

# Effects of microbial phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of available phosphorous

Shahab Ghazi Harsini<sup>1\*</sup>, M. Pourmostafa, R. Mahdavi<sup>1</sup> and M. Salimi<sup>2</sup>

1- Department of animal science, Razi university, Kermanshah, Iran

2- College of nutrition, S.B.Medical University, Tehran, Iran

\* Corresponding author: sghazi@razi.ac.ir

Abbreviated title: Housing system, storage and egg quality

## Summary

An experiment employing a factorial arrangement of four levels of available phosphorus (AP) (0.3125, 0.25 [recommended by the NRC], 0.1875, and 0.125%), and two levels (0 and 450 FTU/kg diet) of microbial phytase was carried out with Hy-Line W-36 layers from 37 to 46 wk of age. Reduction of AP from 0.25 to 0.125% (50% NRC) decreased feed intake, egg production, egg mass and increased feed conversion ratio, percentage of broken and soft-shell eggs ( $P < 0.05$ ) but phytase supplementation completely prevented these deficiencies. Feeding 0.1875% AP without phytase had no effect on performance and egg quality ( $P > 0.05$ ). Phytase supplementation and reduction of AP decreased excretion of phosphorus. Phosphorous excretion was lower in birds fed 0.125% AP diet with phytase but high in birds fed 0.3125% AP diet without enzyme ( $P < 0.05$ ). Supplementation of phytase, different levels of AP and their interactions had no significant influence on eggshell weight, eggshell thickness, eggshell calcium content, eggshell and tibia ash, Haugh unit, yolk index, egg shape index ( $P > 0.05$ ). Microbial phytase seems to be more efficient in diets with low levels of AP supplementation. However, the best results with phytase were achieved by those laying hens fed a lowest level of AP (0.25%).

Keywords: laying hen, available phosphorous, phytase, egg quality, phosphorous excretion

## **Introduction**

It has been well documented that majority of phosphorus in plant sources exist as phytate complex which decreased bioavailability of P, Zn, Fe, Ca and K. Phytate-P makes it relatively unavailable for poultry because they do not contain sufficient amounts of endogenous phytase necessary to hydrolyze the phytic acid complexes (Nelson., 1976). The poor digestive utilization of phytate-P by laying hen and its consequences on diet cost, environment and bioavailability of minerals and proteins have led to efforts directed toward improving phytate digestion (Dilger et al., 2004).

Previous studies with laying hens fed corn-soybean meal diets have showed that phytase supplementation improved performance (Lim et al., 2003), tibia ash, P Selle and Ravindran, 2007), protein digestibility and reduce P excretion (Selle and Ravindran, 2007). The objective of the present study was to determine effects of microbial phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed corn-wheat-soybean meal based diets with different Levels of available phosphorous.

## **Materials and methods**

Two hundred sixteen Hy-Line W36 laying hens were randomly assigned to one of eight dietary treatments in an experiment that was conducted from 37 to 46 wk of age. Each treatment was replicated three times; each replicate comprised three adjoining cages housing three birds per cage. Diets were fed in a factorial arrangement of four levels of NPP (0.125, 0.1875, 0.25 and 0.3125%) and two levels of microbial phytase (0 and 450 units (FTU) per kilogram). The experimental diets were formulated to meet National Research Council (1994) nutrient requirement of laying hens.

## **Samples collection and laboratory analysis**

Records of weekly feed intake and feed conversion ratio and daily egg production (%), egg mass, broken and soft-shell eggs and egg weight were kept during the experiment and were summarized every 5 wk. Specific gravity of eggs was determined by using salt solutions (1.062, 1.070, 1.082, 1.090 and 1.102 units). Shell thickness was a mean value of measurements at three places on the egg (air cell, equator, and sharp end) measured by using dial pipe gauge. Haugh

units were calculated with the HU formula, based on the height of albumen determined by a micrometer and egg weight (Eisen et al., 1962). The eggshell samples were washed and dried (15h at 65°C) and then were ground and analyzed for ash and Ca. Calcium was determined by Atomic absorption spectrophotometry.

All data were analyzed according to a completely randomized design with a 4×2 factorial arrangement of treatments. The General Linear Models procedure of SPSS13 was used to analyze the data. The treatment means were compared using Duncan's Multiple Range Test.

## **Results and Discussion**

### **Production performance**

Feed intake, feed conversion ratio, egg production, egg weight, Egg mass, Broken and soft-shell eggs production are shown in Table 2. The level of available phosphorous had significant effect on feed intake related to 0.125% of AP compared with other treatment ( $P < 0.01$ ). Decreasing the level of dietary AP (to 0.125%) has shown to decrease feed intake in laying hens. The negative effect of AP deficiency on feed intake has also been demonstrated by others (Lim et al, 2003; Panda et al, 2005). There was no significant effect of enzyme supplementation on feed intake ( $P > 0.05$ ). This finding agrees with the previous studies (Boling et al, 2000; Liebert et al, 2005). Contrary to the this study, Lim et al, (2003), and Panda et al, (2005), reported that feed consumption increased by phytase supplementation. These discrepancies may be attributed to breed, age of hens, level of dietary Ca, type and level of enzyme.

