ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 6 (4): 189-195, 2012
ISSN 1819-1878 / DOI: 10.3923/ajas.2012.189.195

Effects of Combination of Citric acid and Microbial Phytase on the Serum Concentration and Digestibility of some Minerals in Broiler Chicks

Yahya Ebrahimnezhad, Naser Maheri-Sis, Abolfazl Aghajanzadeh-Golshani, Jamshid Ghiasi Galekandi, Masoud Sarikhan and Akbar Darvishi
Department of Animal Science, Faculty of Agriculture, Shabestar Branch, Islamic Azad University, Shabestar, East Azerbaijan, Iran

Corresponding Author: Yahya Ebrahimnezhad, Department of Animal Science, Faculty of Agriculture, Shabestar Branch, Islamic Azad University, Shabestar, East Azerbaijan, Iran

ABSTRACT

This experiment was conducted to evaluate the combined effects of Citric Acid (CA) 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, 2.5 and 5% CA and 0 and 500 FTU MP). Four replicate of 15 chicks per each were fed dietary treatments. 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 interaction effect of CA and MP on concentration of copper, zinc and manganese in serum of broilers fed with low available phosphorus diets was significant ($p<0.05$). Adding 2.5% CA into low available phosphorus diets increased digestibility of zinc in comparison to diets without CA and with 5% CA in broiler. Adding CA into low available phosphorus diets increased manganese digestibility on based corn-soybean meal diets ($p<0.01$).

Key words: Citric acid, microbial phytase, zinc, copper, manganese

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, 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. Akyurek *et al.* (2005) demonstrated that phytase supplementation did not affect broiler performance whereas it significantly increased calcium and phosphorus retention in broiler chicks. It is known that the phytase (Edwards, 1993; Biehl *et al.*, 1995; Biehl and Baker, 1996; Gordon and Roland, 1997; Bingol *et al.*, 2009; Ziaei *et al.*, 2009; El-Sherbiny *et al.*, 2010) 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 affectively be use to develop the availabilities of phytate in non-ruminant animals. Ebrahim Nezhad *et al.* (2008a) concluded that, Adding 300 FTU kg^{-1} microbial phytase to the corn-soybean meal based diets with

0.1% phosphorus, increased the concentration of serum copper and manganese and phosphorus of tibia ash in laying hens at 53-64 week age.

There is little information to say that if organic acids (except of citric acid) can improve the availability of phytate phosphorus in poultry. Ebrahim Nezhad *et al.* (2008b) reported that, Supplementing low available phosphorus diets with different levels of citric acid, increased the concentration of serum copper and the percentage of tibia ash in laying hens at 53-64 week age. Results obtained in our previous study suggest that contrary to the effects of citric acid in broiler chicks, this organic acid couldn't enhance phytase effectiveness in laying hens, probably due to high levels of Ca in laying hens diets (Ebrahim Nezhad *et al.*, 2007). Ebrahim Nezhad *et al.* (2008c) reported that, the 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 in laying hens. The EDTA is an organic acid which has similar potential with citric acid, and it increases availably of same minerals. Ethylenediaminetetraacetic acid (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 its 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 research was to study the combination effect of citric acid 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, 2.5 and 5% CA and 0 and 500 FTU MP). Four replicates of 15 chicks per each were fed dietary treatments including (1) P-deficient basal diet [0.2% available phosphorus (aP)] (NC); (2) NC + 500 FTU MP per kilogram of diet; (3) NC + 2.5% CA per kilogram of diet; (4) NC + 2.5% CA + 500 FTU MP per kilogram of diet; (5) NC + 5% CA per kilogram and (6) NC + 5% CA + 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 citric acid used in this experiment was monohydrate 92% which was added to the diets after calculating purity percentage.

