aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 3 (5): 293-297, 2008
ISSN 1683-9919

The Effects of Combination of Citric Acid and Microbial Phytase on the Egg Quality Characteristics in Laying Hens

Y. Ebrahim Nezhad, N. Maheri Sis, H. Aghdam Shahryar, M.R. Dastouri,
A. Aghajanzadeh Golshani, A. Tahvildarzadeh and K.A. Najafyan
Department of Animal Science, Islamic Azad University,
Shabestar Branch, Shabestar, Iran

Abstract: In this study, the citric acid and microbial phytase combination effect on the egg quality characteristics in commercial laying hens of Hy-line (W-36) strain in 53-64 week of age is studied. One hundred and ninety two of laying hens were tested. The experimental design was completely randomized design with a 3×2 factorial arrangement with three levels (0, 2 and 4%) of citric acid and two levels (0.0 and 300 FTU kg^{-1}) of microbial phytase in low available phosphorus diets with 6 treatments, 4 replicates and 8 hens in each replicate. The measurements were specific gravity, eggshell thickness, Hough unit, eggshell weight and eggshell ash. The results showed that interaction of citric acid and phytase had significant ($p<0.05$) effect on specific gravity, eggshell thickness and eggshell weight. Adding of 4% citric acid to low available phosphorus diets decreased the Hough unit and eggshell ash in comparison with other groups, significantly ($p<0.05$). 300 unit kg^{-1} phytase addition in low available phosphorus diets, decreased the Hough unit, significantly ($p<0.05$). Also, phytase addition in low available phosphorus diets, was increased eggshell ash, significantly ($p<0.05$).

Key words: Citric acid, microbial phytase, laying hens, egg, available phosphorus

INTRODUCTION

Phosphorus is an essential and costly mineral material in poultry nutrition. Animal feed additives are rich of phosphorus and the availability of this mineral material in these foods supposed 100% approximately, while the availability of phosphorus in plant feed additives is ouly 30% (National Research Council, 1994).

The portion of phosphorus of the grain, grain by products and plant feed additives available phosphorus are in form of acid phytic or it's salt form (phytate) that restrict the phosphorus besides could combine with proteins and carbohydrates and decrease digestibility and bioavailability of them because of so many eclectic charge. There is low availability of phosphorus in phytate because of low activity of phytase enzyme in poultry digestive system. Thus the noticeable amount of phosphorus available in poultry foods excretes to environment. From previous studies deduced that organic acids may increase the utilities of phosphorus. Adding the mixture of citric acid and sodium citrate in (1:1 ratio) to rat diets with lack of calcinm and phosphorus inhibit from rickets diseases (Shohl, 1937).

Pileggi *et al*. (1956) study is confirmed the previous studies (Shohl, 1937) and showed that the best results of citrate is observed when the rat is feed with phytate containing diets and there is no useful response after adding of citrate to diets with lack of phytate. Boling *et al*. (2000a) reported that in lying hens that feed with corn-soybean meal the citric acid didn't affect utility of phosphorus.

Snow *et al*. (2004) showed that there are additive effects of citric acid, phytase, 1-α-hydroxycholecalciferol in response to tibia ash and phosphorus utility in broilers generally and combination of citric acid with phytase and 1-α-hydroxycholecalciferol with phytase could have much

Corresponding Author: Y. Ebrahim Nezhad, Department of Animal Science, Islamic Azad University, Shabestar Branch, Shabestar, Iran

interaction effect on phytate phosphorus utility. Therefore deduced that the citric acid may decrease the pH of digestive juices in small intestine and prevent to phytate insoluble salts complex which resist to interior phytase enzymes hydrolysis due to be ready of phytic acid to hydrolysis with interior and exterior phytase enzymes (Maenz *et al*., 1999; Applegate *et al*., 2003).

Boling *et al*. (2000b) hypothesized that citric acid complex with calcium and reduces the formation of more stable calcium-phytate complexes and ready the diet phytate to endogenous phytase. Thus the goal of this study is to detect the effects of combination of citric acid and microbial phytase on the egg quality characteristics in laying hens.

MATERIALS AND METHODS

One hundred and ninety two, 53 week old Hy-line (W-36) laying hens were examined in this study in age of we used 53 weeks to 64 weeks. Hens were distributed in completely randomized design included 6 treatments with 4 replications and 8 hens in each replicates. This experiment was carried out at December 2004. Microbial phytase, was the product of BASF company? (Natuphos® 500, BASF Crop., Mt. Olive, NJ) and including 10000 unit active phytase per gram. This product was informed of white granules which derived from *Aspergillus niger* and citric acid is a monohydrated granule. The citric acid used in this experiment was monohydrate 92%, which was added to the diets after calculating purity percentage. The ME of citric acid was assumed to be 2600 Kcal kg^{-1} (Boling *et al*., 2000b).