Feed conversion ratio was not affected by AP levels ( $P > 0.05$ ). The adverse effect of the lower AP level on feed conversion ratio was completely alleviated by phytase. This result is in agreement with other study (Van der Klis et al, 1997; Jalal and Scheideler, 2001). The information of this study indicates the AP requirements can be reduced considerably further in the presence of phytase in the diet without adverse effect on feed conversion ratio. Panda et al (2005), Francesch and Brufau (2005) reported did not observe any beneficial effect on feed conversion ratio from phytase supplementation. There was no effect of phytase on egg production ( $P > 0.05$ ), but the effect of AP level was significant ( $P < 0.05$ ). However, hens fed 0.125% AP without enzyme showed significantly lower egg production ( $P < 0.05$ ). Egg weight was not significantly influenced by AP levels or phytase supplementation ( $P > 0.05$ ). Egg weight

was not affected by phytase supplementation, although phytase numerically improved egg weight when dietary levels of AP were low. These results are similar to those reported by Francesch and Brufau (2005). A significant effect of dietary AP on egg mass was observed ( $P < 0.05$ ). As dietary AP increased from 0.125 to 0.3125% in the diets, egg mass significantly increased from 48.08 to 52.75, resulting in a 9.76% increase of egg mass. Egg mass wasn't significantly affected by phytase supplementation ( $P > 0.05$ ). In the absence of phytase, broken and soft-shell egg was significantly higher for the group that was receiving the least (0.125%) available phosphorous ( $P < 0.05$ ). Broken and soft-shell egg was significantly ( $P < 0.01$ ) reduced with phytase supplementation only for the group fed the lowest AP regimen (0.125%). Decreasing the levels of AP to 50% of the NRC recommendation (0.125%) resulted in broken and soft-shell egg production higher than other dietary treatments and improved by phytase supplementation. This finding could be the result of phytase on the release of phytate-bound minerals (P and Ca) and increase their eggshell retention that is increased eggshell quality. Jalal and Scheideler (2001) and Lim et al. (2003) showed that the addition of phytase to low AP regimens decreased broken and soft-shell egg production.

## **Egg quality**

Specific gravity, Haugh unit, eggshell thickness and weight were not significantly influenced ( $P > 0.05$ ) by dietary treatments (Table 3). Jalal and Scheideler (2001), Panda et al. (2005) and Francesch and Brufau (2005) reported that specific gravity wasn't influenced by AP levels and phytase supplementation. Similarly, other investigators (Um and Paik 1999; and Panda et al, 2005) have reported that Haugh unit was not affected by using phytase.

## **Tibia and Excreta**

Tibia ash percentage was affected by dietary AP level (Table 3). The main effect of AP levels for tibia P was significant ( $P < 0.01$ ). Tibia P was greater with highest AP level (0.3125%), whereas tibia ash of birds fed the higher AP regimens (0.3125%) was lower than the other treatments ( $P < 0.01$ ). The excreta P was affected by AP level ( $P < 0.01$ ) (Table 3). However, P excretion was not affected by phytase supplementation ( $P > 0.05$ ). As dietary AP increased from 0.125 to 0.3125% in the diets, excreta P significantly ( $P < 0.01$ ) increased from 0.75 to 1. Tibia ash was significantly increased due to the use of 0.25% AP but was decreased when the diet contained 0.3125% AP without microbial phytase. This finding may be due to adverse effects of additional P on tibia ash. Liebert et al. (2005) observed no phytase effect on

tibia ash. Gordon and Roland (1997) showed that the tibia ash could be increased by means of dietary supplementation with phytase.

Tibia P was significantly higher for hens consuming 0.3125% AP. No effect of adding phytase and no interaction between AP and phytase were observed. The higher AP intake as compared to the NRC (1994) suggested requirement may have resulted in a higher tibia P. This finding agrees with the previous studies (Musapuor et al, 2005).

The lowest and highest P excretion belonged to the groups fed 0.125% and 0.3125 AP respectively. Supplemented phytase decreased P excretion only numerically. As dietary AP reduced from 0.25 to 0.125% in the diets, excreta P significantly decreased from 0.99 to 0.75 resulting in a 24.24% reduce of P excretion. Therefore environmental pollution with P could be decreased by using diets containing lower levels AP with phytase. Several investigators reported a beneficial effect on P excretion from phytase supplementation (Van der Klis et al., 1997; Boling et al, 2000; Keshavarz 2000; Musapuor et al, 2005; and Panda et al, 2005; Francesch and Brufau, 2005).

