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Effects of combination of ethylene diamine tetraacetic acid and microbial phytase on digestibility of calcium, phosphorous and mineralization parameters of tibia bone in broilers

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Abstract

This experiment was conducted to evaluate the combined effects of ethylene di amine tetra acetic acid (EDTA) and microbial phytase (MP) on digestibility of calcium and phosphorus and mineralization parameters of tibia bone in broilers chicks. This experiment was conducted using 360 Ross-308 male broiler chicks. In a completely randomized design with a 3×2 factorial arrangement (0, 0.1 and 0.2% EDTA and 0 and 500 FTU MP). Four replicate of 15 chicks per each were fed dietary treatments including (i) P-deficient basal diet [0.2% available phosphorus (aP)] (NC); (ii) NC + 500 FTU MP per kilogram of diet; (iii) NC + 0.1% EDTA per kilogram of diet; (iv) NC + 0.1% EDTA + 500 FTU MP per kilogram of diet; (v) NC + 0.2% EDTA per kilogram; and (vi) NC + 0.2% EDTA + 500 FTU MP per kilogram of diet. The content of calcium, phosphorus and length of tibia bone, digestibility of calcium and phosphorus was evaluated. The results showed that interaction effect of EDTA×MP on tibia calcium content to low available phosphorus diets was significant ($p<0.01$). Adding of EDTA to P-deficient diets, was increased tibia phosphorus content of broilers in compared with control group ($p<0.001$). Adding MP to P-deficient diets, based on corn-soybean meal, cause increased digestibility of phosphorus and also, length of tibia in broilers ($p<0.0001$). From this study it could be deduced that adding of MP to low available phosphorus diets can cause improvement of utilization of phytate phosphorus. Also adding EDTA as a chelator to diet can improve tibia mineralization parameters in broilers.

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Introduction

The environment contamination with phosphorus which is caused by animals, recently, has been an important issue. Mono gastric 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). Maens *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 was to evaluate of combination effect of ethylene di amine tetra acetic acid (EDTA) and microbial phytase on digestibility of calcium and phosphorus and mineralization parameters of tibia bone in broilers chicks and its effect on efficacy of microbial phytase in corn-soybean diets with low available phosphorus level.

Materials and methods

A total of 360 feather sexed male day old Ross 308 broiler chicks were used in this experiment. Chicks were weighed individually and randomly assigned to battery pens so that pens had equal initial weight and weight distribution. The chicks fed *Ad-libitum* and exposed to the 23 hours light and 1 hours darkness during a day. The experiment was carried out using a completely randomized design with a 3×2 factorial arrangement (0, 0.1 and 0.2% EDTA and 0 and 500 FTU MP). Four replicates of 15 chicks per each were fed dietary treatments including (i) P-deficient negative control diet [0.2% available phosphorus (aP)] (NC); (ii) NC + 500 FTU MP per kilogram of diet; (iii) NC + 0.1% EDTA per kilogram of diet; (iv) NC + 0.1% EDTA and 500 FTU MP per kilogram of diet; (v) NC + 0.2% EDTA per kilogram; and (vi) NC + 0.2% EDTA + 500 FTU MP per kilogram of diet. All diets meet or exceed NRC (1994) recommendation except for aP (Table 1). The same ingredients were used for formulation of diets during 0–21 and 21–49 days of age (diet composition for period of 21–49 days of age are not presented). The supplied MP (Natuphos_500; BASF, Mt. Live, Nj) had 1000 FTU active phytase per gram. The ethylene di amine tetra acetic acid used in this experiment was dehydrating EDTA-2Na 99%, which was added to the diets after calculating purity percentage.

At day 44, chrome oxide (Cr_2O_3) was added to all diets at 0.1% level as a detectable marker for specifying of calcium (Ca^{++}) and phosphorus (P). To determine of digestibility of calcium and phosphorus, special sacs fastened to back of two chickens that their weights were close to mean weight of cage and their feces were collected for three days. Samples of digested materials were freeze immediately after collection and then were dried in oven at 60 degree of centigrade. After drying of

samples of digested materials, they grinded and use 1 mm pore filter to homogenized them. After preparing phosphorus concentration by Photometric method (AOAC, 1995) and calcium concentration by Atomic absorption set (AOAC, 1995) for feeds and feces samples was measured in Animal Nutrition Laboratory of Islamic Azad University-Shabestar branch and was expressed as percent. Chrome (Cr) concentration in feeds and feces samples were measured by method explained by Fenton and Fenton (1979) and by using of spectrophotometer. Digestibility of minerals was calculated by following formula (Ravindran *et al.,* 2000):

$$\text{Digestibility of nutrients (\%)} = 100 - \left(100 \times \frac{\text{Chrome concentration in feed (\%)}}{\text{Chrome concentration in feces (\%)}} \times \frac{\text{Nutrient concentration in feces (\%)}}{\text{Nutrient concentration in feed (\%)}}\right)$$

At day 49, two chickens that their weights were close to mean weight of cage, killed by cervical dislocation and their left tibia were taken. After collecting, tibia length measured and then, fat from soft tissue of tibia (Soxhlet method), 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).

