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ebrahimnezhad@gmail.com

The effects of combination of ethylene diamine tetra acetic acid and microbial phytase on the concentration of some minerals of serum and parameters of mineralization of tibia in commercial laying hens

Yahya Ebrahim-Nezhad[1*], Ebrahim Jafari Helan[2], Abolfazl Aghajanzadeh Golshani[1], Ali Tahvildarzadeh[1]

1- Department of Animal Science, Islamic Azad University, Shabestar Branch, Shabestar, IRAN

2- Master Science of Animal Science, Tarbiate modares University, Tehran, IRAN

[*]Corresponding author: E-mail:ebrahimnezhad@gmail.com

Abstract:

This experiment was conducted to evaluate the combined effect of ethylene diamine tetra acetic acid (EDTA) and microbial phytase on the concentration of minerals of serum and parameters of mineralization of tibia in Hy-line commercial layers (W-36) in 53-64 weeks of age. 192 of laying hens were tested. The experimental design was completely randomized design with a 3×2 factorial arrangement with three levels (0, 0.1 and 0.2 %) of EDTA and two levels (0.0 and 300 FTU/kg) of microbial phytase in low available phosphorus diets with 6 treatments, 4 replicates and 8 hens in each replicate. The concentration of zinc, cupper and manganese of serum and ash, calcium and phosphorus of tibia was evaluated. The results showed that adding of EDTA into low available phosphorus diets significantly affected the concentration of serum zinc and tibia ash calcium ($p<0.05$). Interaction between EDTA and microbial phytase significantly affected the concentration of copper and manganese in serum and tibia ash percentage ($p<0.01$). Using 300 FTU/kg microbial phytase into low available phosphorus diets increased tibia ash phosphorus percentage ($p<0.01$).

***Key words*:** Ethylene diamine tetra acetic acid, microbial phytase, laying hens, zinc, copper, manganese.

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; Biehl et al., 1995; Angel et al., 2001; Edwards, 2002; Snow et al., 2004) and citric acid (Boling et al., 2000; Boling-Frankenbach et al., 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 hydrolyzation 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 is to evaluate the effects of combination of EDTA and microbial phytase on the concentration of some of minerals of serum and ash, calcium and phosphorus of tibia in commercial laying hens and effect of EDTA on microbial phytase efficacy in low available phosphorus corn-soybean meal based diets.

Material and methods

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. The experimental design was completely randomized design with a 3×2 factorial arrangement with three levels (0, 0.1 and 0.2 %) of EDTA and two levels (0.0 and 300 FTU/kg) of microbial phytase in low available phosphorus diets with 6 treatments, 4 replicates and 8 hens in each replicate. 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*. The ethylene diamine tetra acetic 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:

1) Control (C) with 0.1% available phosphorus 2) C + 300 FTU/kg of microbial phytase 3) C + 0.1% EDTA 4) C + 0.1% EDTA + 300 FTU/kg of microbial phytase 5) C + 0.2% EDTA 6) C + 0.2% EDTA + 300 FTU/kg 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 50 cm length, 50 cm wide and 50 cm height. 4 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 $^{\circ}$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 hours light and 8 hours darkness during a day. In order to adaptation to new diets they were fed during 1 week before the experiment.

At the end of experiment, two birds were selected from each replication and five milliliter blood was taken from wing puncture. Blood samples centrifuged for 15 minutes (3000 rpm/min) and serum was separated.

The concentration of zinc, copper and manganese measured by using ICP (Inductively Coupled Plasma Emission Spectrometer, Model JY-24, Jobin Yvon, Longjumeau,Cedex,France). Then, hens killed by cervical dislocation and left tibia

ebrahimnezhad@gmail.com

was taken. After collecting fat from soft tissue of tibia (Soxhlet method; AOAC, 1995), it was kept in alcohol for about 15 minutes and dried in 100° C and weighted. Dried tibia was burned in muffle furnace for eight hours and in 550° C. Tibia ash was calculated as percentage of dry weight. Then the calcium and phosphorus content of samples were measured (AOAC, 1995).