The 6 experimental diets were:

1) Control (C) with 0.1% available phosphorus (C), 2) C + 300 FTU kg^{-1} of microbial phytase, 3) C + 2% citric acid, 4) C + 2% citric acid + 300 FTU kg^{-1} of microbial phytase, 5) C + 4% citric acid 6) C + 4% citric acid + 300 FTU kg^{-1} of microbial phytase.

The diets had similar nutrient level except of phosphorus were regulated with National Research Council (1994) recommendation. The ingredients of diets are showed in Table 1. The used cages had

Table 1: Ingredients and nutrient composition (g kg^{-1}) of experimental diets during laying (53-64) week of age

	Treatments					
Ingredients	1	2	3	4	5	6
Corn	664.40	663.80	632.70	632.10	601.10	600.50
Soybean meal (44%)	211.20	211.30	211.70	217.30	223.40	223.50
Soybean oil	12.10	12.30	15.70	19.90	19.40	16.60
Calcium carbonate	81.20	81.20	81.20	81.20	81.20	81.20
Oyster shell	20.00	20.00	20.00	20.00	20.00	20.00
Salt	1.50	1.50	1.50	1.50	1.50	1.50
Sodium bicarbonate	3.60	3.60	3.60	3.60	3.60	3.60
Premix[a]	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine	1.00	1.00	1.00	1.00	1.00	1.00
Citric acid (92%)	-	-	21.90	21.90	43.80	43.80
Phytase[b]	-	0.30	-	0.30		0.30
Analysis (data on dry matter)						
Calculated						
ME (kcal kg^{-1})	2817.00	11.78	11.78	11.78	11.78	11.78
Crude protein (g kg^{-1})	150.00	150.00	150.00	150.00	150.00	150.00
Available P (g kg^{-1})	1.00	1.00	1.00	1.00	1.00	1.00
Total P (g kg^{-1})	3.20	3.20	3.20	3.20	3.20	3.20
Calcium (g kg^{-1})	38.00	38.00	38.00	38.00	38.00	38.00
Methionine+cystine (g kg^{-1})	6.00	6.00	6.00	6.00	6.00	6.00
Lysine (g kg^{-1})	7.40	7.40	7.40	7.40	7.40	7.40

[a]: Vitamin and mineral mix supplied kg^{-1} diet: vitamin A, 9000 IU; vitamin D_3, 2000 IU; vitamin E, 18 IU; vitamin K_3, 2 mg; Vitamin B_1, 1.8 mg; Vitamin B_2, 6.6 mg; Vitamin B_6, 4 mg; vitamin B_{12}, 0.015 mg; Nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 0.1 mg; choline chloride, 250 mg; ethoxyquin, 0.125; Mn, 100 mg; Zn, 10 mg; cu, 100 mg; Se, 0.22 mg; I, 1 mg; Fe, 50 mg

[b]: Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 300 FTU microbial phytase per kilogram of diet

50 cm length, 50 cm width and 50 cm height. Four hens were kept in each cage and every 2 cage were assumed as experimental unit. The experiment was done is 6 period, each 15 days sequential period. Average temperature in all 6 periods was constant (19°C). In order to evaluate the condition of flock, first data collecting supplied in 1 month before starting the experiment and it was found that there were no differences in performance of treatments before the experiment. The hens fed *ad libitum* and exposed to the 16 h light and 8 h darkness during a day. In order to adaptation to new diets they were fed during 1 week before the experiment.

In experimental period every 15 day the characteristics of egg shell such as specific gravity, Hough units, dry shell weight, thickness and weight of eggshell ash is measured.

For measuring egg specific gravity the produced eggs from each experimental unit in 2 days before the end of any period collected and then used floatation method in salty water solution. At first 16 salty water solution with concentration 1.05, 1.054, 1.058 1.062, 1.066, 1.70, 1.074, 1.078, 1.082, 1.086, 1.090, 1.094, 1.098, 1.102, 1.106, 1.100, 1.140 supplied. These solutions supplied with adding common salt to water and adjusted by Hydrometer (an instrument to determine liquids specific weights with desired level of differences). The eggshell thickness measured with eggshell meter (OGAWA SEIKI Co. Ltd., 3rd Ed., OSK, 13469), which have ability to measuring egg shell thickness up to 1.1 mm.

