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Full Length Research Paper

Effects of combination of ethylenediaminetetraacetic acid and microbial phytase on the serum concentration and digestibility of some minerals in broiler chicks

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This experiment was conducted to evaluate the combined effects of ethylene diamine tetraacetic acid (EDTA) and microbial phytase (MP) on the serum concentration and digestibility of some minerals in broiler 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 replicates of 15 chicks per each were fed dietary treatments which included; P-deficient basal diet [0.2% available phosphorus (aP)] (NC), NC + 500 FTU MP per kilogram of diet, NC + 0.1% EDTA per kilogram of diet, NC + 0.1% EDTA + 500 FTU MP per kilogram of diet, NC + 0.2% EDTA per kilogram and NC + 0.2% EDTA + 500 FTU MP per kilogram of diet. The concentration of zinc, copper and manganese of serum and their digestibility and also digestibility of apparent metabolizable energy (AME_n) was evaluated. The results showed that phytase supplementation of P-deficient diets significantly increased zinc concentration of serum ($P < 0.05$). Interaction effect of EDTA+MP on serum concentration of copper and manganese and also digestibility of zinc was significant ($P < 0.05$). EDTA supplementation of P-deficient diets significantly increased manganese digestibility in broiler chicks ($P < 0.01$).

Key words: Ethylene diamine tetraacetic acid, microbial phytase, zinc, copper, manganese.

INTRODUCTION

The environmental 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 phytate orphytic acid forms. Commonly, phytase, which has known activity in the intestine of poultry, is not 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 its 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 be affectively use to develop the availabilities of phytate in non-ruminant animals.

There is little information to show if organic acids (except of citric acid) can improve the availability of phytate phosphorus in poultry. EDTA is an organic acid which has similar potential with citric acid and it increases availability of some 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^{2+} in turkey chicks (Kratzer et al., 1959) and chicks (O'Dell et al., 1964). Means 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 its ligand to the phytate. Consequently, it bounds the

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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
Dicalcium 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.

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 and also to determine if the supplements affect the qualitative traits of egg of chicks which were fed with corn- soybean meal based diets.

The research also involved the study of combination effect of ethylenediamine tetraacetic acid (EDTA) and microbial phytase on the serum concentration and digestibility of some minerals in broilers 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 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 which included; P-deficient negative control diet [0.2% available phosphorus (aP)] (NC), NC + 500 FTU MP per kilogram of diet, NC + 0.1% EDTA per kilogram of diet, NC + 0.1% EDTA and 500 FTU MP per kilogram of diet, NC + 0.2% EDTA per kilogram and NC + 0.2% EDTA + 500 FTU MP per kilogram of diet. All diets met or exceeded 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 ethylenediaminetetraacetic 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 zinc (Zn^{2+}), copper (Cu^{2+}), manganese (Mn^{2+}) and also apparent metabolizable energy. To determine digestibility of minerals, special sacs were fastened to the back of two chickens that their weights were close to mean weight of cage and their faeces were collected for three days. Samples of digested materials were frozen immediately after collection and then dried in an oven at 60°C. Dried samples of digested materials were grinded and 1 mm pore filter was used to filter. Also samples of foods were grinded and filtered in this leach. Gross energy of feed samples and feces samples were measured by automatic colorimeter (Automatic Colorimeter, AC-300 and Model 789-400) in nutrition laboratory of Tabriz University and then apparent metabolizable energy were calculated. Minerals concentration for feeds and feces samples was measured by atomic absorption in nutrition of Islamic Azad University-Shabestar branch and was expressed as percent. Chrome (Cr) concentration in feeds

Table 2: The Effect of EDTA and microbial phytase on the serum concentration and digestibility of some mineral in broiler chicks