Data were statistically evaluated by the analysis of variance procedure of SAS software (SAS Institute, 1990), involving a factorial arrangement of main factor (EDTA and phytase levels) in a completely randomized design. Significant differences between mean values were separated by the GLM procedure of SAS software (SAS Institute, 1990). Statistical significance was considered ($p<0.05$).

Results and discussion

The effect of EDTA and MP on tibia calcium and phosphorus, calcium and phosphorus digestibility and also, tibia length are shown in Table 2. Results showed that a significant interaction between EDTA×MP was observed for tibia calcium in 49 days of age ($P<0.0022$). The addition of EDTA at the 0.2% level to the diets containing low available phosphorus did not complement by MP, increased tibia calcium to the levels of 0 and 0.1% but, no significant difference was observed between 0 and 0.1% levels, on the other hand, the addition of EDTA at the 0.1% level to the diets containing low available phosphorus complemented by MP, increased tibia calcium to the diets containing MP and the diet containing of EDTA at the 0.2% level and MP but, the influence was not significant. In this experiment, the addition of EDTA at the 0.1% level to the diets containing low available phosphorus complemented by MP, increased tibia calcium to the diets containing low available phosphorus did not complement by MP but, the influence was significant ($P<0.05$). The addition of EDTA at the 0.2% level to the diets containing low available phosphorus complemented by MP, decreased tibia calcium to the diet of EDTA at the 0.2% level but, the influence was significant ($P<0.05$). It seems, the addition of EDTA to the diets containing low available phosphorus, increased tibia calcium. However, the addition of EDTA at the 0.2% level to the diets containing low available phosphorus complemented by MP, decreased tibia calcium. This indicates that if the level of EDTA in diet increases, it may prevent the activity of MP. EDTA acted as chelator of calcium and caused the availability of calcium to increase. This is reflected in the calcium of tibia. It has been determined; chelaters and similar compounds caused the increasing of (Zn^{++}) available in broilers (Nielsen *et al.,* 1966) and turkey poults (Vohra and Kratzer., 1965). The main effect of EDTA on tibia phosphorus was indicated, the addition different levels of EDTA to the diets containing low available phosphorus, increased tibia phosphorus to the control group ($P<0.001$). It seems that, EDTA increases phytate phosphorus utilization in the diets containing low available phosphorus and so it was concluded that, chelating minerals by EDTA inhibits formation of phytate-minerals insoluble and soluble complexes and causes phytate hydrolysis by endogenous phosphatases.

Table 1. Composition and nutrient content of the diet during starter (0–21 days) period.

Ingredients (%)	Treatment					
	1	2	3	4	5	6
Corn	62.32	62.22	62.12	62.02	61.92	61.82
Soybean meal (44%)	33.72	33.74	33.76	33.78	33.80	33.82
Soybean oil	0.29	0.32	0.35	0.38	0.41	0.44
Oyster shell	2.26	2.26	2.26	2.26	2.26	2.26
Di calcium phosphate	0.3	0.3	0.3	0.3	0.3	0.3
Common salt	0.41	0.41	0.41	0.41	0.41	0.41
Premix [a]	0.5	0.5	0.5	0.5	0.5	0.5
DL-Met	0.2	0.2	0.2	0.2	0.2	0.2
EDTA (99%)	-	-	0.1	0.1	0.2	0.2
Phytase [b]	-	0.05	-	0.05	-	0.05
Nutrients (Calculated)						
ME (Kcal/kg)	2875	2875	2875	2875	2875	2875
CP (%)	20.25	20.25	20.25	20.25	20.25	20.25
Ava. P (%)	0.2	0.2	0.2	0.2	0.2	0.2
T. P (%)	0.45	0.45	0.45	0.45	0.45	0.45
Ca (%)	0.9	0.9	0.9	0.9	0.9	0.9
Met + Cys (%)	0.85	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.07	1.07	1.07	1.07	1.07	1.07

a- Supplied per kilogram of diet: vitamin A, 9000 IU; Cholecalciferol, 3000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; vitamin B12, 0.015 mg; thiamin, 1.8 mg; riboflavin, 6.6 mg; folicacid, 1 mg; biotin, 0.10; niacin, 35 mg; pyridoxine, 4 mg; choline chloride, 250 mg; ethoxyquine, 0.125. _Supplied per kilogram of diet: manganese sulphate, 100 mg; copper sulphate, 10 mg; selenium (sodium selenate), 0.2 mg; iodine (EEI), 1 mg; zinc sulfate, 100 mg; Fe, 50 mg.

b- Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 500 FTU microbial phytase per kilogram of diet.

Table 2. Effect of EDTA and MP on digestibility of calcium, phosphorus and mineralization parameters of tibia bone in broilers.