For estimation weight of eggshell at first extracted the egg contents then dried the eggshell in fresh air for 48 h and weighted and fit as percentage of egg weight. For eggshell ash measuring us put dried eggshell into oven for 12 h with 600°C heat and changed it to ash and estimated the percentage of it in eggshell.

Because of doing this research within 6 period of sampling we used, the period effect as an one factor inside the model when the analysis of data done and because of no significant effects of period the all data of 6 period pooled together and inside designed model with General Linear Models (GLM) procedures of SAS® (SAS, 1990) software was employed and significant differences between treatments were separated using Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

The results showed that interaction effect of citric acid and microbial phytase on egg specific gravity is significant statically ($p<0.001$). Comparison between means of citric acid and phytase interaction effects showed that adding citric acid to the diets with lack of available phosphorus, decreased egg specific gravity significantly ($p<0.05$). Reason of why adding citric acid to diets rich by phytase caused decrease of egg weight, is unknown. While adding citric acid to the low available phosphorus diets increased the egg weight in comparison with diets with no citric acid ($p<0.05$).

The interaction effect of citric acid and microbial phytase on thickness and eggshell weight is significant statistically ($p<0.01$, $p<0.001$), respectively. Comparison between mean of interaction effect showed that adding citric acid to low available phosphorus diets rich by phytase had no significant effect on thickness and weight but adding citric acid to diets rich with phytase increased the thickness and weight numerically with no statistically effect. It seems that adding citric acid to diets of hens had no effective output on microbial phytase act while adding citric acid to low available phosphorus diets increase thickness and eggshell weight significantly ($p<0.05$), But there is no significant effect between various levels of citric acid with respect to effect of citric acid on these trait (Table 2).

Notice that measuring the specific gravity and eggshell thickness are good index for calcium metabolism hence it seems that the increase of specific gravity, thickness and eggshell weight with addition of citric acid to diet is depend on chelate characteristics of calcium by citric acid (Erdman, 1979), thus decrease the banding of calcium to phytate molecule and insoluble phytate-

Table 2: The effect of citric acid and microbial phytase on the egg quality characteristics of laying hens (53-64) at whole period

Treatments		Specific	Eggshell thickness	Hough	Eggshell weight	Eggshell ash
Citric acid (%)	Phytase (U kg^{-1})[1]	gravity	(mm)	unit	(%)	(%)
0	0 (Control)	1.060^{d}	0.264^{b}	79.03^{ab}	9.62^{b}	57.65^{c}
0	300	1.070^{abc}	0.285^{a}	76.33^{bc}	10.63^{a}	71.73^{a}
2	0	1.072^{ab}	0.284^{a}	81.99^{a}	10.77^{a}	65.62^{b}
2	300	1.068^{bc}	0.284^{a}	73.11^{c}	10.57^{a}	66.19^{ab}
4	0	1.073^{a}	0.289^{a}	75.03^{bc}	10.76^{a}	62.67^{bc}
4	300	1.066^{c}	0.279^{a}	71.85^{c}	10.38^{a}	66.50^{ab}
SEM pooled		0.001	0.004	1.87	0.22	2.01
Main effects						
Citric acid	0	1.065^{b}	0.274^{b}	77.68^{a}	10.12^{b}	64.69
	2	1.070^{a}	0.284^{a}	77.55^{a}	10.67^{a}	65.90
	4	1.070^{a}	0.284^{a}	73.44^{b}	10.57^{a}	64.58
Phytase	0	1.068	0.279	78.65^{a}	10.38	64.65^{b}
	300	1.068	0.282	73.76^{b}	10.53	68.14^{a}
Probabilities						
Citric acid		0.0003	0.0094	0.0245	0.0001	0.1169
Phytase		0.8439	0.1991	0.0008	0.1388	0.0308
Citric acid×Phytase		0.0001	0.0003	0.1478	0.0001	0.1942

-: Means in columns with no common superscript differ significantly ($p<0.05$), [1]: Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 300 U microbial phytase per kilogram of diet

calcium complex formation therefore it is possible to more calcium absorption from intestine and precipitation as calcium bicarbonate on eggshell. Boling *et al*. (2000b) reported that adding various level of citric acid to the diets with 0.1% available phosphorus had no effect on egg specific gravity. This result had no agreement with others.

