poultry (Sharpley *et al.*, 1993). Recently the most effective factor in environment contamination is especially contamination by phosphorus. Phytase is a phosphatase that is capable of catalyzing the release of phosphorus from phytate (Nelson, 1967). A number of studies have indicated that supplementing laying diets with microbial phytase results in improved performance (Van der Klis *et al.*, 1996), particularly when dietary levels of non phytate P (NPP) are low (Gordon & Roland, 1997). The results of a number of research studies with laying hens have shown that a diet with 0.1-0.13% available phosphorus (AP) in the presence of 100 to 300 units phytase can result in comparable performance to the control group which were fed a normal level of 0.4-0.45% available phosphorus (Ravindran *et al.*, 2000). Use of phytase enzyme effects on bio-availability of phytate phosphorus and reduces excreta phosphorus in

poultry. This factor is effective in reducing phosphorus pollutant in environment. Present study reports a laying trial in which hens were fed at two levels of P, three levels of phytase enzyme and two levels of Ca, in a factorial arrangement.

## MATERIALS AND METHODS

Twelve diets were fed to Hy-line W-36 hens from 30 to 42 week of age. The treatments consisted of a  $3 \times 2 \times 2$  factorial arrangement with three levels of Natuphos® phytase (0, 500 & 1000 FTU<sub>kg</sub><sup>-1</sup> diet), two levels of Ca (2.275 & 3.25%) and two levels of NPP (0.175 & 0.25%). Each treatment was randomly assigned to four replicate cages for a total of 48 cages. Each cage was an experimental unit and contained 4 hens. The experimental diets were formulated to meet National Research Council (1994) nutrient requirement of laying hens (Table I). Records of daily egg production and weekly feed consumption were kept during the experiment. At 42 weeks of age, four birds from each dietary treatment were killed and their left tibia was removed. They were solvent-extracted to remove fat and then dried and ashed. At 42 weeks of age chromic oxide was added to all diets as an analytical marker. Diets and excreta were analyzed for P (AOAC, 1995) and chromium (Fenton & Fenton, 1979) for determination of P digestibility. Analysis of variance was performed on the data using the General Linear Models of SAS® software (SAS Institute, 1995).

**Table I. Ingredients and nutrient composition of experimental diets**

Ingredient (%)	0.25%AP, 3.25% Ca	0.25%AP, 2.27%Ca	0.175%AP, 3.25%Ca	0.175%AP, 2.27%CA
Corn	65.86	72.27	66.30	72.48
Soybean meal	21.37	20.13	21.28	20.09
Fat	3.1	0.50	2.92	0.50
Oyster shell	7.95	5.39	8.18	5.62
Dicalciumphosphate	0.70	0.71	0.30	0.31
Vitamin premix <sup>1</sup>	0.30	0.30	0.30	0.30
Mineral premix <sup>2</sup>	0.30	0.30	0.30	0.30
Salt	0.35	0.34	0.35	0.34
DL-Methionine	0.07	0.06	0.07	0.06
<b>Nutrient Composition</b>				
ME, kcal/kg	2900	2905	2900	2911
Protein (%)	15	15	15	15
Calcium (%)	3.25	2.27	3.25	2.275
Nonphytate P (%)	0.25	0.25	0.175	0.175
Sodium (%)	0.15	0.15	0.15	0.15
Arg. (%)	0.921	0.906	0.92	0.906
Lys. (%)	0.746	0.729	0.745	0.728
TSAA (%)	0.58	0.58	0.58	0.58
Try. (%)	0.197	0.192	0.197	0.192

<sup>1</sup>Vitamin mix supplied the following per kilogram of diet: vitamin A, 10000 IU; vitamin D<sub>3</sub>, 500 IU; vitamin E, 10 IU; B1, 2.2 mg; B2, 4 mg; B3, 8 mg; B6, 2 mg; B9, 0.56 mg; B12, 15 mg; H2, 0.15 mg.

<sup>2</sup>Mineral mix supplied the following per kilogram of diet: Mn, 800 mg; Zn, 60 mg; Fe, 50 mg; Cu, 5 mg; Co, 0.1 mg; I, 1 mg; Se, 0.1 mg; Choline chloride, 200 mg.

## RESULTS AND DISCUSSION

Eggshell quality measurements were not consistently affected by the dietary treatments. Other researchers have reported mixed results of phytase supplementation on eggshell quality measurements (Gordon & Roland, 1998). Addition of 1000 FTU<sub>kg</sub><sup>-1</sup> phytase to the diet significantly increased feed intake and feed conversion of laying hens. There were no significant effects of enzyme supplementation on egg weight and egg production (Table II). The inclusion of phytase to the diets possibly increased feed intake by liberating the phytate phosphorus. These findings are in agreement with those of Keshavarz (2000). Supplementing diets with 1000 FTU<sub>kg</sub><sup>-1</sup> phytase resulted

in an increased bone ash weight and percentage and bone mineral content (P). At the 0.175% NPP level, hens with phytase had higher bone phosphorus percent than when diets were not supplemented with phytase. The improvement in bone ash and bone phosphorus content associated with phytase supplementation could be attributed to phytate P liberation. There was a highly significant positive influence of phytase supplementation on P digestibility at 42 weeks of age as expected. Other study (Um & Paik, 1999) found that supplementation of phytase increased P retention by increasing the liberation of bound phytate P.