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

Ingredients	Treatment					
	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%)	-	-	1	1	2	2
Phytase [b]	-	0.3	-	0.3	-	0.3
Calculated analysis (data on dry matter)						
ME (Kcal/kg)	2817	2817	2817	2817	2817	2817
Crude protein (g/kg)	150	150	150	150	150	150
Available P (g/kg)	1	1	1	1	1	1
Total P (g/kg)	3.2	3.2	3.2	3.2	3.2	3.2
Calcium (g/kg)	38	38	38	38	38	38
Methionine+cystine (g/kg)	6	6	6	6	6	6
Lysine (g/kg)	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.

The statistical model for the design is as follows:

$$y_{ijk} = m + a_i + b_j + (ab)ij + e_{ijk}$$

i=1, 2, 3 j=1, 2 k=1, 2, 3, 4

ebrahimnezhad@gmail.com

y_{ijk} = The observation from the *k*th replicate receiving the *i*th EDTA and the *j*th microbial phytase; m = the overall treatment mean responce; a_i =the mean effect of EDTA at level *i*; b_j = the mean effect of microbial phytase at level *j*; ab_{ij} = the mean effect of the interaction between EDTA at level *i* and microbial phytase at level *j* e_{ijk} = the random experimental error

General linear Models (GLM) procedures of SAS® (SAS 1990) software was employed and significant differences between treatments were separated using Duncan's multiple range test (Duncan's, 1955).

Results and Discussion

Results showed that addition of 0.2% EDTA to treatment contains low available phosphorus diets increased serum zinc with respect to control group ($p<0.05$), while there is no significant difference between different levels of EDTA and addition 0.1% EDTA to diet increased the serum zinc concentration with respect to control group ($p>0.05$). EDTA is strong chelators and appeared that it improved some of minerals absorption in poultry diet (Table 2). The previous investigation showed that addition of EDTA to turkey's poults (Kratzer et al., 1959) and chickens (O'Dell et al., 1964; Vohra and Kratzer, 1965) contains plant proteins, improved zinc absorption.

Interaction effect of EDTA and microbial phytase on copper and manganese concentration in serum with low available phosphorus diets is significant ($p<0.01$), and the trend of different levels of EDTA in different levels of phytase hadn't similar function. A comparison between means of treatment composition showed that addition of different levels of EDTA to not supplemented low available phosphorus diets with phytase, increased serum copper concentration ($p<0.05$), while addition different levels of EDTA to supplemented diets with phytase, decreased serum copper concentration with respect to low available phosphorus diets that only added phytase to that diets ($p<0.05$). There isn't any reason for reduction of serum copper concentration with adding microbial phytase to diet and EDTA, but it seems that because EDTA is an organic acid probably with decreasing the gastrointestinal tract pH, inhibited from suitable activity of microbial phytase and endogenous phosphatases. The comparison between mean of treatment composition showed that addition different levels of EDTA to low available phosphorus diets, increased serum manganese concentration with respect to control group ($p<0.05$).

Adding 300 FTU/kg microbial phytase to diet, increased serum manganese concentration with respect to control group ($p<0.05$) but adding 0.1 and 0.2% EDTA to the diets contains microbial phytase, didn't affect serum manganese concentration. The affinity a chelate with a metal ion, quantitatively described as stability coefficient (SC), and EDTA have higher SC with all of minerals and this is the reason of metal sweeper of EDTA and when EDTA is available in system could bind all cations even complete with most of chelators (Kratzer et al., 1959).

The results from this study aren't in agreement with Maenz et al. (1999). These researchers showed that addition of EDTA to the culture medium contain microbial phytase, increased efficacy of microbial phytase in hydrolyzation of phytate phosphorus in canola meal, while in our experiment the results showed that the efficacy of microbial phytase didn't affect by EDTA. The interaction between EDTA and microbial phytase on tibia ash in low available phosphorus diets is significantly

ebrahimnezhad@gmail.com

($p<0.01$). The comparison between mean of treatment showed that addition different levels of EDTA to low available phosphorus diets increased tibia ash with respect to control group, but there isn't any significant difference between different levels of EDTA on the trait.