The main effect of citric acid and phytase on Hough unit is significant statistically ($p<0.05$, $p<0.001$) respectively, while there is no significant effect in interaction between them. The results showed that adding 4% citric acid to diet, decreased Hough unit in comparison with diets containing 2% of citric acid and diets with no citric acid, but there are no significant differences between 0 and 2% levels of citric acid statistically. Furthermore adding phytase to low available phosphorus diets decreased Hough unit. The reason of why high level of citric acid and addition of phytase to diets decreased Hough unit is unknown. By the way it seems that because of effect of egg height and egg weight on Hough unit, thus as amount as the egg weight increased Hough unit decreased and also in this study adding citric acid and microbial phytase increased egg weight and logical result could be ascribed this decrease to increase of egg weight.

UM and Paik (1999), Jalal and Scheideler (2001) and Lim *et al*. (2003) reported that phytase addition to laying hens diets had no effect on Hough unit. The results of eggshell ash data analysis showed that main effect of phytase on this trait is significant statistically ($p<0.05$), although the main effect of citric acid and interaction effect of citric acid and phytase is no significant statistically. Adding phytase to the low available phosphorus diets increased eggshell ash 5.3%. These represented that phytase affect increase of eggshell ash with causing phosphorus to be released from phytic acid and other minerals from phytine. This result is in agreement with Rama Rao *et al*. (1999) and UM and Paik (1999) findings.

CONCLUSION

From this study it could be deduced that:

- Addition 2% citric acid to low available phosphorus diets improves quality characteristics such as specific gravity, eggshell thickness, eggshell weight, eggshell ash and Hough unit.

- Addition citric acid to low available phosphorus diets rich with microbial phytase couldn't affect activity output of microbial phytase in laying hens at 53-64 week age.
- Addition 300 FTU kg^{-1} microbial phytase to diets containing 1% available phosphorus diets based on corn-soybean meal is enough protect of egg quality characteristics laying hens in 53-64 week age.

ACKNOWLEDGMENT

The authors gratefully acknowledge the excellent financial and technical assistance Islamic Azad University-Shabestar branch, of East Azerbaijan.

REFERENCES

Applegate, T.J., R. Angel and H.L. Classen, 2003. Effect of dietary calcium, 25-hydroxy cholecalciferol, or bind strain on small intestiual phytase activity in broiler chickens. Poult. Sci., 82: 1140-1148.

Boling, S.D., D.M. Webel, I. Marromichalis, C.M. Parsons and D.H. Baker, 2000a. The effects of citric acid on phytate phosphorus utilization in young chicks and pigs. J. Anim. Sci., 78: 682-689.

Boling, S.D., M.W. Douglas, J.L. Snow, C.M. Parsons and D.H. Baker, 2000b. Citric acid does not improve phosphorus utilization in laying hens fed a corn-soybean meal diet. Poult. Sci., 79: 1335-1337.

Duncan, D.B., 1955. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

Erdman, Jr., J.W., 1979. Oilseed phytates: Nutritioual implications. J. Anı. Oil Chem. Soc., 56: 736-741.

Jalal, M.A. and S.E. Scheideler, 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci., 80: 1463-1471.

Lim, H.S., H. Namkung and I.K. Paik, 2003. Effect of phytase supplementation on the performance, egg quality and phosphorus excretion of laying hens fed different levels of dietary calcium and non phytate phosphorus. Poult. Sci., 82: 92-99.

Maenz, D.D., C.M. Engele-Schann, R.W. Newkirk and H.L. Classen, 1999. The effect of minerals and mineral chelators on the formation of phytate-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol., 77: 177-192.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Edn. Natioual Academy Press Washington DC., USA.

Pileggi, V.J., H.F. Deluca and J.W. Steenbock, 1956. Citrate in the prevention of rickets in Rats. Arch. Biochem. Biophys., 60: 52-57.

Rama Rao, S.V., V.R. Reddy and V.R. Reddy, 1999. Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. Anim. Feed Sci. Technol., 79: 211-222.

SAS, 1990. SAS/STAT User's Guide: Statistics. Release 6.04 Edn. SAS Institute Inc., Cary, NC.

Shohl, A.T., 1937. The effect of the acid-base content of the diet upon the performance and cure of rickets with special reference to citrates. J. Nutr., 14: 69-83.

Snow, J.L., D.H. Baker and C.M. Parsons, 2004. Phytase, citric acid and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poult. Sci., 83: 1187-1192.

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.