Hens fed low Ca (2.275%) had significantly lower feed intake and excreta phosphorus percentage than hen fed with diets high in Ca. Although the extent of Ca withdrawal practiced in this study is not at this point recommended commercially, but hens consuming the 2.275% Ca diet performed as well as hen fed diets containing higher level of Ca. In current study, the use of a NPP regimen of 0.175%, which was used for the age periods of 30-42 weeks was adequate to support all the production traits, in spite of negative effect on feed intake and bone ash measurements at 0.175% NPP. Apparently, under condition of 0.175% AP diets, the P could be utilized for production and was not deposited in the bones. From our results it is concluded that supplemental phytase has beneficial effects on the performance of laying hen. It is recommended that laying hen diets be formulated to provide 0.175% NPP, 2.275% Ca, with supplemental phytase to hen early in the production cycle. Microbial phytase supplementation with low-Ca, low-P diet can decrease the level of phytate. P excretion in the manure and limit soil and water contamination.

## REFERENCES

- AOAC, 1995. *Animal feeds, Official Methods of Analysis*, P. 27. Association of Official Analytical Chemists, Washington, DC
- Fenton, T.W. and M. Fenton, 1979. Determination of chromic oxide in feed and faeces. *Canadian J. Animal Sci.*, 58: 631-4

**Table II. The effect of supplemental phytase, P and Ca, levels on digestibility P, production traits and bone ash measurements in layer hen**

Diet	Excreta ash (%)	Excreta phosphorus (%)	Phosphorus digestibility (%)	Egg weight (g)	Egg production (%)	Feed intake (g/h/d)	Feed conversion ratio (g:g)	Bone ash (%)	Bone phos. (%)
<b>Phytase(FTU/kg)</b>									
0	37.63	1.13a	34.59a	57.02	83.40	95.28c	2.01b	61.55b	7.87b
500	39.09	0.97b	42.88b	57.32	83.72	96.61b	2.02ab	62.35ab	8.82a
1000	37.69	0.94b	48.12c	57.37	83.51	99.46a	2.08a	62.54a	8.60a
<b>Calcium (%)</b>									
2.27	37.58	0.87b	42.24	57.20	82.61	94.88b	2.10	62.19	8.45
3.25	38.68	1.15a	41.49	57.27	82.48	99.35a	2.11	62.10	8.41
<b>Phosphorus (%)</b>									
0.175	37.14	1.05	40.36	57.09	83.40	96.32b	2.03	61.75b	8.45
0.25	39.13	0.97	43.04	57.39	83.69	97.91a	2.05	62.54a	8.41
Pooled SEM	-	0.043	-	4.32	16.18	2.08	0.015	3.23	1.71

<sup>abc</sup>means with in columns with no common superscript differ (p<0.05).

- Gordon, R.W. and D.A. Roland, 1997. Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. *Poultry Sci.*, 76: 1172–7
- Gordon, R.W. and D.A. Roland, 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. *Poultry Sci.*, 77: 290–4
- Keshavarz, K., 2000. Non-phytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poultry Sci.*, 78: 699–706
- Liu, B.L., A. Rafiq, Y.m. Tzeng and A. Rob, 1998. The induction and characterization of phytase and beyond. *Enzyme Microb. Technol.*, 22: 415–24
- National Research Council, 1994. *Nutrient Requirements of Poultry*, 9th revised ed. National Academy Press Washington, D.C., U.S.A
- Nelson, T.S., 1967. The utilization of phytate phosphorus by poultry-A review. *Poultry Sci.*, 46: 862–971
- Ravindran, V., S. Cabahug, P.H. Selle and W.L. Bryden, 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *British Poultry Sci.*, 41: 193–200
- SAS Institute, 1995. *SAS/STAT® User's Guide: Statistics*, 1996 Edition. SAS Institute, Inc., Cary, NC
- UM, J.S. and I.K. Paik, 1999. Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. *Poultry Sci.*, 78: 75–9
- Sharple Y., A.N.T. Ce. Daniel and D.R. Edwards, 1993. Phosphorus movement in the landscape. *J. Prod. Agric.*, 6: 492–500
- VAN der Klis, J.D., H.A.J. Versteegh and P.C.M. Simons, 1996. *Natuphos in laying hen nutrition*, Pages 71–82 in: BASF Technical Symposium Phosphorus and Calcium Management in layers, Atlanta, GA, January 23, 1996

(Received 18 June 2005; Accepted 03 December 2005)