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

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Abstract: This experiment was carried out in order to survey the combination effect of Ethylenediaminetetraacetic Acid (EDTA) and microbial phytase on the egg quality characteristics in commercial laying hens of Hy-line (W-36) strain in 53-64 week of age. In this experiment 192 laying hens were examined. The experimental design was completely randomized design with a 3×2 factorial arrangement with three levels (0.0, 0.1 and 0.2%) of ethylenediaminetetraacetic acid and 2 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 criteria used to assess were specific gravity, eggshell thickness, Hough unit, eggshell weight and eggshell ash. The results showed that interaction effect of ethylenediaminetetraacetic acid × phytase on specific gravity, Hough unit and eggshell weight was significant ($p<0.05$). Adding of ethylenediaminetetraacetic acid to low available phosphorus diets had not influence on the egg quality characteristics in laying hens. Phytase addition in low available phosphorus diets, increased eggshell weight and eggshell thickness significantly ($p<0.05$). Also, phytase addition in low available phosphorus diet did not improve eggshell ash.

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

INTRODUCTION

The environment contamination with phosphorus which is caused by animals, recently, has been an important issue. Monogastric animals consume diets based on oil seed meals and crops. These diets contain high amounts of phosphorus in phytase or phytic acid forms. Commonly, phytase, which has known activity in the intestine of poultry, isn't available (Nelson, 1976). Various feed additives are used in order to increase the use of phosphorus and decrease the excretion of phosphorus in poultry and swine. It is known that the phytase (Edwards, 1993; Biehl *et al*., 1995; Biehl and Baker, 1996; Gordon and Roland, 1997) vitamin D and it's products (Edwards, 1993, 2002; Biehl *et al*., 1995; Angel *et al*., 2001; Snow *et al*., 2004) and citric acid (Boling *et al*., 2000, 2001; Rafacz *et al*., 2003; Snow *et al*., 2004) can affectively use to develop the availabilities of phytate in non-ruminant animals.

There is little information to say that if organic acids (except of citric acid) can improve the availability of phytate phosphorus in poultry. The EDTA is an organic acid which has similar potential with citric acid, and it increases availably of same minerals. EDTA is a strong chelate and it improves the absorption rate of minerals of diets in poultry.

Previous studies indicated that, supplementing diets which contain plant protein with EDTA, improved absorption of (Zn^{++}) in turkey chicks (Kratzer *et al*., 1959) and chicks (O'Dell *et al*., 1964). Maenz *et al*. (1999) showed that EDTA increased the hydrolysation of phytate phosphorus from canola meal when associated with microbial phytase *in vitro* experiments. It seems that EDTA comparatively links to the calcium and decreases it's ligand to the phytate. Consequently it bounds the formation of insoluble calcium-phytate complexes and makes phytate of the diet sensitive to the endogenous and exogenous phytase.

The aim of this study was to evaluate the effect of EDTA and microbial phytase on the availability of phytate phosphorus and the effect of EDTA on the efficacy of microbial phytase in low available phosphorus diets. Also to see that if these supplements affect the qualitative traits of egg which fed soybean meal corn based diets.

MATERIALS AND METHODS

A total of 192, 53 week old Hy-Line (W-36) laying hens were examined in this study in age of we used 53 weeks to 64 week. Hens were distributed in completely randomized design included 6 treatments with 4 replications and 8 hens in each replicates. Microbial

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

	Treatment					
Ingredients	1	2	3	4	5	6
Corn	664.4	663.8	663.4	662.8	662.4	661.8
Soybean meal (44%)	211.2	211.3	211.4	211.5	221.6	221.7
Soybean oil	12.1	12.3	12.4	12.5	12.7	12.8
Calcium carbonate	81.2	81.2	81.2	81.2	81.2	81.2
Oyster shell	20	20	20	20	20	20
Salt	1.5	1.5	1.5	1.5	1.5	1.5
Sodium bicarbonate	3.6	3.6	3.1	3.1	2.6	2.6
Premix [a]	5	5	5	5	5	5
DL-Methionine	1	1	1	1	1	1
EDTA (99%)	-	-	0.1	0.1	0.2	0.2
Phytase [b]	-	0.3	-	0.3		0.3
Calculated analysis (data on dry matter)						
ME (Kcal kg^{-1})	2817	11.78	11.78	11.78	11.78	11.78
Crude protein (g kg^{-1})	150	150	150	150	150	150
Available P (g kg^{-1})	1	1	1	1	1	1
Total P (g kg^{-1})	3.2	3.2	3.2	3.2	3.2	3.2
Calcium (g kg^{-1})	38	38	38	38	38	38
Methionine+cystine (g kg^{-1})	6	6	6	6	6	6
Lysine (g kg^{-1})	7.4	7.4	7.4	7.4	7.4	7.4

a-Vitamin and mineral mix supplied/kg 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

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*. The ethylenediaminetetraacetic acid used in this experiment was dehydrating EDTA-2Na 99%, which was added to the diets after calculating purity percentage.