At day 44, chromium oxide (Cr_2O_3) was added to all diets at 0.1% level as a detectable marker for specifying of zinc (Zn^{++}), copper (Cu^{++}), manganese (Mn^{++}) and also apparent metabolizable energy. To determine digestibility of minerals, 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

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

	Treatment					
Item	1	2	3	4	5	6
Ingredients (%)						
Corn	62.32	62.22	58.36	58.26	54.41	54.31
Soybean meal (44%)	33.72	33.74	34.48	34.5	35.24	35.26
Soybean oil	0.29	0.32	0.75	0.78	1.21	1.24
Oyster shell	2.26	2.26	2.25	2.25	2.24	2.24
Di calcium phosphate	0.3	0.3	0.31	0.31	0.32	0.32
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	0.21	0.21
Citric acid (92%)	-	-	2.73	2.73	5.47	5.47
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 kg^{-1} of die; 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, Folic acid: 1 mg, Biotin: 0.10, Niacin: 35 mg, Pyridoxine: 4 mg, Choline chloride: 250 mg, Ethoxyquine: 0.125, Supplied kg^{-1} 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 kg^{-1} of diet, ME: Metabolisable energy, CP: Crude protein, Ava. P: Available Phosphorous, TP: Total phosphorous

use 1 mm pore filter to homogenize them. 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 at percent. Chromium (Cr) concentration in feeds and feces samples were measured by method explained by Fenton and Fenton (1979) and by using spectrophotometer. Digestibility of minerals was calculated by following formula (Ravindran *et al.*, 2000):

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

At the end of experiment, two birds were selected from each replication and 5 mL blood was taken from wing puncture. Blood samples centrifuged for 15 min (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).

Statistical analysis: Data were statistically evaluated by the analysis of variance procedure of SAS software (SAS Institute, 1990), involving a factorial arrangement of main factor (citric acid and microbial 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 CA and MP on concentration of zinc, copper and manganese in serum and also digestibility of these minerals and apparent metabolizable energy are presented in Table 2. The results showed that interaction effect between CA×MP on the percentage of zinc, copper and manganese at the end of breeding period in broilers that fed with low available phosphorous diets were significant $p<0.01$, $p<0.0001$ and $p<0.05$, respectively, Also adding of CA into low available phosphorus diets that supplemented MP, decreased the concentration of zinc in serum $p<0.05$. However, supplementing these diets with 5% CA without adding MP, increased zinc concentration in serum in comparison to diets without MP and 2.5% CA ($p<0.05$). The concentration on copper in serum increased when low available phosphorus diets were supplemented by CA in comparison to the control group ($p<0.05$), although adding 2.5% CA into low available phosphorous diets supplemented with MP, decreased copper in serum ($p<0.05$). Also, adding CA into low available phosphorous diets, increased manganese in serum in comparison to the control group ($p<0.05$). Likewise, manganese concentration in serum increased when 5% CA added to low available phosphorous diets supplemented with MP ($p<0.05$). These results showed that CA did not have effect on the efficiency of MP in low available phosphorous diets. In addition, it can be said that adding 2.5% CA into low available phosphorous diets supplemented with MP, caused reduced activity of MP. The exact reason of this reaction in not clear, but it might be because of the lack of response of MP activity to CA supplementation in low available phosphorous diets, maybe reflective of the fact that reduced pH in Gastrointestinal tract (GIT) caused by adding CA leads to lower MP efficiency, although CA is an organic acid that metabolized in body rapidly and it does not have much effect on the pH of intestine. These results are in agreement with the findings of Radcliffe *et al.* (1998) who observed that adding 1.5-3% CA to the piglet's diets had significantly decreased the MP activity in the gastric digesta of piglets after slaughtered. Brenes *et al.* (2003) reported, adding 2% CA into low available phosphorous diets, had no effect on the efficacy of MP that results of recent experiment was in agreement with findings of these researchers whereas Deepa *et al.*, (2011) indicated that, the addition of phytase improved the growth performance and increased Ca, P and N retention in chicks. Also, inclusion of CA along with phytase caused an increase in mineral utilization. These researchers demonstrated that, proper combination of CA and phytase may represent a practical solution to improve growth performance, phytate P and other mineral utilization. Adding CA into low available phosphorous diets increased the digestibility of zinc and manganese in comparison to the control group. Also there was a significant difference between different levels of CA added to these diets. For instance, adding 5% CA, decreased digestibility of some minerals in comparison to 2.5% CA ($p<0.05$). In this experiment, adding different levels of CA into low available phosphorous diets, increased manganese digestibility (40%) in comparison to the control group ($p<0.05$). It seems that, reducing the pH of gastric due to CA added to the diet, would reduced the passage rate time from intestine and it provides enough time for absorbing of nutrient from intestine. Han *et al.* (1998) suggested that, may be organic acids like CA, increases solubility of phosphorous in digesta and also transitions time of digesta from intestine, consequently increases total absorbance of phosphorous.