Treatment		Zn (µg L^{-1})	Cu (µg L^{-1})	Mn (µg L^{-1})	Zn dig. (%)	Cu dig. (%)	Mn dig. (%)	AME_n (Kcal Kg^{-1})
EDTA (%)	Phytase (FTU Kg^{-1})[1]							
0	0 Control)	187.2	36.4 [b]	103.2 [d]	23.9 [c]	24.1	26.1 [c]	3104.7
0	500	373.4	134.3 [a]	109.1 [b]	33.9 [bc]	26.1	28.6 [c]	3045.7
0.1	0	217.9	107.0 [a]	107.3 [bc]	86.8 [a]	22.6	61.4 [ab]	3117.7
0.1	500	292.2	99.3 [a]	107.0 [bc]	44.7 [b]	19.0	50.7 [b]	3173.4
0.2	0	247.0	134.9 [a]	106.6 [c]	79.8 [a]	22.0	75.3 [a]	3172.5
0.2	500	254.3	48.6 [b]	139.1 [a]	73.0 [a]	25.4	53.0 [b]	3075.6
SEM Pooled		40.3	12.7	0.7	5.6	1.7	4.8	111.8
Main effects								
EDTA	0	280.3	85.3 [b]	106.2 [b]	28.9 [b]	25.1	27.4 [b]	3075.2
	0.1	255.1	103.1[a]	107.2 [b]	65.7 [a]	20.9	56.1 [a]	3124.1
	0.2	250.7	91.7 [ab]	122.9 [a]	76.4 [a]	23.7	64.1 [a]	3145.5
Phytase	0	217.4 [b]	92.7	105.7 [b]	63.5 [a]	22.9	54.3 [a]	3131.6
	500	306.7 [a]	94.0	118.4 [a]	50.6 [b]	23.5	44.1 [b]	3098.2
Probabilities								
EDTA		0.7352	0.0249	0.0001	0.0001	0.0861	0.0001	0.8153
Phytase		0.0188	0.4702	0.0001	0.0153	0.6795	0.0225	0.7209
EDTA × Phytase		0.1222	0.0001	0.0001	0.0018	0.1568	0.0675	0.7810

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

and feces samples was measured by method of Fenton and Fenton (1979) and by use 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 the end of the experiment, two birds were selected from each replicate and five milliliter blood was taken from wing puncture. Blood samples were centrifuged for 15 min (3000 rpm/min) and serum was separated. The concentration of zinc, copper and manganese were measured by using ICP (Inductively Coupled Plasma Emission Spectrometer, Model JY-24, Jobin Yvon, Longjumeau, Cedex, France).

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 concentration of zinc, copper and manganese in serum and also digestibility of these minerals and apparent metabolizable energy are shown in Table 2. The results showed that addition of MP at rate of 500 FTU/kg to diet increase zinc concentration in serum up to 41% ($p < 0.05$). Interaction effects of EDTA and MP on digestibility of zinc at the end of breeding period in broiler were significant ($p < 0.01$). Addition of EDTA to diets that contained low level of available phosphorus increased digestibility of zinc but addition of EDTA especially in 0.1% level to diets that contained low level of available phosphorus supplemented with MP decreased digestibility of zinc. It is reported that phytase can improve availability of some minerals including zinc by breaking up phytate and absorption of these minerals can be influenced negatively by phytate of diet (Saha et al., 1994).

In fact in poultry diets in which availability of zinc is low because of high level of phytate, addition of phytase can increase efficacy of zinc (Sebastian et al., 1996). Interaction effect of EDTA and MP on concentration of copper and manganese in serum of broilers fed with low level of available phosphorus was significant ($p < 0.01$) and different levels of EDTA in different levels of MP did not affect equivalent. Comparison of mean of different treatments showed that addition of different levels of EDTA to diets not supplemented with MP and contained low level of available phosphorus increased concentration of copper in serum whereas addition of EDTA at 0.2% level to diets supplemented with MP decreased concen tration of copper in serum in comparison with diets that contained low available phosphorus supplemented with MP alone or 0.1% EDTA and MP.

Comparison of mean of different treatments groups for manganese concentration in serum showed that addition

of different level of EDTA to diets not supplemented with MP and contained low available phosphorus increased manganese concentration in serum but addition of EDTA especially at 0.1% level to diets that contained low available phosphorus supplemented with MP decreased concentration of manganese in serum. Reason for why addition of EDTA to diets that contained low available phosphorus supplemented with MP decreased concentration and digestibility of mineral is unknown.

Addition of EDTA to diets that contained low available phosphorus increased the digestibility of manganese in broiler ($p < 0.05$). EDTA is a strong chelator which appeared to improve some of the minerals absorption in poultry diet. Previous investigation showed that addition of EDTA to turkey's pullets (Kratzer et al., 1959) and chickens (O'Dell et al., 1964; Vohra and Kratzer, 1965) that contains plant proteins, improved zinc absorption.

The affinity to chelate with metal is quantitatively described as stability coefficient (SC). EDTA has higher SC with all of the minerals and this is the reason for metal sweeper of EDTA and when EDTA is available in system it could bind all cations (Kratzer et al., 1959).