The 6 experimental diets were:

- Control (C) with 0.1% available phosphorus ©.
- C + 300 FTU kg^{-1} of microbial phytase.
- C + 0.1% EDTA.
- C + 0.1% EDTA + 300 FTU kg^{-1} of microbial phytase.
- C + 0.2% EDTA.
- C + 0.2% EDTA + 300 FTU kg^{-1} of microbial phytase.

The diets had similar nutrient level except of phosphorus were regulated with NRC (1994) recommendation. The ingredients of diets are showed in Table 1. The used cages had 50 cm length, 50 cm wide 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 Edn., 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 Institute, 1990) software was employed and significant differences between treatments were separated using Duncan's multiple range test.

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

	Treatment					
EDTA (%)	Phytase (FTU kg^{-1})	Specific gravity	Eggshell thickness (mm)	Hough unit	Eggshell weight (%)	Eggshell ash (%)
0	0 Control)	1.060^{c}	0.264	79.03^{a}	9.62^{b}	65.65
0	300	1.070^{a}	0.284	77.40^{ab}	10.63^{a}	71.73
0.1	0	1.065^{bc}	0.269	81.19^{a}	10.27^{ab}	67.95
0.1	300	1.068^{ab}	0.277	72.99^{bc}	10.31^{ab}	65.81
0.2	0	1.064^{bc}	0.263	79.41^{a}	10.08^{b}	69.07
0.2	300	1.068^{ab}	0.273	71.35^{c}	10.20^{b}	71.38
SEM Pooled		0.0013	0.0044	1.68	0.23	2.27
Main effects						
EDTA	0	1.065	0.274	78.21	10.12	68.69
	0.1	1.067	0.273	77.09	10.29	66.88
	0.2	1.066	0.268	75.38	10.14	70.22
Phytase	0	1.063^{b}	0.265^{b}	79.88^{a}	9.99^{b}	67.56
	300	1.069^{a}	0.278^{a}	73.91^{b}	10.38^{a}	69.64
Probabilities						
EDTA		0.3510	0.1529	0.1559	0.3293	0.2475
Phytase		0.0001	0.0001	0.0001	0.0002	0.2019
EDTA×Phytase		0.0055	0.1498	0.0417	0.0002	0.1224

-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

RESULTS AND DISCUSSION

In Table 2, the effects of EDTA and microbial phytase on the qualitative traits of egg in commercial layers are shown. Results showed that the interaction effect of EDTA and microbial phytase significantly affected egg weight ($p<0.01$). Adding different levels of EDTA to the low available phosphorous diets which is supplemented with phytase, but supplementing low available phosphorus diets with phytase increased the egg weight ($p<0.001$).

The egg shell thickness increased in comparison to the control group when 300 FTU per kg phytase was added to the low available phosphorus diets ($p<0.0001$). Otherwise, different levels of EDTA didn't have significant effect. It seems that phytase releases available phosphorus from phytic acid and other minerals from phytin. It improves the weight and egg shell thickness.

Ram Rao *et al.* (1999) and Um and Paik (1999) reported the same results. Lim *et al.* (2003) indicated that reduction in available phosphorus in diet (from 0.25-0.15%) in 31-40 week of age, didn't affect the egg shell thickness in layers. They also reported that supplementing low available phosphorus diets with phytase, didn't affect the egg shell thickness. It wasn't the same with other researches. It might be because of this fact in this study reduction rate of available phosphorus in diets was from 0.3-0.1% (instead of 0.25-0.15%).

Hough unit decreased when 300 FTU per kg phytase added to the low available phosphorus in comparison to the groups which didn't received any phytase. It is likely because Hough unit affected with the height of albumin and egg weight, the more the egg weight increases, the Hough unit decreases. In this study supplementing diets with microbial phytase increased the egg weight (the data doesn't published), it might be decreased the Hough unit. Same researchers reported no significant effect of adding phytase to the diets of layers on the Hough unit (Lim *et al.*, 2003; Snow *et al.*, 2004).

The eggshell weight significantly affected by EDTA×phytase interaction ($p<0.001$). Supplementing low available phosphorus diets with 0.2% of EDTA and phytase decrease the eggshell weight in comparison to the diets with no EDTA and supplemented with phytase ($p<0.05$). There was no significant difference between groups which were fed diets with phytase and different levels of EDTA. This indicates that supplementing diets which contain microbial phytase with EDTA doesn't increase the efficacy of microbial phytase.

CONCLUSION

From this study it could be deduced that:

- Supplementing low available phosphorus diets with different levels of EDTA have no effect on qualitative traits of egg (specific gravity, eggshell weight, eggshell thickness, egg shell ash and Hough unit).
- Addition EDTA to low available phosphorus diets rich with microbial phytase couldn't affect efficacy of microbial phytase in laying hens at 53-64 week age.
- Addition 300 FTU kg^{-1} microbial phytase to diets containing 0.1% available phosphorus diets based on corn-soybean meal improves the eggshell quality of laying hens in 53-64 week age.

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