Digestibility of copper and apparent metabolizable energy was not affected by treatments (Table 2).

Table 2: The Effects of CA and MP on the serum concentration and digestibility of some minerals in broiler chicks

Treatment								
MP (FTU kg^{-1})	CA (%)	AME_n (kcal kg^{-1})	Manganese dig. (%)	Copper dig. (%)	Zinc dig. (%)	Manganese (µg L^{-1})	Copper (µg L^{-1})	Zinc (µg L^{-1})
0	0	3104.7	26.1	24.1	23.9	103.2^{d}	36.4^{d}	187.2^{c}
500	0	3045.7	28.6	26.1	33.9	109.1^{c}	134.3^{b}	373.4^{a}
0	2.5	3201.1	47.2	24.5	67.0	119.9^{b}	44.5^{c}	173.0^{c}
500	2.5	3179.2	43.9	24.2	70.4	116.3^{b}	28.2^{d}	264.1^{bc}
0	5	2954.2	56.4	24.7	42.3	207.0^{a}	190.9^{a}	399.4^{a}
500	5	3003.4	37.0	22.3	46.0	206.6^{a}	133.1^{b}	297.2^{ab}
SEM pooled	99.7	5	1.6	5	1.4	9.9	31.8	
Main effects								
0	CA	3075.2	27.4^{b}	25.1	28.9^{c}	106.2^{c}	85.3^{b}	280.3^{ab}
2.5		3190.1	45.5^{a}	24.4	68.7^{a}	118.1^{b}	36.3^{c}	218.5^{b}
5		2978.8	46.7^{a}	23.5	44.2^{b}	206.8^{a}	162.0^{a}	348.3^{a}
0	MP	3086.6	43.2	24.5	44.4	143.4	90.6	253.2^{b}
500		3076.1	36.5	24.2	50.1	144.0	98.5	311.6^{a}
Probabilities								
	CA	0.1479	0.0035	0.6038	0.0001	0.0001	0.0001	0.0054
	MP	0.8989	0.1252	0.8640	0.1861	0.6005	0.0934	0.0444
	CA×MP	0.8605	0.1175	0.3895	0.7640	0.0188	0.0001	0.0022

Means in columns with no common 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, AME: Apparent metabolisable energy, dig.: Digestibility

CONCLUSION

From the present study it can be concluded that supplementing low available phosphorus diets with different levels of CA, increases the digestibility of zinc and manganese in broiler chicks. Adding different levels of CA to the low available phosphorus diets which supplemented with MP, did not improved the efficacy of MP on the bioavailability of zinc, copper, manganese and Apparent metabolizable energy (AME_n) in broiler chicks. Adding 500 FTU kg^{-1} MP to the corn-soybean meal based diets with low available phosphorus, do not affect the bioavailability of zinc, copper and manganese in broiler chicks.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent financial and technical assistance of Shabestar Islamic Azad University, and Shahid Sardari's Educational and Research Center of Agricultural Jahad Ministry of East Azerbaijan.

REFERENCES

Akyurek, H., N. Senkoylu and M.L. Ozduven, 2005. Effect of microbial phytase on growth performance and nutrients digestibility in broilers. Pak. J. Nutr., 4: 22-26.

Angel, R., A.S. Dhandu, T.J. Applegate and M. Christman, 2001. Phosphorus sparing effect of phytase, 25-hydrocycholecalciferol and citric acid when fed to broiler chicks. Poult. Sci., 80: 133-134.

Biehl, R.R. and D.H. Baker, 1996. Efficacy of supplemental 1 alpha-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus or amino acid deficient corn-soybean meal diets. J. Anim. Sci., 74: 2960-2966.

Biehl, R.R., D.H. Baker and H.F. Deluca, 1995. 1 alpha-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.