Addition of 500 FTU/kg of MP to diets that contained low available phosphorus in broilers decreased digestibility of manganese up to 23.12% ($p < 0.05$). The reason why addition of MP to diets decreased digestibility of manganese is unknown. These results do not agree with results of Saha et al. (1994) and Sebastien et al. (1996). Also results of this study on increase in efficacy of MP by the addition of EDTA in diets that contained low available phosphorus do not agree with results of Maenz et al. (1999). These researchers showed that addition of EDTA to the culture medium that 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 was not affected by EDTA.

Digestibility of copper and digestibility of apparent metabolizable energy were not affected in the treated groups. From this study it could be deduced that (1) supplementing low available phosphorus diets with different levels of EDTA increases the bioavailability of zinc and manganese in broiler chicks, (2) addition of different levels of EDTA to the low available phosphorus diets which was supplemented with MP, did not improve the efficacy of MP on the bioavailability of zinc, copper, manganese and apparent metabolizable energy (AME_n) in broiler chicks, and (3) the addition of 500 FTU/kg MP to the corn-soybean meal based diets with 0.2% phosphorus, increases the bioavailability of zinc inbroiler chicks.

REFERENCES

Angel R, Dhandu AS, Applegate TJ, M. Christman (2001). Phosphorus sparing effect of phytase, 25-hydroxycholecalciferol, and citric acid when fed to broiler chicks. Poult. Sci., 80 :133-134.

Biehl RR, Baker DH (1996). Efficacy of supplemental 1α-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus or amino acid deficient corn-soybean meal diets. J. Anim. Sci., 74: 2960-2966.

Biehl RR, Baker DH, Deluca HF (1995). 1-α-hydroxylatedcholecalciferol compounds act additively with microbial phytase to improve phosphorous, zinc, and manganese utilization in chicks fed soy-based diets. J. Nutr. 125: 2407-2416.

Boling SD, Webel DM, Marromichalis I, Parsons CM, Baker DH (2000). The effects of citric acid on phytate phosphorus utilization in young chicks and pigs. J. Anim. Sci., 78: 682-689.

Boling-Frankenbach SD, Snow JL, Parsons CM, Baker DH (2001). The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci., 80: 783-788.

Edwards HM Jr (1993). Dietary 1, 25-dihydroxycholecalciferol supplementation increases natural phytate phosphorous utilization in chickens. J. Nutr. 123: 567-577.

Edwards, H.M., Jr. 2002. Studies on the efficacy of cholecalciferol and derivatives for stimulating phytate utilization in broiler. Poult. Sci. 81: 1026-1031.

Fenton TW, Fenton M (1979). Determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 58: 631-634.

Gordon RW, Roland DA Sr (1997). Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. Poult. Sci. 76: 1172-1177.

Kratzer FH, Alfred JB, Davis PN, Marshall BJ, Vohra P (1959). The effect of autoclaving soybean protein and the addition of ethylene di amine tetra acetic acid on biological availability zinc for turkey poults. J. Nutr. 68: 313-316.

Maenz DD, Engele-Schann CM, Newkirk RW, Classen HL (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 edition National Academy Press Washington, D.C., U.S.A.

Nelson TS (1976). The hydrolysis of phytate phosphorus by chicks and laying hens. Poult. Sci. 55: 2262-2267.

O'Dell BL, Yohe JM, Savage JE (1964). Zinc availability in the chick as affected by phytate, calcium and ethylenediamine teraacetate. Poult. Sci. 43: 415-419.

Rafacz, K.A, Martinez C, Snow JL, Baker DH, Parsons CM (2003). Citric acid improves phytate phosphorus utilization in two breeds of chicks fed a corn-soybean meal diets. Poult. Sci. 82:(Suppl.1) : 142. (Abst.).

Ravindran V, Cabahug S, Selle PH, Bryden WL (2000). Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41: 193-200.

Saha PR, Weaver CM, Mason AC (1994). Mineral bioavailability in rats from intrinsically labeled whole wheat flour of various phytate levels. J. Agric. Food Chem., 44: 2531-2535.

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

Sebastian S, Touchburm SP, ER Chavez, Lague PC (1996). The effect of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed a corn – soybean diets. Poult. Sci. 75: 729-736.

Snow JL, Baker DH, Parsons CM (2004). Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poult. Sci. 83: 1187-1192.

Vohra P, Kratzer FH (1965). Influence of various chelating agents on the availability of zinc. J. Nutr. 82: 249-256.