Table3. Effect of dietary available phosphorus (AP) level and phytase on feed intake, feed conversion, egg production, egg weight, egg mass and broken and Soft-shell egg

Variable	% Available phosphorus (AP)				Phytase U/kg	
	0.125	0.1875	0.25	0.3125	0	450
Feed Intake (g/hen/day)	93.67 <sup>b</sup>	97.80 <sup>a</sup>	97.22 <sup>a</sup>	96.68 <sup>a</sup>	96.13	96.56
Feed conversion ratio	2.00	1.90	1.87	1.84	1.95	1.86
Egg production (%)	75.50 <sup>b</sup>	81.40 <sup>a</sup>	81.93 <sup>a</sup>	81.93 <sup>a</sup>	81.71	81.66
Egg weight (g)	63.64	63.45	63.76	64.42	63.75	63.89
Egg mass (g/day)	48.08 <sup>b</sup>	51.63 <sup>ab</sup>	52.24 <sup>a</sup>	52.77 <sup>a</sup>	50.18	52.17
Broken and Soft-shell eggs (%)	2.48 <sup>a</sup>	0.65 <sup>b</sup>	0.42 <sup>b</sup>	0.71 <sup>b</sup>	1.66 <sup>a</sup>	0.47 <sup>b</sup>

Table4. Effect of dietary available phosphorus (AP) level and phytase on egg quality tibia and excreta charectristics

Variable	% Available phosphorus (AP)				Phytase U/kg	
	0.125	0.1875	0.25	0.3125	0	450
Specific Gravity (unit)	1.085	1.086	1.085	1.086	1.085	1.085
Eggshell thickness (mm)	0.345	0.328	0.343	0.333	0.335	0.339
Eggshell weight (%)	10.31	10.52	10.53	9.98	10.26	10.41
Egg shell Ca (%)	32.72	31.98	32.02	31.82	32.53	31.74
Haugh unit	98.67	97.65	99.08	98.67	98.49	98.54
Shape index (%)	74.75	74.54	74.23	75.52	75.49	74.03
Tibia ash (%)	58.38 <sup>a</sup>	59.28 <sup>a</sup>	60.67 <sup>a</sup>	53.48 <sup>b</sup>	57.32	58.59
Tibia P (%)	13.98 <sup>b</sup>	13.88 <sup>b</sup>	13.82 <sup>b</sup>	14.11 <sup>a</sup>	13.95	13.94
Tibia Ca (%)	32.06	30.55	30.19	30.73	30.21	31.56
Excreta P (%)	0.75 <sup>b</sup>	0.91 <sup>a</sup>	0.99 <sup>a</sup>	1.04 <sup>a</sup>	0.95	0.90

## References

- Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. (2000) The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. *Poultry Science*. 79: 224-230
- Carlos, A.B., and H.M.Edwards. (1998) The effects of dihydroxycholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. *Poultry Science* 77: 850-858
- Dilger, R. N., Onyango, E. M., Sands, J.S.and Adeola, O. (2004) Evaluation of microbial phytase in broiler diets. *Poultry Science* 83: 962–970.
- Eisen, E. J., B. B. Bohren, and H. E. McKean, (1962) The Haugh unit as a measure of egg albumen quality. *Poultry Science*. 41: 1461–1468.
- Francesch, M. and Brufau, J. (2005) Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on maize-or barley-based diets. *British Poultry Science*. 46: 340-348.
- Gordon, R. and D. Roland. (1997) Performance of commercial laying laying hens fed various phosphorus levels, with and without supplemental phytase. *Poultry Science*. 76: 1172–1177.
- Jalal, M.A. and S.E. Scheideler. (2001) Effect of supplementation of two different sources of phytase on egg production parameters in laying hens. *Poultry Science*. 80: 1463-1471.
- Keshavarz, K. (2000). Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poultry Science*. 79: 748-763
- Lim, H. S., H. Namkung, and I. K. Paik. 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorus excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorus. *Poultry Science*. 82:92–99.
- Liebert, F. and A. Sunder. (2005) Performance and nutrient utilization of laying hen fed low- phosphorous diets supplemented with microbial phytase. *Poultry Science*. 84:1576-1583
- Musapuor, A., J. Pourreza, A. Samie and H. Moradi Shahrabak. 2005. The Effects of Phytase and Different Level of Dietary Calcium and Phosphorous on Phytate Phosphorus Utilization in Laying Hens. *International Journal of Poultry Science*. 48 : 560-562.
- Nelson, T. S., J. Wodzinski, and J. H. Ware. (1976). Effect of supplemental phytase on the utilisation of phytate phosphorus by chicks. *Journal of Nutrition* 101:1289-1294.
- NRC. (1994). Nutrient requirements of poultry. National Academy of Science. Washington, DC.
- Panda, A.K., Rama Rao, S. V., Raju, M. V.L.N., and Bhanja, S.K. (2005) Effect of microbial phytase on production performance of white leghorn layers fed on a diet low in non-phytate phosphorus. *British Poultry Science*. 46: 464-469.
- Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Animal Feed Science and Technology* 135: 1–41.
- 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. *Poult. Sci.*, 78: 75-79.
- Van der Klis, J. D., H.A.J. Versteegh, P.C.M. Simons, and A. K. Kies, 1997. The efficacy of phytase in corn-soybean meal based diets for laying hens. *Poultry Science*. 76:1535–1542.

Wu, G., Z. Liu, M. Bryant, R. A. Voitle, and D. A. Roland. 2006. Comparison of natuphos and phyzyme as phytase sources for commercial layers fed corn- soy diet. *Poultry Science*. 85:64-69.