Bingol, N.T., M.A. Karsli, D. Bolat, I. Akca and T. Levendoglu, 2009. Effects of microbial phytase on animal performance, amount of phosphorus excreted and blood parameters in broiler fed low non-phytate phosphorus diets. Asian J. Anim. Vet. Adv., 4: 160-166.

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

Boling-Frankenbach, S.D., J.L. Snow, C.M. Parsons and D.H. Baker, 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci., 80: 783-788.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro and C. Braro, 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol., 110: 201-219.

Deepa, C., G.P. Jeyanthi and D. Chandrasekaran, 2011. Effect of phytase and citric acid supplementation on the growth performance, phosphorus, calcium and nitrogen retention on broiler chicks fed with low level of available phosphorus. Asian J. Poult. Sci., 5: 28-34.

Ebrahim Nezhad, Y., M. Shivazad, R. Taherkhani and K. Nazeradl, 2007. Effects of citric acid supplementation on phytate phosphorus utilization and efficiency of microbial phytase in laying hen. J. Boil. Sci., 7: 638-642.

Ebrahim Nezhad, Y., N.M. Sis, A.A. Gholshani, Y. Saedi and R. Aminvakili, 2008a. The effects of combination of citric acid and microbial phytase on the concentration of some minerals of serum and parameters of mineralization of tibia in commercial laying hens. AJAVA., 3: 375-380.

Ebrahim Nezhad, Y., N.M. Sis, H.A. Shahryar, M.R. Dastouri, A.A. Golshani, A. Tahvildarzadeh and K.A. Najafyan, 2008b. The effects of combination of citric acid and microbial phytase on the egg quality characteristics in laying hens. Asian J. Anim. Vet. Adv., 3: 293-297.

Ebrahim Nezhad, Y., G.H. Bibalani, E.J. Helan, K.H. Nezhad, J.D. Sharaf and R. Ebrahim-Nezhad, 2008c. The effects of combination of ethylenediaminetetraacetic acid and microbial phytase on the concentration of some minerals of serum in laying hens. AJAVA., 3: 351-356.

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

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

El-Sherbiny, A.E., H.M.A. Hassan, M.O. Abd-Elsamee, A. Samy and M.A. Mohamed, 2010. Performance, bone parameters and phosphorus excretion of broilers fed low phosphorus diets supplemented with phytase from 23 to 40 days of age. Int. J. Poult. Sci., 9: 972-977.

Fenton, T. and M. Fenton, 1979. Determination of chromic oxide in feed and feces. Can. J. Anim. Sci., 58: 631-635.

Gordon, R.W. and D.A. Roland, 1997. Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. Poult. Sci., 76: 1172-1177.

Han, Y.M., K.R. Roneker, W.G. Pond and X.G. Lei, 1998. Adding wheat middlings, microbial phytase and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. J. Anim. Sci., 76: 2649-2656.

Kratzer, F.H., J.B. Alfred, P.N. Davis, B.J. Marshall and P. Vohra, 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, 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 slurry of canola meal. Anim. Feed Sci. Technol., 77: 177-192.

NRC, 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC.

Nelson, T.S., 1976. The hydrolysis of phytate phosphorus by chicks and laying hens. Poult. Sci., 55: 2262-2264.

O'Dell, B.L., J.M. Yohe and J.E. Savage, 1964. Zinc availability in the chick as affected by phytate, calcium and ethylenediamineteraacetate. Poult. Sci., 43: 415-419.

Radcliffe, J.S., Z. Zhang and E.T. Kornegay, 1998. The effects of microbial phytase, citric acid and their interaction in a corn-soybean meal-based diet for weanling pigs. J. Anim. Sci., 76: 1880-1886.

Rafacz, K.A., C. Martinez, J.L. Snow, D.H. Baker and C.M. Parsons, 2003. Citric acid improves phytate phosphorus utilization in two breeds of chicks fed a corn-soybean meal diets. Poult. Sci., 82: 142-142.

Ravindran, V., S. Cabahug, G. Ravindra, P.H. Selle and W.L. Bryden, 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci., 41: 193-200.

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

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.

Ziaei, N., M. Shivazad, S.A. Mirhadi and A. Gerami, 2009. Study the effects of reduced calcium and phosphorous diets supplemented with phytase on laying performance of hens. Pak. J. Biol. Sci., 12: 792-797.