Adding 300 FTU/kg microbial phytase to diet increased the tibia ash percentage up to 8.75% with regard to control group ($p<0.05$). Adding 0.2% EDTA to diets contain microbial phytase with respect to treatment containing 0.1% EDTA plus 300 FTU/kg microbial phytase and treatment contains 300 FTU/kg microbial phytase increased tibia ash numerically but not significant statistically. The effect of EDTA on laying hens ash tibia calcium in low available phosphorus diets is significant ($p<0.05$).

Addition 0.1% EDTA to the control diet and diets contains 0.2% EDTA on low available phosphorus diets, increased the tibia ash calcium percentage. The main effect of microbial phytase on laying hens tibia ash phosphorus percentage on low available phosphorus diets is significant ($p<0.05$). Adding 300 FTU/kg microbial phytase to the laying hens diets with lack of available phosphorus, increased tibia ash phosphorus up to 12.45% in comparison with diets with lack of microbial phytase supplement.

This represent that microbial phytase would increase utilization of phytate phosphorus with releasing of phosphorus from phytic acid molecule and increase of phosphorus availability for birds. These results confirmed the results of Sebastian et al. (1996), Rama Rao et al. (1999) and Brenes et al. (2003) in broiler chicks.

From this study it could be deduced that:

1) Supplementing low available phosphorus diets with different levels of EDTA, increases the concentration of serum zinc, copper and manganese and calcium bioavailability in laying hens at 53-64 week age.
2) Adding different levels of EDTA to the low available phosphorus diets which supplemented with microbial phytase, didn't improved the efficacy of microbial phytase in laying hens at 53-64 week age.
3) Adding 300 FTU/kg microbial phytase to the corn-soybean meal based diets with 0.1% phosphorus, increases the concentration of serum copper and manganese and phosphorus of tibia ash in laying hens at 53-64 week age.

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ebrahimnezhad@gmail.com

Table 2: The Effect of EDTA and microbial phytase on the concentration of zinc, copper and manganese of serum and ash, calcium and phosphorus of tibia in laying hens (53-64) at whole period

Treatment		Zinc ($\mu g\ L^{-1}$)	Copper ($\mu g\ L^{-1}$)	Manganese ($\mu g\ L^{-1}$)	Tibia ash (%)	Tibia ash Calcium (%)	Tibia ash phosphorus (%)
EDTA (%)	Phytase (FTU/kg)[1]						
0	0 (Control)	199.4	211.86 ^{e}	297.81 ^{b}	42.51 ^{c}	38.10	16.61
0	300	148.4	254.10 ^{a}	310.47 ^{a}	46.59 ^{ab}	38.75	20.20
0.1	0	221.8	240.25 ^{b}	309.65 ^{a}	47.04 ^{ab}	40.00	17.50
0.1	300	173.3	232.15 ^{cd}	309.34 ^{a}	44.75 ^{b}	39.75	18.55
0.2	0	230.0	233.48 ^{bc}	308.31 ^{a}	46.68 ^{ab}	37.00	17.15
0.2	300	237.0	224.77 ^{d}	308.12 ^{a}	47.14 ^{a}	39.00	19.80
SEM Pooled		20.42	2.54	1.78	0.72	0.63	0.89
Main effects							
EDTA	0	173.9 ^{b}	232.98 ^{ab}	304.14 ^{b}	44.55 ^{b}	38.42 ^{b}	18.40
	0.1	197.5 ^{ab}	236.20 ^{a}	309.49 ^{a}	45.89 ^{ab}	39.87 ^{a}	18.02
	0.2	233.5 ^{a}	229.12 ^{b}	308.22 ^{a}	46.91 ^{a}	38.00 ^{b}	18.47
Phytase	0	217.0	228.53 ^{b}	305.26 ^{b}	45.41	38.66	17.08 ^{a}
	300	186.2	237.01 ^{a}	309.31 ^{a}	46.16	39.16	19.51 ^{b}
					Probabilities		
EDTA		0.0293	0.0399	0.0201	0.0151	0.0210	0.8648
Phytase		0.0811	0.0007	0.0125	0.2184	0.1391	0.0039
EDTA × Phytase		0.2997	0.0001	0.0023	0.0014	0.2294	0.3813

-Means in columns with dissimilar superscript differ significantly ($P<0.05$).

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

ebrahimnezhad@gmail.com

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