Treatment		Tibia calcium (%)	Tibia phosphorous (%)	Calcium Dig. (%)	Phosphorous Dig. (%)	Tibia length (Cm)
EDTA (%)	Phytase(FTU Kg^{-1})[1]					
0	0 Control)	35.5 [d]	13.2	44.7	20.2	9.7
0	500	39.1 [abc]	13.9	83.6	35.8	10.2
0.1	0	37.1 [cd]	16.2	54.8	22.1	9.8
0.1	500	39.6 [ab]	16.7	58.7	33.5	10.4

0.2	0	40.8 [a]	18.6	74.8	24.8	9.5
0.2	500	38.1 [bc]	17.9	67.4	35.9	9.8
SEM Pooled		0.7	0.9	9	3	0.2
Main effects						
EDTA	0	37.3 [b]	13.6 [b]	64.1	28	9.9
	0.1	38.4 [ab]	16.5 [a]	56.8	27.8	10.1
	0.2	39.5 [a]	18.3 [a]	71.1	30.2	9.6
Phytase	0	37.8	16	58.1	22.4 [b]	9.6 [b]
	500	39	16.2	69.9	35 [a]	10.1 [a]
				Probabilities		
EDTA		0.0375	0.0010	0.3134	0.6760	0.0782
Phytase		0.0750	0.8303	0.1334	0.0002	0.0093
EDTA × Phytase		0.0022	0.6875	0.0584	0.6874	0.7798

[1]Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 500 FTU microbial phytase per kilogram of diet.
-Means in columns with no common superscript differ significantly (P<0.05)

In this experiment, the increasing of hydrolysis of phytate phosphorus by EDTA caused the releasing of phosphorus from phytic acid; this is reflected in tibia phosphorus. This result agrees with the results of Maens *et al.* (1999), in terms *in vitro*. The researchers demonstrated that, adding calcium chloride at the culture, hydrolysis of phytate was violated. So that, at the absence of calcium, EDTA did not affect on hydrolysis of phytate phosphorus. But, at the absence of chelator, the addition of 5 mmol calcium chloride prevent the hydrolysis of plytate phosphorus and the addition of 5 mmol EDTA at culture, the effects of excess calcium to the preventing from the hydrolysis of pytate phosphorus was thwarted by MP. The same scientists showed the addition of 5 mmol EDTA to canola meal complemented by MP, the level of inorganic phosphorus increased from 74 to 96 nm per gram of canola meal at culture. Ebrahim-Nezhad *et al.* (2008) reported that, adding EDTA to low available phosphorus diets of the laying hens (53-64 wk), did not affect tibia phosphorus. It seems no effect of EDTA on tibia phosphorus, caused to high amount of diet calcium (3.8%) in the laying hens to the amount of diet calcium (0.9%) in the broilers.

The main effect of EDTA and MP, also their interaction was not significant on calcium available. The main effect of MP was significant on phosphorus available in diets containing low available phosphorus (P<0.0002). The addition of 500 units per kilogram of MP to the low available phosphorus diets, increased phosphorus available the amount of 36%. The increasing of phosphorus retention with MP to the diet by scientists (Qian *et al.,* 1996; Sebastian *et al.,* 1996; Ravindran *et al.,* 2000; Selle *et al.,* 2000 and Viveros *et al.,* 2002) demonstrated. Perney *et al.* (1993) and Ahmad *et al.* (2000) stated the excretion of phosphorus at the diets containing low available phosphorus with the addition MP reduced. It may due to increased availability phosphorus also, phosphorus is apart from the phytic acid complex, and at the same time, was released from molecule by MP. It seems, when phosphorus is limiting, to maintain physiological takes, more phosphorus remains in the body, as a result, less phosphorus is excreted through the feces.

The main effect of EDTA was not significant on length of tibia in fed broilers with diets containing low available phosphorus (P<0.0782). The main effect of MP was significant on length of tibia in fed

broilers with diets containing low available phosphorus (P<0.0093). In this experiment, the addition of MP to the diets containing low available phosphorus increased the length of tibia the amount of 5%. The release of phosphorous from phytic acid with the addition of MP to the diets containing low available phosphorus, caused to increase the length of tibia in broilers. As it turns out, phosphorus is a key component of bone, and therefore the releasing and increasing of available phosphorus of diet with the addition MP to the diets containing low available phosphorus caused to increase bone-building process and the increasing of bone length to the diet of without MP. From the results can be concluded that, adding different levels EDTA to the low available phosphorus diets based on corn-soybean meal, improved tibia calcium and phosphorus in broilers. Also the addition of 500 units of MP, to the diets containing low available phosphorus, diets based on corn-soybean meal, caused to release of phosphorous from phytic acid and improved mineralization parameters of bone and phosphorus digestibility in broilers. In other hand, adding different levels EDTA to the low available phosphorus diets which supplemented with MP, did not improve the efficacy of MP in broilers.

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